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[Authors]. [Abstract Title]. Program No. XXX.XX. 2016 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2016. Online.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.01/A1

Topic: A.07. Developmental Disorders

Support: NIMH Grant R00MH090238

NIMH Grant R01DC014702

NIMH Grant R01MH102603

The Uehara Memorial Foundation

a March of Dimes Basil O'Connor Starter Scholar Research Award (5-FY13-19)

Once Upon a Time Foundation

Friends of the Alzheimer's Disease Center at UT Southwestern, CREW Dallas

Title: Post-translational modification of FOXP1 in the developing brain

Authors: *N. USUI, D. J. ARAUJO, M. CO, M. HARPER, G. KONOPKA;
Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Mutations in the gene encoding the transcription factor forkhead box P1 (*FOXP1*) result in brain developmental abnormalities and developmental disorders such as autism spectrum disorders (ASD) and intellectual disability (ID). However, the molecular mechanisms regulating FOXP1 function during human brain development and how disruption to these functions leads to developmental disorders are largely unknown. Our previous work has demonstrated that sumoylation of the related transcription factor FOXP2 is an important post-translational modification that regulates FOXP2 function and ultimately affects cerebellar development and related behaviors. We therefore assessed whether sumoylation of FOXP1 might similarly regulate its function in the brain. Here, we demonstrate that FOXP1 is sumoylated during brain development, and this modification peaks during embryonic brain development, at the time points relevant to genetic pathways implicated in ASD. The site of FOXP1 sumoylation at K636 is evolutionally conserved from mouse to human. We demonstrate that SUMO-1/2 and PIAS2/3 are the SUMO proteins and E3 SUMO ligases carrying out sumoylation of FOXP1. We further show a requirement for sumoylation of FOXP1 in neuronal development, in particular during embryonic brain development. Together, these data highlight one of the molecular mechanisms regulating the role of an ASD-relevant gene, *FOXP1*, during brain development.

Disclosures: N. Usui: None. D.J. Araujo: None. M. Co: None. M. Harper: None. G. Konopka: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: IVIC-VENEZUELA PO-1114

Title: Counterbalanced neurogenesis after cortical devascularization. a functional interpretation of local circuits

Authors: L. V. VARGAS-SATURNO, Jr[1], *C. A. AYALA-GROSSO[2];
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Abstract: Constitutive neurogenesis occurring in the subgranular (SGZ) and subventricular (SVZ) zones of the brain contribute to the turnover of neurons in circuits of hippocampus and olfactory bulb. Adaptive neurogenic response may occur as a result of intrinsic signals generated by cell components of the niche and, or, as a consequence of extrinsic signalling that include changes in blood supply or neurotransmission. Alteration of cerebral blood flow by ischemia or brain trauma may induce proliferation in neurogenic areas. We asked whether cortical devascularisation in an established animal model of cortical cholinergic denervation may induce a functional a response in neurogenic niches. C57BL/6L male mice of 25g, under deep anesthesia induced by ketamine, xylacine (75/7.5 mg/Kg in saline solution) after intra peritoneal administration, were craneotomized at 1 mm caudal from fronto-parietal suture and 1 mm medial lateral from midline to generate a window of 4 by 4 mm side. Lesion consists of removal of meninge and surface vasculature; control (sham) animals suffered craniotomy but not removal of meninges and blood vessels. After surgery, BrdU (50 mg/Kg), every 2 h, three doses during 48h period were administered to sham and lesioned animals. Animals were intracardially perfused with 4% p-formaldehyde at 1, 4, 7 and 10 days post surgery. Free floating sagital brain sections were analyzed under immunohistochemistry protocols. BrdU+ proliferative cells or coexpression with neuronal/glial progenitors phenotypic markers were counted to determine subpopulation of cells in the neurogenic niche. We established a significant increase of BrdU+ cells at 4 days postsurgery in the ipsilateral and contralateral SVZ and rostral migratory stream (RMS) in lesioned animals with respect to control. We determined a significant increase of BrdU+/Nestin+ and BrdU+/DCX+ in the contralateral SVZ and RMS with respect to ipsilateral neurogenic

hemisphere. We detected BrdU+/DCX+ in the lesioned cortex. No BrdU=/DCX+ cells were determined in the contralateral cortex. Counterbalanced neurogenesis may be explained by dysfunctional local circuits and projections of cholinergic and glutamatergic corticofugal efferents. In summary, dysbalanced cholinergic and glutamatergic neurotransmission was concurrent with proliferation and potentially migration of neural progenitors from SVZ and RMS to injured hemisphere.

Disclosures: L.V. Vargas-Saturno: None. C.A. Ayala-grosso: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.03/A3

Topic: A.07. Developmental Disorders

Support: NIH R01 AG038710

NIH R01 AG021173

NIH R01 NS046673

NIH R01 AG030197

NIH R01 AG044420

NSFC 81571176

NSFC 81202419

Title: SNX27 deletion causes hydrocephalus through impaired ependymal cell differentiation and ciliogenesis

Authors: *X. WANG[1], Y. ZHOU[1], I.-C. TSENG[2], T. HUANG[2], Y. ZHAO[2], H. LUO[1], X. ZHANG[1], G. BU[1], W. HONG[3], H. XU[2];
[1]Inst. of Neurosciences, Xiamen Univ., Fujian, China; [2]Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA; [3]Inst. of Mol. and Cell Biol., Singapore, Singapore

Abstract: Ependymal cells line the cerebral ventricle epithelium and regulate cerebrospinal fluid (CSF) production and circulation. Although apical cilia in ependymal cells play a key role in the development of hydrocephalus, the underlying genetic factors and molecular mechanisms remain largely unknown. SNX27 regulates endosome-plasma membrane receptor trafficking to maintain

proper brain function, and modulates γ-secretase cleavage of APP and Notch. Here, we find that *Snx27*-deficient mice exhibit a marked reduction in ependymal cell and cilia density, abnormal cortical development, and severe postnatal hydrocephalus. SNX27 deficiency results in increased differentiation of radial glial cells into premature neurons and decreased production of ependymal cells. Inhibition of Notch intracellular domain (NICD) signaling using γ-secretase inhibitors reversed ependymal cells/cilia loss and dilation of lateral ventricles in *Snx27*-deficient mice, supporting the notion that Notch activity negatively regulates ependymal cell differentiation and ciliogenesis. Our study suggests that SNX27 is essential for ependymal cell differentiation and ciliogenesis, causing defective CSF flow and hydrocephalus.

Disclosures: X. Wang: None. Y. Zhou: None. I. Tseng: None. T. Huang: None. Y. Zhao: None. H. Luo: None. X. Zhang: None. G. Bu: None. W. Hong: None. H. Xu: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: SFI Grant 09/SRC/B1794s1

SFI Grant 13/IA/1787

NUIG Grant RSU002

Title: Ulk4 regulates the size of neural stem cell pool

Authors: ***S. SHEN**[1], M. LIU[2], Z. GUAN[3], Q. SHEN[4], F. FLINTER[5], L. DOMÍNGUEZ[2], J. AHN[6], D. A. COLLIER[7], T. O'BRIEN[2];
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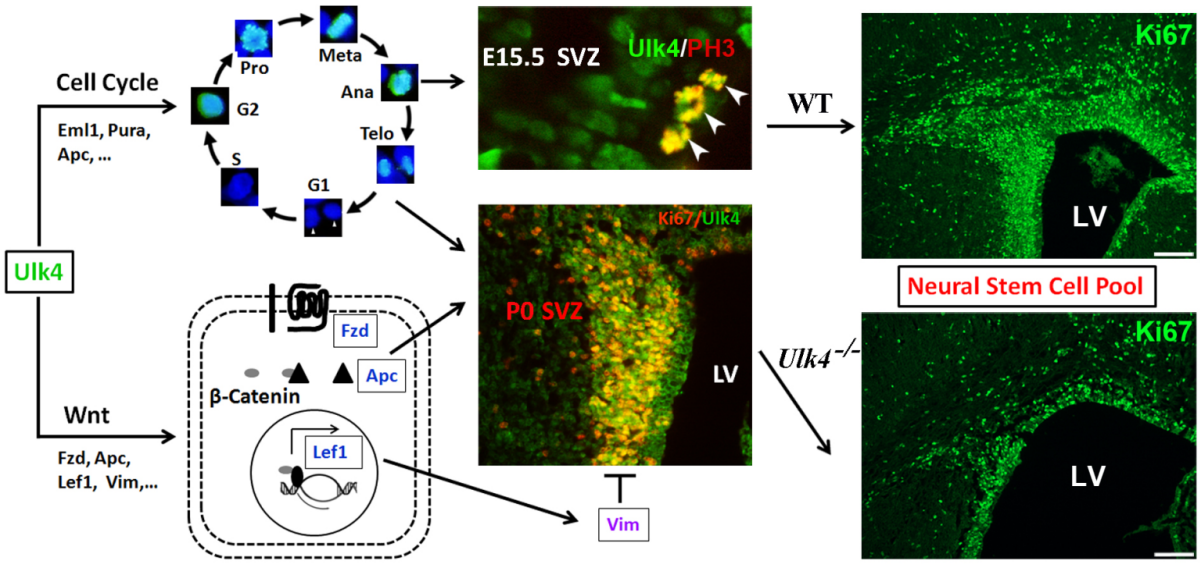
Abstract: The size of neural stem cell (NSC) pool at birth determines the starting point of adult neurogenesis. Aberrant neurogenesis is associated with major mental illness, in which *ULK4* is proposed as a rare risk factor. Little is known about factors regulating the NSC pool, or function of the ULK4. Here we show that *Ulk4*$^{tm1a/tm1a}$ mice display a dramatically reduced NSC pool at

birth. Ulk4 is found to express in a cell cycle-dependent manner and peaked in G2/M phases, and targeted disruption of the *Ulk4* perturbs mid-neurogenesis and significantly reduces cerebral cortex in postnatal mice. Pathway analyses of dysregulated genes in *Ulk4*$^{tm1a/tm1a}$ mice reveal Ulk4 as a key regulator of cell cycle and NSC proliferation, partially through regulation of the Wnt signaling. In addition we have identified hemizygous deletion of *ULK4* gene in 1.2/1000 patients with pleiotropic symptoms including severe language delay and learning difficulties. *ULK4*, therefore, may significantly contribute to neurodevelopmental, neuropsychiatric and neurodegenerative disorders.



Disclosures: S. Shen: None. M. Liu: None. Z. Guan: None. Q. Shen: None. F. Flinter: None. L. Domínguez: None. J. Ahn: None. D.A. Collier: None. T. O'Brien: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Paul Allen Family Foundation

Title: Species-specific mechanisms of cortical neural progenitor differentiation.

Authors: *M. B. JOHNSON[1], K. M. GIRSKIS[1], P. P. WANG[1], J. FAN[2], P. V. KHARCHENKO[2], C. A. WALSH[1];
[1]Genet. and Genomics, Boston Children's Hosp., Boston, MA; [2]Biomed. Informatics, Harvard Med. Sch., Boston, MA

Abstract: The human cerebral cortex, the seat of our most highly developed cognitive functions and the largest structure of our brain, depends for its normal development and ultimate size on a precisely controlled balance between self-renewal and differentiation of diverse **neural progenitor cells** (**NPC**). The canonical NPC type of the mammalian cortex is the **radial glial cell (RGC)** with its distinct morphology: a cell body integrated into the neuroepithelium at the ventricular surface, and a long radial process that guides migrating neurons into the developing cortical plate. In addition to producing neurons directly, RGC also generate **intermediate progenitors (IP)** and **outer radial glia (ORG)**. The mechanisms that balance the self-renewal and differentiation of each of these NPC subtypes remain relatively unknown, motivating us to apply single-cell RNA-sequencing to these cells to examine in finer detail **the molecular time-course and lineage relationships of mammalian neurogenesis.** From these data, we identified **PPP1R17**, whose species- and progenitor subtype-specific expression is regulated by an enhancer derived from a primate lineage-specific insertion of an L1 repeat element. PPP1R17 is an inhibitory regulatory subunit of the phosphatase complex, and regulates the balance of proliferation and self-renewal in part through downstream modulation of canonical growth factor signaling pathways.

Disclosures: M.B. Johnson: None. K.M. Girskis: None. P.P. Wang: None. J. Fan: None. P.V. Kharchenko: None. C.A. Walsh: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Multiple Sclerosis Society (NMSS)

Title: Integrin linked kinase (ILK) deletion disrupts oligodendrocyte development by altering cell cycle

Authors: *R. HUSSAIN, W. B. MACKLIN;
Dept. of Cell and Developmental Biol., Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: During development, neural precursor cells establish lineage-specific differentiation of glia and neurons by sensing environmental cues through membrane bound receptors and intracellular signaling pathways. This is a series of highly orchestrated events involving active communication between the extracellular environment, cytoplasmic mediators and finally nuclear effectors. Integrin linked kinase (ILK) is a crucial partner in signaling between the extracellular environment and the nucleus. It acts as a serine/threonine kinase as well as a scaffolding molecule. Through its scaffolding function, it interacts with numerous proteins and its kinase activity is involved in phosphorylating protein kinase B (AKT) and glycogen synthase kinase (GSK-3β). The current studies demonstrate that deletion of ILK in early neural precursors severely alters the normal commitment to oligodendrocyte precursor cells (OPCs). We found a significant decrease in the number of OPCs and mature oligodendrocytes throughout postnatal development in Olig1-Cre $^{+/-}$ X ILK $^{fl/fl}$ mice. These changes were accompanied by a marked decrease in phosphorylation of AKT at Ser473 and S6RP at Ser 235/236 and Ser 240/244. The number of myelinated axons was reduced at P30 in corpus callosum and spinal cord, likely because of reduced numbers of oligodendrocytes. Myelin thickness in spinal cord was unchanged, but it was thinner in corpus callosum. Intriguingly, we found a decrease in key proteins involved in cell cycle regulation; Cyclin D1/D3 and cyclin dependent kinase 2/4 (cdc2/cdc4) by western blot and immunohistochemistry along with upregulation of the inhibitory signaling protein p27 Kip1. In conclusion, ILK deletion impairs the developmental profile and proliferation potential of OPCs by interfering with cell cycle, but in general in cells that generate myelin, the myelin is relatively normal. This suggests that ILK has a greater impact on OPC proliferation than on myelination per se. Supported by the National Multiple Sclerosis Society.

Disclosures: R. Hussain: None. W.B. Macklin: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS084398

Title: Sustained genomic abnormalities in embryonic neural progenitor cells (NPCs) may underlie cellular pathologies of Lysophosphatidic Acid induced congenital hydrocephalus in mice.

Authors: *W. S. MCDONALD, Y. YUNG, N. STODDARD, M. LEE, J. CHUN;
The Scripps Res. Inst., La Jolla, CA

Abstract: Post-hemorrhagic hydrocephalus (PHH) is a common neurological disorder afflicting newborns and children characterized by increased head size, cortical thinning, ventricular cerebrospinal fluid (CSF) accumulation and is frequently comorbid with other neural malformations and disorders such as cranial deformity, brain tissue loss, psychiatric and cognitive impairments. There are no curative therapies, with major treatments limited to palliative, surgical approaches that utilize shunts to remove excess CSF. Although the etiology of PHH isn't fully characterized, we identified the involvement of lysophosphatidic acid (LPA), a bioactive and blood-borne lysophospholipid, through its cognate G protein-coupled receptor LPA_1, in PHH pathogenesis (Yung et al., 2011). Our LPA-induced model of PHH recapitulates many pathological features of hemorrhagic injury mechanism identified in clinical hydrocephalus cases including cortical cell loss or dysfunction, NPC lineage alterations (including ependymal cell loss), ciliary dysfunction and/or loss, choroid plexus dysfunction and intracranial fluid imbalances. The existence of genomic mosaicism in normal brain through aneuploidies suggested a link between LPA exposure and genomic alterations, in view of an identified 8-16% of congenital hydrocephalus cases associated with aneuploidy, which predict a higher risk of poor clinical outcomes as well as other neurological disorders (*i.e.,* schizophrenia, and Down syndrome). To determine the effects of fetal exposure to LPA on NPC genomic abnormalities, E13.5 mouse embryos received varied dosing of intraventricular LPA, followed by DNA content analyses by flow cytometry and cytogenetics approaches in embryonic cortices from E14.5 to P0. Our results show that within 24 hours of LPA exposure, acute changes in chromosomal content are observed in cortical progenitor cells. Maintained genomic NPC aberrations were also observed at later ages. The results suggest that LPA exposure at critical periods during embryonic development alters survival, fate and functions of NPCs and neurons, which may promote CNS pathologies associated with hydrocephalus.

Disclosures: W.S. McDonald: None. Y. Yung: None. N. Stoddard: None. M. Lee: None. J. Chun: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS073112

Shriners Hospitals for Children Research Grant 85410

Title: Novel subcortical heterotopia caused by loss of basal complex protein, Lgl1, through dysregulated cell proliferation and polarity

Authors: *A. SIMMONS[1], R. PARK[1], A. PELUZZO[1], S.-H. CHO[1], V. VASIOUKHIN[2], S. KIM[1];
[1]Shriners Hosp. Pediatric Res. Center, Dept. of Anat. and Cell Bio, Temple Univ. Lewis Katz Sch. of Med., Philadelphia, PA; [2]Div. of Human Biol., Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: Lethal giant larvae (Lgl1), a known tumor suppressor, has been shown to have a critical role in cell polarity maintenance and has been proposed to function upstream of cell growth regulation pathways. Due to early neonatal lethality, the role of Lgl1 in cortical development and beyond has not been explored. Here we demonstrate that conditional loss of Lgl1 in the developing cortical neuroepithelium results in severe disruption of cell polarity and the generation of an ectopic proliferating zone, ultimately leading to a novel subcortical heterotopia model with hydrocephalus. The heterotopic cortex forms as a result of hyperproliferation of cortical progenitors and significant disruption to tissue integrity. Loss of Lgl1 increases the apical progenitor pool by reducing cell cycle length with no change to S-phase length. The severely compromised ventricular lining results in rosette formation and a displaced apical membrane serving as a platform for bidirectional growth. Furthermore, the rosettes and ectopic ventricular lining are concentrated with apical protein Pals1, suggesting its function in proliferation at the ectopic locations. Remarkably, concurrent loss of Lgl1 and Pals1 results in removal of ectopic proliferation but partial restoration of cortical tissue in the decorticated *Pals1* CKO through accelerated cell proliferation. This finding provides unique insight on the interplay between polarity complex proteins in regulation of cortical progenitor proliferation. Thus, our study identifies Lgl1 as a new subcortical heterotopia causing gene by uncovering its role in cell cycle length regulation in combination with its well-established cell polarity function.

Disclosures: A. Simmons: None. R. Park: None. A. Peluzzo: None. S. Cho: None. V. Vasioukhin: None. S. Kim: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Title: Kruppel-like factor 5 maintains neural precursor cells undifferentiated in the developing brain

Authors: *T. FUCHIGAMI[1], Y. HAYASHI[2], A. KURODA[2], T. AZAMI[3], M. EMA[3], S. HITOSHI[2];
[2]Integrative Physiol., [3]Res. Ctr. for Animal Life Sci., [1]Shiga Univ. of Med. Sci., Ohtsu City/Shiga Prefecture, Japan

Abstract: Kruppel-like factor (Klf) family proteins are DNA-binding transcriptional factors, which share highly conserved sequences and redundant functions, for instances, in the regulation of cell cycle, cell differentiation and tissue organization. Among Klf family, Klf5 is indispensable for the blastocyst implantation, segregation into three germ layers and formation of cardiovascular system and optic vesicles. Klf5 shares overlapping function with Klf4, one of the Yamanaka factors, and it not only helps maintain the pluripotency of ES cells but also enhances reprogramming somatic cells to generate iPS cells. Although these precedence studies suggest the broad roles of Klf5 in the organogenesis, its roles in the central nervous system has not been investigated despite of its expression in the developing brain. We have investigated roles of Klf5 in the proliferation and maintenance of neural precursor cells (NPCs) in the developing cortex. Overexpression of Klf5 by *in utero* electroporation promoted the proliferation of the apical progenitors, whereas shRNA-mediated knockdown of *Klf5* showed little effects, possibly due to the redundant functions by other Klf family proteins. Indeed, the knockdown of *Klf2*, *Klf4* and *Klf*5 resulted in the significant reduction of the apical progenitor proliferation and in the impaired migration of NPCs. Furthermore, the self-renewal of NPCs was also reduced in the developing brain of NPCs-specific *Klf5* conditional knockout mice. Our data suggest that Klf5, together with Klf2 and Klf4, plays important roles in the neural development.

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

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant NS46616

Title: Aristaless related homeobox (Arx) interacts with β-catenin, Bcl9, and p300 to regulates canonical wnt signaling

Authors: I.-T. CHO, Y. LIM, *J. A. GOLDEN, G. CHO;
Dept. of Pathology and Lab. Med., Brigham and Women's Hosp., Boston, MA

Abstract: Mutations in the Aristaless Related Homeobox (ARX) gene are associated with a spectrum of structural (lissencephaly) and functional (epilepsy and intellectual disabilities) neurodevelopmental disorders. How mutations in this single transcription factor can give rise such a broad range of phenotypes remains poorly understood. We have hypothesized that ARX regulates unique target genes through distinct interactions with specific transcription factors/cofactors in different cell type. To identify ARX interacting proteins, we used an unbiased proteomics approach and unexpectedly identified several components of the Wnt pathway including Ctnnb1 (β-catenin), BCL9 and LRRFIP2 expressed highly in cortical progenitor cells. The following studies revealed ARX positively regulates Wnt signaling and that the C-terminal domain of ARX interacts with the armadillo repeats in β-catenin to promote Wnt signaling. In addition, BCL9 and P300 cooperate with ARX to modulate Wnt signaling. These data provide new insight into how ARX can uniquely regulate cortical progenitor cell population and links two well-defined networks (ARX and Wnt) that had not previously been associated with each other.

Disclosures: I. Cho: None. Y. Lim: None. J.A. Golden: None. G. Cho: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS075393

Title: Essential role of KIF20A/MKLP2 in regulation of neural progenitors symmetric versus asymmetric division in the developing brain

Authors: *A. GENG[1], R. QIU[2], Q. LU[2];
[1]Developmental & stem cell Bio, [2]Developmental & Stem Cell Bio, Beckman Res. Inst. City of Hope, Duarte, CA

Abstract: The balanced production of progenitors and neurons is critical to embryonic neocortical development, but the governing mechanisms are not well understood, particularly with respect to the role of the cell division machinery, which is expected to control the mode (symmetric self-renewal versus asymmetric differentiation) of progenitor cell divisions. In this study, we investigated the functions of KIF20A/MKLP2, a mitotic kinesin important for ordered progression of cytokinesis, during cerebral cortical development. We show that KIF20A binds to RGS3 and facilitates its translocation into the intercellular bridge, where RGS3 and Gα subunit

coordinate in cell fate regulation. KIF20A is symmetrically expressed by daughter cells in self-renewal and asymmetrically expressed in daughter progenitors during differentiation. Inhibition of KIF20A function blocks the action of Ephrin-B/RGSsignaling and leads to early neuronal differentiation. Genetic knockout of Kif20a caused a loss of progenitors and neurons and resulted in thinner cortex and ventriculomegaly. Interestingly, loss-of-function of KIF20A induced early cell cycle exit and precocious neuronal differentiation without causing substantial apoptosis in mutant progenitor cells, and led to severe impairment in the production of cortical neurons. Collectively, the mitotic kinesin KIF20A/MKLP2 regulates symmetric versus asymmetric cell divisions in the embryonic cerebral cortex in coordination with the regulator of G protein signaling (RGS)-mediated Ephrin-B pathway. Our study demonstrates a crucial role of KIF20A in balancing self-renewal and differentiation during brain development and reveals a potential link of cytokinesis control to regulation of cell fate determination.

Disclosures: **A. Geng:** None. **R. Qiu:** None. **Q. Lu:** None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.12/A12

Topic: A.01. Neurogenesis and Gliogenesis

Title: The function of hominoid specific gene tbc1d3 on cortical folding

Authors: ***Q. HOU**, X. JU, Z.-G. LUO;
Lab. of Synaptic Signaling, Institude of Neurosci., Shanghai city, China

Abstract: It is generally assumed that the expansion of the mammalian neocortex during evolution correlates with the increase in intelligence, but it was unknown whether induction of cortical folding in animals with lissencephalic cortex could enhance cortical functions. We found that expression of the hominoid-specific gene *TBC1D3* in ventricular cortical progenitors of mice via *in utero* electroporation disrupted ventricular adherens junctions and caused delamination of radial glia, leading to an increased number of self-renewing basal progenitors with typical morphology of outer radial glia (oRG), which are most abundant in primates. Furthermore, localized oRG proliferation, resulting from either *in utero* electroporation or transgenic expression of TBC1D3, was often found to underlie cortical regions exhibiting folding. Notably, the transgenic mice showed more rapid motor learning during rotarod training. Thus, we have identified a hominoid gene that regulates the cortical expansion and folding via elevating oRG proliferation, and demonstrated that cortical expansion and folding could cause enhanced cognitive function.

Disclosures: **Q. Hou:** None. **X. Ju:** None. **Z. Luo:** None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Title: Role of cdk inhibitor $p57^{kip2}$ in regulating the fate of embryonic neural progenitor cells

Authors: *Y. HARADA, S. FURUTACHI, D. KAWAGUCHI, Y. GOTOH;
Univ. of Tokyo, Tokyo, Japan

Abstract: Adult neural stem cells (aNSCs) generate neurons that modify cognitive functions such as learning and memory. Our previous studies demonstrate that slowly-dividing neural progenitor cells (NPCs) exist in the embryonic ganglionic eminences and that a fraction of this population is destined to become aNSCs located in the subependymal zone (Furutachi et al., Nat. Neurosci. 2015). We found that these slowly-dividing cells highly express the CDK inhibitor $p57^{kip2}$ (p57) and that p57 is essential for quiescence of aNSC and these slowly-dividing embryonic NPCs. However, it remains unclear whether p57 influences the characteristics of aNSCs and these NPCs other than quiescence. In this study, we found that ectopic overexpression of p57 in the embryonic mouse neocortex led not only to cell cycle arrest but also to maintenance of the undifferentiated state of NPCs. This function of p57 appears to require the CDK domain. Moreover, our results suggested that high level expression of p57 results in the activation of Notch signaling. We are now examining the downstream targets and upstream regulators of p57 in the slowly-dividing embryonic NPCs.

Disclosures: Y. Harada: None. S. Furutachi: None. D. Kawaguchi: None. Y. Gotoh: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.14/B1

Topic: A.01. Neurogenesis and Gliogenesis

Support: SKH-TMU-105-09

Title: Characterization of function and regulation of Plzf in the P19 cell model of neurogenesis

Authors: *H.-C. LIN[1], Y.-H. LEE[4], P.-C. PAO[1], W.-C. CHANG[1,2], Y.-C. LEE[3,2];
[1]Grad. Inst. of Med. Sciences, Col. of Med., [2]Ctr. for Neurotrauma and Neuroregeneration, [3]Ph.D. Program for Neural Regenerative Medicine, Col. of Med. Sci. and Technol., Taipei Med. Univ., Taipei, Taiwan; [4]Dept. of Pharmacology, Col. of Med., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Promyelocytic leukemia zinc finger (*Plzf*), a *kruppel*-like zinc finger gene, is a transcriptional regulator and plays a role in maintenance of stem cells of various lineages, such as hematopoietic stem cells and spermatogonial stem cells. Previous studies showed that Plzf expressed in spatially restricted and temporally dynamic patterns in the central nervous system. Studies also revealed that Plzf has a function in maintenance of neural progenitors and inhibition of neurogenesis. In Drosophila, Plzf expression is regulated by Notch signaling, but the detailed mechanism is not clear. P19 embryonal carcinoma cell is a useful model for investigating the molecular mechanisms of neurogenesis. In our preliminary data, we found that Plzf was dramatically induced at the early stage of neuronal differentiation and diminished at the late stage. The temporal expression of Plzf during neuronal differentiation in P19 cells is similar to the pattern of expression observed in brain development. Using promoter activity assay, we found that E2F family, which is important for neural stem cell self-renewal and embryonic development, may activate *Plzf* gene promoter activity. We also found that BrdU incorporation was decreased in the *Plzf* knockdown cells, indicating that Plzf can regulate the neural stem cell-like properties in P19 cells. Further study such as site-directed mutagenesis, ChIP analysis will be used to examine the role of E2F family in P*lzf* gene regulation.

Disclosures: H. Lin: None. Y. Lee: None. P. Pao: None. W. Chang: None. Y. Lee: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.15/B2

Topic: A.01. Neurogenesis and Gliogenesis

Support: CIHR Innovation Grant

Title: Feed Forward Cytokine signalling in glioblastoma pathogenesis

Authors: *A. JAHANI-ASL[1], M. RUDNICKI[2], A. BONNI[3];
[1]McGill Univ., Montreal, QC, Canada; [2]Ottawa Hosp. Res. Inst., Ottawa, ON, Canada;
[3]Washington Univ. Sch. of Med., St Louis, MO

Abstract: EGFRvIII/STAT3 signaling plays a significant role in glioblastoma pathogenesis. Here, we identify the cytokine receptor OSMR as a direct target gene of the transcription factor STAT3 in mouse astrocytes and human brain tumor stem cells (BTSCs). Strikingly, we find that OSMR functions as an essential co-receptor for EGFRvIII. OSMR forms a physical complex with EGFRvIII and depletion of OSMR impairs EGFRvIII/STAT3 signaling. Conversely, pharmacological inhibition of EGFRvIII phosphorylation inhibits the EGFRvIII/OSMR interaction and activation of STAT3. EGFRvIII/OSMR signaling in tumors operates constitutively, whereas EGFR/OSMR signaling in non-tumor cells is synergistically activated by the ligands EGF and OSM. Finally, knockdown of OSMR strongly suppresses cell proliferation and tumor growth of murine glioblastoma cells and human BTSC xenografts in mice and prolongs lifespan of these mice. Our findings identify OSMR as a critical regulator of glioblastoma tumor growth that orchestrates a feed forward signaling mechanism with EGFRvIII and STAT3 to drive tumorigenesis.

Disclosures: A. Jahani-asl: None. M. Rudnicki: None. A. Bonni: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.16/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: BMBF 01GQ1405

Title: Sphingosine-1-phosphate receptor 1 expression in cerebellum regulates Sphingosine-1 Phosphate release and is a mediator of cerebellar granule cell progenitor proliferation and migration

Authors: C. DUMAN, *J. ALFONSO;
Clin. Neurobio., DKFZ, Heidelberg, Germany

Abstract: Sphingosine-1 phosphate (S1P) is a structurally simple lipid molecule that results in several diverse and potent cellular responses such as proliferation, migration and cell death via five G protein coupled receptors, S1P1-5. We previously identified S1P1 signaling as a critical mediator of neuroblast migration through RMS and into the olfactory bulb during the switch

from tangential to radial migration. Here we investigated the role of S1P1 signaling in early postnatal cerebellar development; an excellent model for neuronal progenitor development and subsequent circuit formation. In the first 2.5 weeks of age, mice continue to develop their cerebellum and house granule cell progenitors (GCPs) in the transient outermost layer of the cerebellar cortex, external granule layer (EGL). These progenitors, after rounds of symmetric division, become post-mitotic and first migrate tangentially along EGL. Tangentially migrating cells later switch to radial migration and using Bergmann glia as scaffold, they migrate through molecular layer (ML) to find their end position of differentiation in the inner granule layer (IGL). We found that S1P1 is strongly expressed in Bergmann glia but not expressed in the GCPs, or the Purkinje neurons. Using CNS specific Nestin-CRE x $S1P1^{flx/flx}$ conditional knock out mouse, BrdU pulse labeling together with acute slice imaging, we studied the proliferation and migration dynamics of GCPs. We found that lack of S1P1 in cerebellum results in significantly increased proliferation of GCPs in EGL at the age P10 and alter migration dynamics into the IGL at subsequent time points *in vivo*. Finally, we observed that in conditional S1P1 knockout mice cerebellum S1P producing enzyme sphingosine kinase 2 (SPHK2) levels are significantly increased. Our data indicate that the S1P1 signaling is potentially playing an indirect role in proliferation of GCPs through increased S1P levels in the cerebellum.

Disclosures: C. Duman: None. J. Alfonso: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.17/B4

Topic: A.01. Neurogenesis and Gliogenesis

Support: NINDS grant R01 AG037506

NINDS grant NS66041

AHA Grant-in-Aid15GRNT25560025

Title: MSCs promote axonal outgrowth via neuronal tPA and synergistically with astrocytic tPA

Authors: *J. QIAN[1], M. CHOPP[1,2], X. SHANG[1], Z. LIU[1];
[1]Henry Ford Hosp., Detroit, MI; [2]Oakland Univ., Rochester, MI

Abstract: We reported that exogenous administration of mesenchymal stromal cells (MSCs) after experimental stroke enhances neurological recovery, that MSCs increase tPA expression in astrocytes both in vivo and in vitro, and intranasal administration of tPA promotes neurological

recovery post stroke in animals. Here, using a microfluidics chamber, we tested the hypothesis that MSCs increase axonal outgrowth directly via neuronal tPA and indirectly via stimulating tPA expression in astrocytes. Primary murine neurons and astrocytes were isolated from WT and tPA-KO cortices of day 17-18 mouse embryos. Mouse MSCs (WT) were purchased from Cognate Inc. Neurons (WT or tPA-KO) were seeded in soma side of Xona microfluidics chambers. Three days later, astrocytes (WT or tPA-KO) and/or MSCs were seeded and cultured in the axonal side of the chambers. To test the effect of MSCs and astrocytes on hypoxic axons, a separate group of microfluidics (same neuronal and astrocyte/MSC conditions as above) were subjected to oxygen deprivation for 2 hours on the 4th day after neuron seeding. At day 6, all cells were stained by anti-Tuj-1 antibody for axon quantification. In a third group, primary neurons, astrocytes and/or MSCs were co-cultured for 3 days to quantify tPA by Western blot. In the microfluidic chamber, WT axons grew faster than tPA-KO axons (n=10-11, $P<0.05$). MSCs promoted WT axonal outgrowth alone (n=8-11, $P<0.05$) and synergistically with WT astrocytes (n=8-10, $P<0.05$) at both normoxia and oxygen deprivation conditions. The synergistic effect was inhibited by the ERK inhibitor, U0126 at 50uM (n=9-10, $P<0.05$). However, MSCs exerted no effect on tPA-KO axonal outgrowth after the same treatments (n=10-12, $P>0.05$). Western blot showed that co-culture of MSCs with WT-astrocytes enhanced tPA expression in astrocytes. We conclude that MSCs promote axonal outgrowth via neuronal tPA and synergistically with astrocytic tPA, suggesting tPA mediates MSC treatment-induced restorative neurite growth which may contribute to neurological recovery after stroke.

Disclosures: J. Qian: None. M. Chopp: None. X. Shang: None. Z. Liu: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.18/B5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS094637

Title: Functional role of ribosome recycling during brain development

Authors: *M. TERREY[1,4], I. DOTU[5], J. H. CHUANG[6], S. L. ACKERMAN[2,4,3];
[1]UCSD, LA Jolla, CA; [2]Cell and Mol. Med., [3]Div. of Biol. Sci., UCSD, La Jolla, CA; [4]Howard Hughes Med. Inst., Chevy Chase, MD; [5]Res. Programme on Biomed. Informatics, Pompeu Fabra Univ., Barecelona, Spain; [6]The Jackson Lab. for Genomic Med., Farmington, CT

Abstract: Gene expression is controlled on multiple levels including transcription, mRNA degradation, protein translation, and degradation. However, gene expression regulated at the level of translation may be affected by mRNA secondary structure, mRNA stability, tRNA concentration, ribosome loading, the interaction between the ribosome and nascent polypeptide, and ribosome recycling. Generally, initiation is thought to be the main regulatory step of translation. However, it has become apparent that translation elongation is also highly regulated to eliminate translational pauses or stalls of ribosomes that may impair protein folding and translation. Ribosomes may stall on mRNAs containing a premature stop codon, cleaved, damaged or structured mRNAs, or as a result of polypeptides that form stable interactions, limited availability of tRNAs, or nutrition stress. Stalled ribosomes need to be recovered for further rounds of translation to limit their energetically costly replacement. While little is known about the components and function of the ribosome recycling pathways in higher eukaryotes, we have previously shown that *Gtpbp2* resolves stalled ribosomes in coding sequences due to tRNA deficiency and thereby, prevents neurodegeneration in mice. GTPBP2 is structurally similar to the mammalian ribosome rescue factor *Hbs1l* that in yeast has been shown to recycle ribosomes that proceed beyond the canonical stop codon. Here we show, the loss of *Hbs1l* is embryonic lethal in mice and its conditional deletion in the developing brain results in developmental defects. However, unlike *Gtpbp2*, loss of *Hbs1l* in post-mitotic neurons does not result in overt phenotypic abnormalities. Despite the structural similarity and cellular co-expression of GTPBP2 and HBS1L, our findings suggest that mammalian ribosome rescue factors may not be functionally redundant and may be necessary during different stages of brain development.

Disclosures: M. Terrey: None. I. Dotu: None. J.H. Chuang: None. S.L. Ackerman: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.19/B6

Topic: A.01. Neurogenesis and Gliogenesis

Support: Intramural Grant Western University of Health Sciences 12356D

R01-RDC007628 NIDCD

Title: Innate overexpression of BDNF improves taste dysfunction following chemotherapy

Authors: C. A. NOSRAT[1], L. PALACIOS[2], K. H. VU[2], S. A. KEZIAN[2], B. S. HENSON[1], *I. VUKMANOVIC NOSRAT[1];
[1]Col. of Dent. Med., [2]Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Basal-cell carcinoma (BCC) is the most common type of cancer of the skin. BCC is associated with mutations in the Hedgehog (Hh) signaling pathway. Genes encoding patched homologue 1 (PTCH1) and smoothened homologue (SMO) are affected. Vismodegib is an approved treatment for advanced BCC and selectively inhibits SMO. It also affects the proliferation and differentiation of taste cells by decreasing the number of taste progenitor cells, leading to a reduction of taste receptor cells. About 55% of patients being treated report dysgeusia. Taste-related adverse events might explain why many patients discontinue taking the drug. This study seeks to find a method to mitigate the negative side effects of vismodegib. We used novel transgenic mouse lines in which an alpha-gustducin promoter drives the overexpression of BDNF in taste buds (Gust-BDNF). BDNF is the most potent neurotrophic factor in the taste system and these mice have denser gustatory innervation, increased number of taste cells, and enlarged taste buds. We first studied if Gust-BDNF mice were able to detect tastants at lower concentrations compared to wild type mice. Furthermore, we evaluated if vismodegib administration affected the taste system in Gust-BDNF mice compared to wild type mice, and to what extent, in order to study if the taste-related side effects would be counteracted by innate overexpression of BDNF in Gust-BDNF mice. Circumvallate epithelia were laser microdissected, total RNA was extracted and real-time PCR was performed. Automated two-bottle choice test was used to evaluate taste preference and avoidance in mice. Additionally, vimodegib was administered orally for 10 weeks and evaluated for taste preference. Our data show that untreated Gust-BDNF mice showed a significant upregulation of sweet and bitter taste receptor genes, as well as some olfactory receptor genes compared to wild type mice. Gust-BDNF mice showed preference for sweet and aversion to bitter tastants at much lower concentrations compared to wild type mice. Most importantly, Gust-BDNF mice were able to detect sucrose after 10 weeks of vismodegib treatment, while wild type mice were not. We propose that BDNF, directly or indirectly, is able to counteract taste disturbances following vismodegib treatment.

Disclosures: C.A. Nosrat: None. **L. Palacios:** None. **K.H. Vu:** None. **S.A. Kezian:** None. **B.S. Henson:** None. **I. Vukmanovic Nosrat:** A. Employment/Salary (full or part-time): Western University of Health Sciences, Karolinska Institutet. 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; Intramural Grant Western University on Health Sciences 12356D, R01-RDC007628 NIDCD.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.20/B7

Topic: A.01. Neurogenesis and Gliogenesis

Support: PO1 HD23315-23

Title: Valproic acid inhibits cortical precursor HDACs and rapidly upregulates cyclin E1 mRNA in embryonic rat cerebral cortex development.

Authors: *R. J. CONNACHER, X. ZHOU, E. DICICCO-BLOOM;
Neurosci. and Cell Biol., Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Valproic acid (VPA) is a neurotherapeutic drug commonly used to treat epilepsy, migraine, and depression. However, VPA exposure during fetal development induces brain malformations, and increases autism risk. Although mechanisms are undefined, evidence suggests VPA modulates epigenetic processes. In previous studies using embryonic day 14.5 (E14.5) and E16.5 cerebral cortical precursors in vitro and in vivo, we have shown VPA stimulates DNA synthesis, cell division, and increases cell numbers, producing enlarged brains by P21. This stimulation corresponds with rapid increases in G1 cyclin proteins D3 and E and levels of acetylated histone 3, consistent with proposed VPA inhibition of histone deacetylases (HDAC). We hypothesize that the proliferation effects of VPA are due to changes in G1-S cyclin levels, which may be a direct result of VPA mediated HDAC inhibition. To define mechanisms we now characterize expression of HDACs in E14.5, E16.5, E19.5 and adult cerebral cortex using RT-PCR. HDAC members of classes I, II, IV, and Sirtuin 1 (class III) are expressed widely during development yet exhibit region- and time specific patterns. Because HDAC 1 and 2 regulate both cell cycle and differentiation, and class I HDAC proteins are primarily targeted by VPA, we assayed levels in cortical cultures: While both were highly expressed, HDAC 1 localized to nucleus and cytoplasm, while HDAC 2 was solely nuclear. After establishing key HDACs are present in cortical precursors, we next determined whether VPA can directly inhibit HDAC activity in E14.5 rat cortical precursors. At a dose that stimulated mitogenesis, VPA inhibited enzymatic activity by 24%, whereas a higher VPA dose that did not stimulate DNA synthesis, inhibited HDAC activity by 42%, suggesting a delicate balance of diverse HDAC targets are involved with stimulatory actions. To begin defining processes underlying rapid increases in DNA synthesis, we examined G1-S phase regulator cyclin E1 mRNA in vitro. VPA elicited increases in mRNA levels by 1.7 fold at 2h, 1.8 at 4h and 1.9 at 8h. Considering that our prior studies showed rapid increases of cyclin E protein by 4 hours, it follows that VPA may directly regulate this gene's expression through epigenetic mechanisms. In sum, VPA can inhibit HDACs in developing cerebral cortex, which in turn may directly target cyclin E1 gene

expression, an issue we will explore by ChIP analysis. Identification of this epigenetic role for VPA on cell cycle progression during cortical neurogenesis may provide insights into its teratogenic effects and possible contributions to autism.

Disclosures: R.J. Connacher: None. X. Zhou: None. E. DiCicco-Bloom: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.21/B8

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH081880

Autism Speaks, Pilot Grant

Simon's Foundation , Pilot Grant

DOD, IDEA deveopment award, #TS150059

Title: Common and unique roles of ASD candidate genes in cortical interneuron development: Increasing evidence for parvalbumin expressing interneurons.

Authors: *D. VOGT[1], K. K. CHO[2], S. E. ROBINSON[2], V. SOHAL[3], J. L. R. RUBENSTEIN[2]; [1]Dept. of Psychiatry, Univ. of California San Francisco, San Francisco, CA; [2]UCSF, San Francisco, CA; [3]UCSF, SanFrancisco, CA

Abstract: There are now multiple genes that are known to contribute to Autism spectrum disorder (ASD) risk. However, the functions of these genes during brain development are largely unknown. Moreover, how human ASD mutations in these genes disrupt brain function is also not understood. One hypothesis proposes that some forms of ASD are caused by an imbalance in brain excitation and inhibition, and that pallial GABAergic interneurons may have a pivotal role in this process. Thus, investigating ASD gene and allele functions in cortical inhibitory interneurons is an ideal experimental system. To this end, I will present work primarily examining the role of key ASD genes that regulate overlapping cell signaling pathways, in particular RAS/MAPK and AKT/mTor signaling. One of these genes, *NF1*, is an inhibitor of RAS signaling and is predicted to also inhibit mTor signaling. Thus, *NF1* dysfunction could potentially generate phenotypes shared with *PTEN* and *TSC1&2,* other syndromic ASD genes. However, deletion of *NF1* in cortical interneuron progenitors led to phenotypes not shared with *PTEN or TSC1* loss of function mice, suggesting unique roles for RAS/MAPK signaling during

development and in ASD. Interestingly, one common phenotype was observed, altered numbers and properties of interneurons that expressed PV. Further analyses revealed that alterations in PV+ interneurons is a common cell autonomous phenotype in another mouse model of ASD, including *CNTNAP2*. These data demonstrate that both unique and complimentary phenotypes are present in multiple models of ASD. Finally, these data could hint that therapies aimed at the PV+ interneuron population may have particular importance for ASD.

Disclosures: D. Vogt: None. K.K. Cho: None. S.E. Robinson: None. V. Sohal: None. J.L.R. Rubenstein: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.22/B9

Topic: A.02. Postnatal Neurogenesis

Support: NINDS (NS076006) for H.T.C.

TSRI CIRM Training Grant #01165 (L-C.H)

Title: Transcriptome profiles of neuronal activity-induced proliferating neural progenitor cells and newly-differentiated immature neurons in optic tectum of xenopus laevis

Authors: *L.-C. HUANG[1], K. VAN KEUREN-JENSEN[2], H. CLINE[1];
[1]Dorris Neurosci. Ctr., The Scripps Res. Inst., La Jolla, CA; [2]Translational Genomics Res. Inst., Phoenix, AZ

Abstract: Neuronal activity-induced neurogenesis has been suggested to play a critical role in maintaining neural circuit functions in adult hippocampus and olfactory bulb. Many factors, of which expression is activity-dependent, are required for neural progenitor cell proliferation, and survival and integration of new-born neurons (Ma et al., 2009). However, the mechanisms governing this neuronal activity-induced neurogenesis during development are largely unknown. Here we utilize RNA-seq to profile the transcriptomes of neuronal activity-induced proliferating neural progenitor cells and newly-differentiated immature neurons from optic tectum of Xenopus laevis in order to provide some insights into the potential mechanisms. Different visual experience protocols bias the fate of neural progenitor cells in the optic tectum towards proliferation or differentiation (Sharma and Cline, 2010; Bestman et al., 2015). Neural progenitor cells and newly-differentiated neurons were isolated using fluorescence-activated cell sorting from Sox2+ neural progenitor cells of animals reared in dark and enhanced visual stimulation for

24 hours, respectively, based on the turboGFP expression driven by Sox2 enhancer. Using RNA-seq, 1,130 differentially expressed transcripts were identified between these two cell types. Gene Ontology analysis using DAVID / PANTHER clusters the differentially expressed transcripts into categories of catalytic activity, transcription factor, receptor-mediated signaling and structural protein. This provides the potential functional roles of these transcripts in neurogenesis. The protein-protein interaction network analysis using STRING and Cytoscape identified the potential key players in neurogenesis based on their connectivity within the protein network. These most connected genes are likely to modulate the expression of many other transcripts. In addition, using ENCODE database, we identified a transcriptional network which may regulate the expression of differentially expressed transcripts in the Sox2+ neural progenitor cells and their neuronal progeny during neural development. This study provides an overview on the transcripts that are differentially expressed between neuronal activity-induced progenitor cells and newly-differentiated neurons, and a series of datamining and bioinformatic analyses reveal an overview of their potential roles in neurogenesis.

Disclosures: L. Huang: None. K. Van Keuren-Jensen: None. H. Cline: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.23/B10

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY022030-4

Title: TGF-beta signaling suppresses the formation of Muller glia derived progenitor cells in the avian retina

Authors: *L. J. TODD, N. MENDONCA, I. PALAZZO, N. SQUIRES, A. FISCHER;
The Ohio State Univ., Columbus, OH

Abstract: Müller glia are the major type of support cell in the vertebrate retina and are the only retinal glia type derived from neuroepthelial stem cells. In response to retinal injury or growth factor treatment, Müller glia can give rise to proliferating neurogenic progenitors with the ability to produce new neurons. The ability of Müller glia-derived progenitor cells (MGPCs) to regenerate neurons is robust in lower vertebrates, but is diminished birds and mammals. Therefore, understanding the mechanisms that orchestrate the ability of Müller glia to transition into MGPCs is imperative to harnessing the regenerative capacity of the retina. A network of signaling pathways underlying MGPC formation is beginning to be uncovered. TGFβ signaling

has been implicated as a negative regulator of MGPC formation in the zebrafish and the juvenile rodent retina (Lenkowski, et al. 2013; Close, et al. 2005). This study investigates whether TGFβ/SMAD-signaling influences the formation of MGPCs in the chick retina *in vivo*. We observed that SMAD2, a readout of TGFβ signaling, is specifically expressed in Müller glia and its nuclear localization is dynamically regulated following NMDA damage, when MGPCs are known to form. We found that intraocular injections of recombinant TGFβ2 ligand inhibited the formation of proliferating MGPCs after retinal damage. By comparison, inhibition of TGFβ signaling at the level of TGFβ receptor and the SMAD3 transcription factor significantly increased numbers of proliferating MGPCs in NMDA damaged retinas. Furthermore, we find that inhibition of SMAD3 in combination with FGF2 treatment leads to significant increase in the number of proliferating MGPCs compared to numbers seen in retinas treated with FGF2 alone. Lastly, evidence is provided that TGFβ signaling is coordinated with MAPK and mTOR signaling, and cross-talk between the pathways may influence the formation of proliferating MGPCs. We conclude that TGFβ/SMAD signaling plays a negative regulatory role during the formation of proliferating MGPCs in the *in vivo* chick retina.

Disclosures: L.J. Todd: None. N. Mendonca: None. I. Palazzo: None. N. Squires: None. A. Fischer: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.24/B11

Topic: A.01. Neurogenesis and Gliogenesis

Support: 532NS084749

K99ES022992

EY011261

Dart Neuroscience LLC.

Hahn Family Foundation

Title: Nutrient-restrictions causes reversible neuronal G2 arrest in the Xenopus visual system.

Authors: *C. R. MCKEOWN, A. C. GAMBRILL, E. M. FITCHETT, C. K. THOMPSON, H. T. CLINE;
Mol. and Cell. Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Access to nutrients is critical for normal brain development, yet the impact of nutrient restriction on brain development and the consequences of nutrient restriction and availability on circuit formation and behavior remain largely unknown. Here we examine the effects of nutrient restriction and availability on neurogenesis in Xenopus laevis tadpoles. We are specifically interested in understanding the nutrient- responsive molecular and cellular mechanisms required for neural progenitor cell proliferation, as well as neuronal differentiation and circuit integration in the developing Xenopus visual system. Nutrient restriction (NR) beginning after yolk depletion results in a reversible block in neural progenitor cell (NPC) proliferation and a developmental stasis of tadpoles, from which animals recover if food is provided within with ~8 days of NR. In contrast to NR, nutrient enrichment (NE) significantly increases NPC proliferation in the optic tectum. Upon re- introduction to food after NR, neural progenitors initiate proliferation within 16hrs, rescuing cell proliferation rates as well as optic tectum size, comparable to non- NR counterparts. By continually labeling proliferating cells with BrdU for 24hrs concurrent with the re- introduction of food, followed by phospho- histone H3 immunofluorescence, we find that after a period of NR the nutrient- induced proliferating cells directly enter M- phase. These data suggest that NR causes a G2 arrest and that NE recruits these arrested progenitors to re- enter the cell cycle upon re- exposure to food. Consistent with this observation, we find that cellular levels of the G1/S-transition marker phospho-cdc2 are down-regulated upon NR and elevated with the reintroduction of nutrients. Furthermore, we show that in NE animals, phospho-cdc2 is apically localized in progenitors on the ventricular surface of the tectum. This localization is lost upon NR and restored just 12hrs after re-introduction to food. Using immunofluorescence and Western blotting, we determined that the mTOR inhibitor, Rapamycin, inhibits NE- induced proliferation, indicating that nutrient-induced proliferation requires mTOR signaling. Using electrophysiological assays, we find that NR decreased the intrinsic excitability of optic tectal neurons and impaired integration of newly generated neurons into the tectal circuit. These studies indicate that nutrient availability plays a key role in regulating neuronal proliferation, differentiation, and circuit connectivity in the developing tadpole visual system, specifically by recruiting quiescent progenitor cells to escape G2 arrest and re-enter the cell cycle.

Disclosures: C.R. McKeown: None. A.C. Gambrill: None. E.M. Fitchett: None. C.K. Thompson: None. H.T. Cline: None.

Poster

208. Regulation of Neurogenesis

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 208.25/B12

Topic: A.02. Postnatal Neurogenesis

Support: Irving Scholar Award

R01MH091844

RO1 MH91427

Title: Neural stem cell responses to chronic stress are autonomously sustained and sexually dimorphic

Authors: M. NAVARRO-SOBRINO[1], A. BURGOS[2], A. GARCIA-GARCIA[1], E. LEONARDO[1], *A. DRANOVSKY[1];
[1]Psychiatry, Columbia Univ. / NYSPI, New York, NY; [2]Neurosci., Columbia Univ., New York, NY

Abstract: Adult stem cells are crucial for tissue homeostasis. In the adult hippocampus, neural stem cells (NSCs) appear to proliferate in response to stressful experiences. However, the functional capacity of accumulating cells and their impact on hippocampal structure and function remain unknown. We used multicolor fluorescent reporter mice to prospectively identify NSCs vs progenitors and purify them from the adult hippocampus. In vitro, social isolation increased the autonomous proliferative capacity of NSCs and the resulting cells exhibited increased potential for neurogenesis. In vivo, NSCs stockpiled during impoverished conditions were recruited for enhanced neurogenesis when conditions improved. Remarkably, these experience-dependent changes in NSC function occurred in male, but not in female mice. Together, our results indicate a sexually dimorphic, cell intrinsic mechanism by which resident NSCs contribute to normal tissue homeostasis and experience-induced brain plasticity. We suggest that NSCs present an avenue for investigating mechanisms underlying sex differences in stress resilience.

Disclosures: M. Navarro-Sobrino: None. A. Burgos: None. A. Garcia-Garcia: None. E. Leonardo: None. A. Dranovsky: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.01/B13

Topic: B.12. Glial Mechanisms

Support: NIH Grant MH094268

Title: Astrocyte aldehyde dehydrogenase ALDH7A1 detoxifies the brain: metabolic impairment can disrupt higher brain function in neuropsychiatric disorders.

Authors: *T. E. FAUST[1,2], T. CASH-PADGETT[1], W. XIN[3,2], S. SAHA[4], S. DESHPANDE[1], D. WOOD[1], C. DAVIS[5], A. BONCI[3], H. JAARO-PELED[1], A. SAWA[1];
[1]Psychiatry, Johns Hopkins Univ. Dept. of Psychiatry and Behavioral Sci., Baltimore, MD; [2]Neurosci., Johns Hopkins University, Sch. of Med., Baltimore, MD; [3]Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD; [4]Johns Hopkins Univ., Baltimore, MD; [5]Sleep and Performance Res. Center, and Program in Neurosci., Washington State University-Spokane, Spokane, WA

Abstract: The aldehyde dehydrogenase (ALDH) protein family is highly enriched in astrocytes and ALDHs are widely used as astrocyte-specific markers. Loss of function mutations in ALDH family members are known to cause a variety of human brain disorders. In particular, ALDH7A1 mutations cause pyridoxine-dependent epilepsy (PDE), a disease characterized by neonatal seizures and cognitive impairments. The seizures can be treated by large dietary intake of vitamin B6 (B6), but cognitive impairments persist. The leading hypothesis in the field is that seizures are caused by a functional B6 deficiency created by metabolic blockage of the lysine degradation pathway. How B6 deficiency causes seizures and the source of the untreated cognitive deficits remain open questions. This study attempts to address these two questions in parallel.

We hypothesize that B6 deficiency causes seizures due to reduced cofactor availability for production of neurotransmitters such as GABA. B6 may also directly act as an antioxidant. We generated *Aldh7a1*$^{-/-}$ mice and found metabolic blockage of the lysine degradation pathway and increased oxidative stress in prefrontal cortex (PFC). Similar to PDE patients, *Aldh7a1*$^{-/-}$ mice display reduced seizure threshold and deficits in higher brain function, but we did not detect any sign of spontaneous seizure activity by EEG. We found changes in GABAergic signaling and the intrinsic excitability of layer V PFC neurons, which may underlie the observed change in seizure threshold. We are investigating whether B6 treatment can rescue the deficits in seizure threshold and oxidative stress in *Aldh7a1*$^{-/-}$ mice.

We hypothesize that the persistence of cognitive deficits in B6-treated PDE patients is due to the continued dysfunction of ALDH7A1 in astrocytes. In other tissues, ALDH7A1 is needed for production of osmolytes, regulation of NAD+/NADH redox ratio, and degradation of reactive aldehydes. ALDH7A1 immunostaining is highly specific for astrocytes and we found that *Aldh7a1*$^{-/-}$ mice have an altered NAD$^+$/NADH ratio and higher ROS levels in astrocytes. To investigate the effects of astrocyte-specific deletion of *Aldh7a1* in the absence of systemic B6 deficiency, we are using *Emx1-Cre*$^+$ x *Aldh7a1*$^{F/F}$ mice for behavioral assays and *GLAST-CreER*$^+$ x *Aldh7a1*$^{F/F}$ mice for cellular assays. Intriguingly, we have also observed decreased ALDH7A1 expression in biopsied neural tissue from patients with schizophrenia and in PFC tissue of animals treated with phencyclidine, suggesting that decreased expression of ALDH7A1 may contribute to deficits in higher brain function in psychiatric disorders.

Disclosures: T.E. Faust: None. T. Cash-Padgett: None. W. Xin: None. S. Saha: None. S. Deshpande: None. D. Wood: None. C. Davis: None. A. Bonci: None. H. Jaaro-Peled: None. A. Sawa: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.02/B14

Topic: B.12. Glial Mechanisms

Support: NIH Grant F31 MH106208

NIH Grant R01 NS037585

Title: Genetic approaches for achieving astrocyte specific transgene expression

Authors: *J. DUNPHY, T. PAPOUIN, M. TOLMAN, K. FLICK, P. G. HAYDON; Neurosci., Tufts Univ., Boston, MA

Abstract: Over the last 10 years, several molecular genetic techniques have been used to study the role of gliotransmission in the brain, including a mouse model that attenuates gliotransmitter release by expressing the cytoplasmic domain of VAMP2 in astrocytes (dnSNARE). Recently the cell-type specificity of the GFAP promoter and the conditional tetracycline responsive system has been called into question, and with it, a myriad of independently-verified astrocytic functions that have been discovered using dnSNARE mice. In this mouse line, the 2 kilobase human GFAP promoter drives expression of the tetracycline transactivator (tTA) in astrocytes. Since tTA is only permitted to interact with the tetracycline operator (tetOff) when doxycycline is removed from the diet, dnSNARE and the EGFP reporter expression is conditional.
To assess the validity of the claim of widespread transgene expression in neurons, we performed quantitative immunohistochemistry in dnSNARE mice and single-gene controls, both on and off doxycycline. Immunolabeling and colocalization analysis through 40μm thick z-stacks taken at 1μm intervals from dnSNARE mice revealed no single EGFP+ neuron among the 5,887 NeuN+ cells that were counted. Rather, 100% of the 1,636 EGFP+ cells that were counted had a distinct bushy morphology and co-localized with GFAP and DAPI, indicating that they were astrocytes.
Other cell-type specific molecular genetic approaches have been used to assess gliotransmission, including alternative promoters, and other conditional (such as cre/loxP) or region specific (virus-mediated) expression systems. We found that adeno-associated viruses (AAVs) that paired an abbreviated version (0.7 kilobase) of the GFAP promoter with the AAV5 serotype were effective in transducing astrocytes with the EGFP reporter. All of the 1271 EGFP+ cells counted colocalized with the GFAP marker. On the other hand, neuronal specificity was achieved by pairing the human Synapsin promoter (hSyn) with an AAV9 serotype virus. 99.15% of EGFP+ cells were neurons, with the remaining cells having microglial morphology (and not colocalizing with GFAP).
Several different approaches can be used in tandem to ensure that experimental results are

interpreted correctly. In this study, we evaluated many approaches that are currently being used to achieve astrocyte-specificity.

Disclosures: **J. Dunphy:** None. **T. Papouin:** None. **M. Tolman:** None. **K. Flick:** None. **P.G. Haydon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaCure.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.03/B15

Topic: B.12. Glial Mechanisms

Support: PHC PROCOPE 2015-2016

IDEX Attractivity Grant 2013-2015

FP7 Career Integration Grant n° 334455

IASP Early Career Research grant 2012

Title: Astrocyte-neuron involvement in oxytocinergic modifications of pain and anxiety.

Authors: J. WAHIS[1], S. GOYON[1], M. ELIAVA[2], V. GRINEVICH[2], P. POISBEAU[3], *A. CHARLET[1];
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Abstract: Oxytocin (OT) is a hypothalamic hormone and neuropeptide well known for its numerous roles in social interaction, anxiety and pain modulation, among others. Particularly, OT can modulate the local circuitry of the central amygdala (CeA) in order to decrease the fear response and promote analgesia. Here, we study the action of OT on the calcium dynamics of astrocytes and the relevance of the astrocyte-neuron interaction to the OT neuromodulatory effect in the CeA. Through calcium imaging and patch clamp experiments on acute slices of rat amygdala, we characterize the response of astrocytes and neurons upon specific activation of OT-receptor, decipher the intracellular mechanisms involved and identify putative gliotransmitter(s) of the OT message. Finally, through a neuropathic pain model, we test the relevance of those mechanisms on pain threshold and anxiety levels. Taken together, our results provide new insights into the mechanisms underlying the OT action in the CeA and its role in pain and anxiety processing.

Disclosures: J. Wahis: None. S. Goyon: None. M. Eliava: None. V. Grinevich: None. P. Poisbeau: None. A. Charlet: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.04/B16

Topic: B.12. Glial Mechanisms

Support: NIA Intramural Support

Title: Neuronal extracellular vesicles modify astrocyte lysosome function, promote trophic factor production and inhibit secretion of pro-inflammatory factors.

Authors: *E. R. HUTCHISON[1], E. EITAN[1], C. SUIRE[1], K. MAROSI[1], R. SPANGLER[1], Z. LIAO[2], K. W. WITWER[2], M. P. MATTSON[1];
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Abstract: Astrocytes support many aspects of neuronal physiology, from basic cellular metabolism and neurotransmitter uptake to more complex actions such as synaptic pruning. Under pathological conditions, including injury or age-related neurodegenerative diseases such as Alzheimer's, astrocytes take on a reactive phenotype in which they migrate to pathological sites and acquire a pro-inflammatory status. Extracellular vesicles (EVs) are a population of circulating vesicles containing protein, RNA and DNA. EVs have been shown to deliver functional proteins, mRNA and ncRNAs, such as microRNAs, into recipient cells and thus confer altered cellular function. Neuronal excitation has previously been found to increase EV release from neurons and recent work from our lab has demonstrated that EVs can mediate the intercellular spread of pathology through the delivery of toxic proteins such as Aβ. Here we examine the functional effects of cortical neuron EVs on cultured astrocytes and demonstrate that these EVs suppress lysosome function in recipient astrocytes. mRNA profiling experiments revealed that astrocytes respond to neuronal EVs by increasing transcripts of trophic factors such as BDNF and decreasing mRNA levels of pro-inflammatory cytokines such as IL1β and TNFα. These findings were confirmed at the protein level using ELISA assays for TNFα and IL6. To identify the mechanism behind this phenotypic shift, we are performing both miRNA profiling and RNASeq analysis on neuronal EVs and investigating the relationship between these EV-enriched RNA species and neuronal activity, aging-related stressors including inflammation, DNA damage and lysosomal stress. We are currently examining the role of this novel form of neuron-astrocyte interaction in the context of neurodegeneration and Alzheimer's disease.

Disclosures: E.R. Hutchison: None. E. Eitan: None. C. Suire: None. K. Marosi: None. R. Spangler: None. Z. Liao: None. K.W. Witwer: None. M.P. Mattson: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.05/B17

Topic: B.12. Glial Mechanisms

Support: Institut Nationalde la Santé et de la Recherche Médicale(INSERM)

Fondation pour la Recherche Médicale (FRM; DEQ20140329488)

Agence Nationale de la Recherche (ANR)

ERA-NET Neuron

China scholarship council (csc)

Université Sorbonne Paris Cité (USPC)

Centre National de la Recherche Scientifique (CNRS)

Title: Release of glutamate and ATP induced by optogenetic activation of astrocytes

Authors: W. SHEN, L. NIKOLIC, C. MEUNIER, *E. AUDINAT;
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Abstract: Astrocytes can modulate synaptic transmission and neuronal excitability through the release of gliotransmitters, such as glutamate, D-serine or ATP. However, the mechanisms and conditions leading to the release of gliotransmitters are still highly debated. We therefore used a transgenic approach allowing optogenetic activation of astrocytes to study gliotransmission in the hippocampus. We crossed Cx30-cre-ERT2 (kindly provided by Frank W Pfrieger) and Floxed-ChR2-EYFP mice (Ai32; Jackson lab) to induce the selective expression of ChR2 in astrocytes. We confirmed by immunocytochemistry that EYFP was specifically expressed in the vast majority of hippocampal and cortical astrocytes. In acute hippocampal slices, we also confirmed that blue light stimulations induced in astrocytes direct membrane currents resembling those of ChR2-induced currents in other cell types. Using two-photon imaging, we observed that low intensity light pulses of more than one second reliably triggered calcium responses in EYP positive astrocytes. These calcium responses were largely inhibited in thapsigargin-treated slices, indicating the involvement of intracellular calcium stores. We then recorded principal neurons of

CA1 and of dentate gyrus regions to analyze the neuronal consequences of ChR2 activation in astrocytes. Light pulses of more than 1 second reliably induced a sequence of depolarizing-hyperpolarizing responses that were not blocked by TTX (1 μM). The hyperpolarizing current was abolished by the adenosine A1 receptor antagonist DCPCX (200 nM), suggesting a release of ATP or adenosine by astrocytes. The depolarizing component was fully blocked by antagonists of NMDARs (50 μM D-AP5, 40 μM MK-801, 50 μM 7Cl-KYN) but not of AMPA-KAR receptors (10-20 μM NBQX). This component was potentiated by the blocker of glutamate transporters TBOA (100 μM) but was not affected by application of glycine or D-serine, which in our conditions failed also to induce any change in the baseline membrane current. Pharmacological manipulations further indicated that light-induced NMDAR-mediated responses were due to the activation of GluN2B-containing extra-synaptic NMDARs by a low concentration of glutamate. Remarkably, light-induced calcium responses in astrocytes and NMDAR-mediated currents in neurons were largely inhibited by PPADS (100 μM), a broad spectrum antagonist of P2 receptors and by MRS 2179, a selective antagonist of P2Y1 receptors, the predominant P2 receptors expressed in astrocytes. Overall, these results validate the use of ChR2 to trigger gliotransmission and indicate that astrocyte glutamate release is tightly controlled by P2Y1 signaling.

Disclosures: W. Shen: None. L. Nikolic: None. C. Meunier: None. E. Audinat: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.06/B18

Topic: B.12. Glial Mechanisms

Support: IRP/NIDA/NIH/DHHS

Title: Loss of sigma-1 receptor chaperone promotes astrocytosis and enhances Nrf2 antioxidant defense

Authors: *S.-Y. A. TSAI[1], T.-Y. WENG[2], J. CIESIELSKI[1], T.-P. SU[1];
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Abstract: The sigma-1 receptor (Sig-1R) is an endoplasmic reticulum chaperone protein abundantly expressed both in neurons and glia and has been implicated in many neurodegenerative and psychiatric diseases. Here we used Sig-1R KO mice to examine CNS expression profiles of astrocytes and ubiquitinated proteins, which are common hallmarks of many CNS pathologies. Our results showed that Sig-1R KO neurons display increased glial

fibrillary acidic protein (GFAP) expression in primary cortical neuron culture and in the whole brain of fetuses and aged mice with concomitantly increased accumulations of ubquitinated proteins. Immunofluorescence data further supported the observation of astrogliosis in neuron-glia culture. Under proteasome and autophagy inhibitor treatments, the pronounced ubiquitinated proteins were further increased in Sig-1R KO cortical neurons compared with WT, indicating that the Sig-1R plays regulatory roles in both protein degradation systems and its pivotal role in protein quality control. Sig-1R deficiency has been associated with increased oxidative stress. Here, we also found that a transcription Nrf2 (nuclear factor erythroid 2-related factor 2), which functions to overcome the stress condition, was enhanced in the Sig-1R KO neurons, especially when the culture condition was antioxidant deprived. The Nrf2 also exhibited longer stability in the Sig-1R KO neurons. Mutation or deficiency of Sig-1Rs has been observed in neurodegenerative models, and this study indicates the critical roles for the Sig-1R in the maintenance of CNS homeostasis and strongly supports the idea that functional complementation signaling pathways are triggered in the Sig-1R KO pathology. (Supported by the IRP/NIDA/NIH/DHHS).

Disclosures: S.A. Tsai: None. T. Weng: None. J. Ciesielski: None. T. Su: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.07/B19

Topic: B.12. Glial Mechanisms

Support: CIHR Grant 14392

Title: Role of astrocytes in defining functional compartments in the chewing central pattern generator

Authors: *D. RYCZKO, D. VERDIER, A. KOLTA;
Groupe de Recherche sur le Systeme Nerveux Central, Dept. of Neurosci., Univ. Montreal, Montreal, QC, Canada

Abstract: Rhythmic motor activities such as walking, breathing or chewing are generated by specialized neural networks called Central Pattern Generators (CPG). Recent work from our lab shows that astrocytes and their protein S100ß are responsible for neuronal rhythmogenesis in the trigeminal main sensory nucleus (NVsnpr) thought to be part of the chewing CPG (Morquette et al. Nat Neurosci 18:844-54). S100ß, a calcium (Ca^{2+}) binding protein, decreases the extracellular Ca^{2+} concentration, and by doing so, activates a persistent sodium current in NVsnpr neurons,

causing a switch from a tonic to a rhythmic firing mode. It is unclear whether astrocytic coupling is involved in these effects. Morquette et al. showed that intracellular dialysis of an astrocyte with the Ca^{2+} chelator BAPTA prevented rhythmic bursting in the adjacent neuron, but only after prolonged periods of time, indicating that diffusion of BAPTA in the astrocytic syncytium was necessary to prevent neuronal bursting. Thus, we hypothesized that astrocytic syncytia could define functional compartments within which activity of adjacent neurons is synchronised when astrocytes are activated. Such units could be recruited by distinct sets of sensory inputs to the CPG, in order to generate different motor patterns. We tested part of this hypothesis by visualizing such functional units using brainstem slices of mice expressing a Ca^{2+} indicator (GCaMP6f) under the neuronal promoter Thy1. We mapped the Ca^{2+} responses of NVsnpr neurons to different sensory inputs which send their axons in two distinct tracts that can be stimulated separately. We show that each set of inputs can activate a different population of neurons. Using double patch-clamp recordings, we show that neurons located within a spatially restricted domain fire synchronously following sensory stimulation, while neurons slightly further apart are not synchronised by sensory stimulation. Next, we will examine whether syncytium deactivation disrupts their synchrony. This will allow us to determine the role of astrocytic syncytia in synchronizing neurons that are functionally linked by their sensory inputs.

Disclosures: D. Ryczko: None. D. Verdier: None. A. Kolta: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.08/B20

Topic: B.12. Glial Mechanisms

Support: Grand Challenges UCL

Title: Hepatic encephalopathy is associated with impaired hemichannel mediated release of lactate in the cerebral cortex

Authors: *A. HADJIHAMBI[1], P. S. HOSFORD[2], F. DE CHIARA[1], A. HABTESION[1], A. KARAGIANNIS[2], R. JALAN[1], A. V. GOURINE[2];
[1]Liver and digestive health, [2]Neuroscience, Physiol. & Pharmacol., UCL, London, United Kingdom

Abstract: Background and Aims: Astrocytes, the most numerous glial cell, are hypothesized to play an important role in the pathological mechanisms underlying hepatic encephalopathy (HE), although the clinical manifestations are mainly neuronal. Astrocytes are extensively connected

by gap junctions formed of connexins, which may also exist as functional hemichannels allowing the exchange of molecules between the cytoplasm and the extracellular milieu. The astrocyte-neuron lactate shuttle hypothesis is a contested yet valuable concept suggesting that neuronal activity is fuelled (at least in part) by lactate provided by neighboring astrocyte. In pathological conditions, including HE, significant changes in astroglial anatomy and function are known to occur which could impair the astrocyte-neuron communication. In this study, we investigated changes in the hemichannel function and lactate release in the cerebral cortex and the role of ammonia in animal (rat) models of HE (bile duct ligation [BDL] and hyperammonemia) and the effect of ammonia lowering treatment, ornithine phenylacetate (OP). Methods: Microelectrode biosensors were used for real-time measurements of lactate release by cortical slices prepared from the brains of SHAM-operated (n=18), SHAM + high ammonia diet (HA, n=16), BDL (n=18) and BDL animals treated with OP (0.3g/kg) (BDL+OP, n=14). Fluorescent dye loading in cortical slices was also performed. The involvement of hemichannels was tested using blockers, carbenoxolone and 5-Nitro-2-(3-phenylpropylamino)benzoic Acid. Results: Both HA diet and BDL resulted in high plasma ammonia concentrations, which were reduced by OP treatment. Biosensor recordings showed a significant reduction ($p<0.05$) in both tonic and hypoxia induced lactate release in the cortical slices of HA and BDL rats, which recovered with OP treatment. Cortical dye loading indicated a decrease in hemichannel-mediated loading ($p<0.05$) in BDL animals. Conclusions: The results of the present study suggest that HE is associated with significant changes in the hemichannel functionality in the CNS under normal and hypoxic conditions, with ammonia playing a key role. These changes are hypothesized to contribute to the development of an altered neurochemical phenotype and HE pathogenesis.

Disclosures: **A. Hadjihambi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by Grand Challenges UCL. **P.S. Hosford:** None. **F. De Chiara:** None. **A. Habtesion:** None. **A. Karagiannis:** None. **R. Jalan:** None. **A.V. Gourine:** None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.09/B21

Topic: B.12. Glial Mechanisms

Support: FAPESP

CNPq

CAPES

Title: Astrocytes modulates hyperosmolality-induced behavior and hormonal release

Authors: *F. M. VECHIATO[1], R. COLETTI[1], J. B. M. DE LIMA[1], F. LUCIO-OLIVEIRA[2], S. G. RUGINSK[3], L. L. K. ELIAS[1], J. ANTUNES-RODRIGUES[1];
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Abstract: Astrocytes have been identified as the synapse third element, suggesting they are involved in neuronal modulation. During chronic dehydration, hypothalamic magnocellular neurons are activated to synthetize and release vasopressin (AVP) and oxytocin (OT) to neurohypophysis (NH). In this condition, astrocytes morphofunctional changes were also observed suggesting their participation in hydromineral homeostasis; however, the astrocytic role still remains little studied. Objectives: To evaluate astrocytes participation in behavioral and hormonal responses in 48-h water-deprived animals (48hWD), and in hormone release of hypothalamic and neurohypophyseal explants in response to hyperosmolarity. Methods: Male Wistar rats were used (250-300g). In *in vivo* protocols, animals were submitted to 48hWD and injected with fluorocitrate (FCA, 1 nmol/4 μL, icv), a glial metabolism inhibitor. After 2 hours, water intake and corticosterone, AVP and OT release were evaluated. In *in vitro* protocols, medial basal hypothalami (MBH) and NH were pre-incubated in KRBG (Krebs-Ringer bicarbonate buffer, 280 mOsm/Kg H_2O), containing or not FCA (6.6, 26.4 or 105.6 μM) for 90 min. The medium was replaced by KRBG medium (280 or 340 mOsm/Kg H_2O, the latter with mannitol or NaCl), containing or not FCA for 30 min. The medium was collected and AVP, OT and atrial natriurect peptide (ANP) secretion was measured. Results: 48hWD increased water intake ($F_{7,196} = 9.2$; $p < .0001$), which was enhanced by FCA ($F_{7,196} = 6.0$; $p < .0001$). There was an increase of corticosterone ($F_{1,34} = 34.1$; $p < .0001$), AVP ($F_{1,38} = 209.3$; $p < .0001$) and OT ($F_{1,33} = 55.9$; $p < .0001$) release in 48hWD animals; nevertheless, FCA reduced hormonal release of every evaluated hormone, respectively ($F_{1,34} = 5.8$; $p < .05$), ($F_{1,38} = 8.2$; $p < .01$) and ($F1,33 = 11.3$; $p < .01$). Incubation with FCA in basal conditions did not change hormone release in MBH and NH. Mannitol-induced hyperosmolality increased AVP, OT and ANP release in both MBH ($t_{52} = 5.6$; $p < .0001$), ($t_{55} = 8.5$; $p < .0001$), ($t_{52} = 20.2$; $p < .0001$) and NH ($t_{45} = 7.5$; $p < .0001$), ($t_{51} = 7.2$; $p < .0001$). In hyperosmolality, FCA, independently of tested concentration, reduced AVP ($F_{3,58} = 9.7$; $p < .0001$), OT ($F_{3,63} = 9.7$; $p < .0001$) and ANP ($F_{3,64} = 46.7$; $p < .0001$) release by MBH, and AVP ($F_{3,58} = 20.36$; $p < .0001$) and OT ($F_{3,64} = 22.9$; $p < .0001$) release by NH. Similar hormonal findings were obtained when hyperosmolality was induced by NaCl followed or not by incubation with FCA in MBH and NH. Conclusion: Astrocytes play a role in hyperosmolality-induced hormonal and behavioral regulation. Their metabolic integrity is crucial for neuronal homeostatic regulation.

Disclosures: F.M. Vechiato: None. R. Coletti: None. J.B.M. de Lima: None. F. Lucio-Oliveira: None. S.G. Ruginsk: None. L.L.K. Elias: None. J. Antunes-Rodrigues: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.10/B22

Topic: B.12. Glial Mechanisms

Title: Impact of aquaporin-4 deletion on cortical spreading depression in awake mice

Authors: *R. ENGER[1], D. B. DUKEFOSS[1], W. TANG[1], K. H. PETTERSEN[1], D. M. BJØRNSTAD[1], P. J. HELM[1], V. JENSEN[1], R. SPRENGEL[2], K. VERVAEKE[1], O. P. OTTERSEN[1], E. A. NAGELHUS[1];
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Abstract: Cortical spreading depression (CSD) is a phenomenon that gravely disrupts the ion homeostasis mechanisms on which normal brain function so critically depends. Demonstrating the sequence of events triggered by CSD holds the potential of providing new insight in the pathophysiology of a range of neurological disorders as well as the physiological processes underlying normal brain function. Here, we have studied the sequential progression of CSD in awake head-fixed mice by two-photon microscopy. We have compared wildtype mice, and mice with targeted deletion of aquaporin-4 (AQP4) or the inositol 1,4,5-triphosphate receptor type 2 (IP3R2). By use of a novel combination of genetically encoded sensors that permits an unprecedented temporal and spatial resolution we show that CSD leads to brisk Ca^{2+} signals in astrocytes and that the duration of these Ca^{2+} signals is shortened after deletion of AQP4 but not after deletion of IP3R2. The decrease of the astrocytic, AQP4 dependent Ca^{2+} signals coincides in time and space with a decrease in the duration of extracellular glutamate overflow. Our results suggest that in CSD, the glutamate accumulation in the extracellular space is extended through AQP4 dependent glutamate release from astrocytes. The present data point to a salient glial contribution to CSD and identifies AQP4 as a new target for therapy.

Disclosures: R. Enger: None. D.B. Dukefoss: None. W. Tang: None. K.H. Pettersen: None. D.M. Bjørnstad: None. P.J. Helm: None. V. Jensen: None. R. Sprengel: None. K. Vervaeke: None. O.P. Ottersen: None. E.A. Nagelhus: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.11/B23

Topic: B.12. Glial Mechanisms

Support: NIH Grant NS087391

Title: Neuronal exosomal mir-124 upregulates astrocytic GLT1 by suppressing GLT1 mrna-binding micrornas

Authors: *J. M. YELICK, S. SEO, E. BROWN, Y. YANG;
Tufts Univ., Boston, MA

Abstract: Neuron to astrocyte signaling is essential for functional tripartite synapses in the mammalian central nervous system. Emerging evidence has shown that microRNAs are sorted into exosomes, delivered to the extracellular space, and internalized by other cells as an important mechanism of intercellular communication. Previous studies in our lab have shown that neuronal cultures secrete exosomes which contain multiple microRNAs, particularly microRNA-124 (miR-124). We further found that these exosomes significantly up-regulate glutamate transporter GLT1 protein expression in miR-124-dependent manner. In this current study we found that upon injecting fluorescently labeled microRNAs into the mouse sciatic nerve, miR-124 is specifically and actively transported into astrocytes from spinal motor neurons, as compared to c. elegans miR-709 control. In addition, we observed widespread expression of eGFP-tagged CD63, a surface marker on exosomes in our newly developed cre-dependent CD63-eGFP exosome reporter mice, indicating the presence of cell-type specific exosomes in an intact central nervous system.
To identify microRNAs that directly inhibit GLT1 expression, we bioinformatically analyzed microRNAs that were predicted to bind to the 3' untranslated region (UTR) of GLT1 mRNA. We also examined expression level of these microRNAs in cultured cortical astrocytes and astrocytes isolated from the BAC ALDH1L1-eGFP reporter mouse line using AffymetrixTM microRNA array and qRT-PCR. We further found that several of these microRNAs significantly decrease protein expression of GLT1 in cultured astrocytes. Interestingly, these microRNAs are also down-regulated by transferred miR-124, which also suppresses the inhibitory effect of these microRNAs on GLT1 expression. Together, our results have identified new microRNAs that are involved in GLT1 expression regulation, as well as characterized a novel exosome-mediated communication pathway from neurons to astrocytes *in vivo*.

Disclosures: J.M. Yelick: None. S. Seo: None. E. Brown: None. Y. Yang: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.12/B24

Topic: B.12. Glial Mechanisms

Support: Lundbeck Foundation

Title: Ketamine promotes rapid morphological alteration of astrocytes and microvessels in the hippocampus of genetic animal model of depression

Authors: *M. ARDALAN[1,3], J. R. NYENGAARD[3,4], B. ELFVING[2], G. WEGENER[2,5];
[1]Translational Neuropsychiatry Unit, Aarhus C, Denmark; [2]Dept. of Clin. Medicine, Aarhus Univ. Hosp., Translational Neuropsychiatry Unit, Risskov, Denmark; [3]Dept. of Clin. Medicine, Aarhus Univ. Hosp., Stereology and Electron Microscopy Lab., Aarhus, Denmark; [4]Aarhus Univ., Ctr. for Stochastic Geometry and Advanced Bioimaging, Aarhus, Denmark; [5]Sch. of Pharm. (Pharmacology), North-West Univ., Pharmaceut. Res. Ctr. of Excellence, Potchefstroom, South Africa

Abstract: **Background:** Major Depressive Disorder (MDD) is a common medical impairment associated with a huge economic problem in the public health. Recently, astrocytic abnormality has been related to MDD. Astrocytes are likely to have an important role in modulating the excitatory glutamatergic synapses in the hippocampus, which is one of the core brain regions of depression. Ketamine is known as a novel glutamatergic antidepressant drug, with a rapid (within hours) effect, therefore, it is important to unravel rapid effect of ketamine on the alteration in the hippocampal vasculature and astroglial plasticity. In this context, we examined the morphological alteration of astrocytes and microvessels in the hippocampus of genetic rat model of depression.
Methods: Adult male Flinders Sensitive Line (a highly validated genetic animal model of depression) and Flinders Resistant Line rats as control group received a single injection of ketamine (15 mg/kg) or saline intraperitoneally one day before transcardial perfusion. Assessment of depressive-like behavior of animals was performed with the forced swim test (FST) was 30 minutes before perfusion-fixation. Investigation of morphological alteration of hippocampal astrocytes was done on glial fibrillary acid protein (GFAP) stained sections of hippocampus. Quantitative measurement of hippocampal microvasculature was done by applying global spatial sampling method.
Results: The FST showed significant rapid antidepressant like effect of ketamine in FSL rats one day after treatment by showing remarkable reduction in immobility time in comparison with FSL-vehicle group ($p<0.05$). In the hippocampus the size of the astrocytes enhanced significantly in the subregions (CA1.stratum radiatum and molecular layer of DG) ($p<0.05$). Moreover, the

analysis of the astrocytes branches complexity indicated significant positive rapid effect of ketamine on the astrocytic arborization in both FSL and FRL rats one day after injection. In addition to the morphological changes of astrocytes, ketamine had a rapid protective effect on the vascular structure of hippocampus by significant increasing in the total length of microvessels one day after treatment in FSL rats ($p<0.05$).
Conclusion: These data suggest that morphological changes of astrocytes and microvessels in the hippocampus are associated with the rapid antidepressant effect of ketamine and astroglial changes as well as vascular alteration of hippocampus respond rapidly to a single dose of ketamine injection.

Disclosures: M. Ardalan: None. J. R. Nyengaard: None. B. Elfving: None. G. Wegener: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.13/B25

Topic: B.12. Glial Mechanisms

Support: BBSRC BB/L019396/1

MRC: MR/L020661/1

BBSRC BB/K009192/1

Title: Structure-activity relationship study of lactate derivatives and their ability to evoke norepinephrine release in locus coeruleus

Authors: *V. MOSIENKO, D. JANE, S. KASPAROV, A. G. TESCHEMACHER;
Univ. of Bristol, Bristol, United Kingdom

Abstract: Astrocytes serve as main source of L-lactate (LL) which they synthetize through the glycolytic pathway from glucose or stored glycogen. According to the 'lactate shuttle' hypothesis, LL released by astrocytes is taken up by the neurons where it is used as a metabolic substrate. In addition, there is a large body of evidence supporting the hypothesis that LL has a signalling role in the brain. Previously, we demonstrated that bath application of LL or optogenetic stimulation of astrocytes excite noradrenergic neurons and stimulate norepinephrine (NE) release in locus coeruleus (LC) *in vitro*. These effects of LL were concentration-dependent, did not require its import into neurons, and were blocked by D-lactate (DL) and inhibitors of cAMP-dependent signalling. Altogether, our data pointed to the existence of a yet unknown excitatory G-protein coupled receptor (GPCR) for LL. To characterise the pharmacological

profile of this potential GPCR we performed structure-activity studies with LL derivatives while monitoring NE release. We tested compounds in which the potential active moieties of LL were systematically replaced by different functional residues. NE release *in vitro* was monitored using cell-based neurotransmitter fluorescent engineered reporter cells (CNiFERs) which express α1 adrenoceptors and TN-XXL, a FRET-based recombinant $[Ca^{2+}]i$ indicator (Muller et al., 2014). When seeded on organotypic brain slices containing the LC region, fluctuation of NE release upon stimulation by LL and its analogues could be detected by a rise in $[Ca^{2+}]i$ in the CNiFERs. We found that α-hydroxyisobutyric acid was equipotent to LL, and its effect was blocked by DL. However, neither (S)-(+)-mandelic acid nor (L)-(-)-phenyllactic acid were able to evoke NA release. This suggests, that the methyl group alpha to the carboxylic acid group of LL might participate in the interaction with the putative LL receptor. (-)-Methyl-L-lactate was without effect, while 2-hydroxy-2-propanesulfonic acid had a similar action to LL, and this effect was blocked by DL. These data suggest that the carboxyl group has to be in ionized form in order to interact with the receptor, however it can be mimicked by a sulfonic acid. L-Alanine, L- or D-serine did not evoke NE release, while (S)-(-)-2-methoxypropionic acid had a similar effect to LL, which was blocked by DL. These data suggest that the hydroxyl group in LL acts as a hydrogen bond acceptor but hydrogen bond donation is not necessary, and that an amino group cannot substitute for the hydroxyl group. These experiments will help to devise the molecular nature of the putative LL receptor and identify molecular determinants needed for LL binding to it.

Disclosures: V. Mosienko: None. D. Jane: None. S. Kasparov: None. A.G. Teschemacher: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.14/B26

Topic: B.12. Glial Mechanisms

Support: Paul G. Allen Family Foundation Award

NIMH Grant R01MH099595

That Man May See

T32 EY007120

NIH-NEI EY002162

Research to Prevent Blindness Unrestricted Grant

Title: Human astrocyte complexity and function recapitulates in a novel three dimensional neuron coculture system

Authors: *R. KRENCIK[1], K. SEO[1], J. VAN ASPEREN[1], M. E. WARD[3], D. H. ROWITCH[2], E. M. ULLIAN[1];
[1]Ophthalmology, [2]Pediatrics, Univ. of California - San Francisco, San Francisco, CA; [3]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: There is an essential need for an improved *in vitro* model of the complex human brain environment. To overcome this barrier, we have optimized a three dimensional organoid coculture system consisting of induced pluripotent stem cell (iPSC)-derived astroglial and neuronal subtypes genetically engineered for inducible expression of transcription factors. This system addresses the fact that human astrocytes have unique and complex characteristics that are not recapitulated in non-human cellular models or two dimensional cultures. We are using this system to ask several questions such as 1) what cell-cell interactions are necessary to induce structurally complex human astrocytes, 2) what intrinsic mechanisms are at play that give human astrocytes their unique characteristics and 3) what conserved and unique functional characteristics are present in human astrocytes. Recently, we have used iPSC approaches as a model for human astrocytes to comprehend this cellular complexity in normal development and in the context of neuropathology, and we now utilize this improved system for more in depth studies of astrocyte function and dysfunction in disease. Finally, to determine functional diversity of human astrocyte subtypes involved in providing signals to neurons, we used this system to obtain gene expression signatures of human astroglial progenitors regionally patterned *in vitro* and are investigating how this diversity supports regionally-matched neurons. By harmonizing human neurons with a more appropriate experimental environment, we have begun to identify critical signals that human astrocytes contribute towards human neuron circuit homeostasis in development and disease.

Disclosures: R. Krencik: None. K. Seo: None. J. van Asperen: None. M.E. Ward: None. D.H. Rowitch: None. E.M. Ullian: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.15/C1

Topic: B.12. Glial Mechanisms

Title: Characterization of glia calcium signaling in the regulation of neuronal excitability

Authors: *Y. V. ZHANG, J. T. LITTLETON;
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Abstract: Alterations in neuronal excitability can lead to severe neurological disorders like epilepsy. Studies in recent years have begun to reveal the essential role of glia in modulating the function of neural circuits. Previous work in our lab identified Zydeco (Zyd), a cortex glial potassium-dependent sodium calcium exchanger (NCKX), as a critical regulator of acute neural excitability in *Drosophila*. Mutations in *zyd* predispose flies to temperature-sensitive seizures and result in neuronal hyperexcitability at elevated temperatures. Meanwhile, near-membrane microdomain calcium transients found in wildtype cortex glia are abolished in *zyd* mutants, which instead show elevated intracellular glial calcium levels. These observations suggest a calcium-dependent cortex glia-to-neuron signaling cascade acutely regulates neuroexcitability in the *Drosophila* CNS. Characterizing this signaling cascade will greatly contribute to our understanding of how glia and neurons work in concert to maintain normal function of the nervous system. To further define this pathway, we performed an RNAi screen to knock down candidate genes in the *zyd* background using the pan-glial repo-Gal4 driver. Interestingly, we identified many seizure suppressors from the screen that were required in astrocyte-like glia instead of cortex glia, where the Zyd protein acts. This unexpected observation indicates that astrocyte-like glia function to propagate seizure-like activity that arises from a primary defect in cortex glial function. As such, both cortex glia and astrocyte-like glia play critical roles in regulating neuronal excitability in response to seizure-inducing signals arising from elevated calcium within cortex glia. We will present current progress in these studies in an effort to identify the mechanisms that distinct glia subtypes use to regulate neuronal function and excitability.

Disclosures: Y.V. Zhang: None. J.T. Littleton: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.16/C2

Topic: B.12. Glial Mechanisms

Support: F32 EY025915-02

R01 EY026766

Title: Role of soluble adenylate cyclase in reactive astrocytes

Authors: *E. G. CAMERON[1], J. GALVAO[1], C. R. DOUGLAS[2], R. LODHAVIA[2], J. L. GOLDBERG[1];
[1]Ophthalmology, Stanford Univ., Palo Alto, CA; [2]UCSD, San Diego, CA

Abstract: In response to CNS injury or degeneration, astrocytes undergo a process referred to as reactive astrogliosis that leads to changes in tissue architecture and function. Cyclic-AMP (cAMP) has been demonstrated to play a significant role in regulating astrocyte physiology including glutamate uptake, glucose metabolism, differentiation and cytoskeletal remodeling. The extent to which cAMP signaling in astrocytes contributes to such features of reactive astrogliosis *in vivo*, however, remains a promising area of study. In mammals, cAMP is synthesized by a family of transmembrane adenylyl cyclases (tmACs) and by a bicarbonate- and calcium-sensitive soluble AC (sAC). In contrast to tmACs, sAC is insensitive to G-proteins and localizes in the cytoplasm and sub-cellular compartments. sAC promotes proliferation in cancer cells and recently was found to be a critical regulator of transendothelial migration. Moreover, sAC is highly expressed in astrocytes and underlies a key metabolic pathway that shuttles lactate to neurons in response to electrical activity or aglycemic conditions. Reactive astrocytes rapidly proliferate to form a glial scar that sequesters damaged tissue and may promote axon regeneration. Does sAC regulate proliferation and migration in astrocytes during reactive astrogliosis and scar formation? Is sAC-mediated metabolic coupling between astrocytes and neurons stimulated or inhibited by CNS injury? Here, we report that sAC is a key regulator of astrocyte migration and proliferation *in vitro* and characterize the effect of sAC KO on astrocyte reactivity and neuronal survival, *in vivo*. Using an *in vitro* scratch assay, we found that pharmacological inhibition of sAC (with KH7 and 2-HE) suppresses astrocyte proliferation and migration in a concentration-dependent manner. siRNA-mediated knockdown of sAC also inhibited astrocyte migration and reduced proliferation, supporting the hypothesis that these processes are regulated by sAC. Finally, we report our preliminary findings on the effect of sAC knockout on astrocyte reactivity and RGC survival and regeneration after optic nerve crush (ONC) injury in an inducible GFAP-CreERT/sAC$^{fl/fl}$ mouse model. Together these data point towards a critical role for sAC in the glial reaction to CNS injury.

Disclosures: E.G. Cameron: None. J. Galvao: None. C.R. Douglas: None. R. Lodhavia: None. J.L. Goldberg: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.17/C3

Topic: B.12. Glial Mechanisms

Support: Bayer HealthCare "From Targets to Novel Drugs" Focus Grant 2015-03-1272

Pelvic Pain Foundation of Australia Research Grant 2015

Title: Endometriosis-like lesions on peripheral tissues in the mouse induces subtle changes in spinal cord glial reactivity

Authors: *K. DODDS[1], E. A. H. BECKETT[1], S. F. EVANS[2,3], M. R. HUTCHINSON[1,4]; [1]Physiol., [2]Pharmacol., Univ. of Adelaide, Adelaide, Australia; [3]Pelvic Pain SA, Norwood, Australia; [4]ARC Ctr. for Nanoscale Biophotonics, Adelaide, Australia

Abstract: Background: Endometriosis is a female-specific chronic inflammatory condition that often manifests with severe pelvic pain. Recent studies have implicated glia and their interactions with the central nervous system ("neuro-immune communication") in facilitating persistent pain development. This has not yet been studied in the context of endometriosis. Thus, we sought to develop a minimally-invasive mouse model of disease to investigate whether peripheral endometriosis lesions can induce central spinal glial adaptations. Methods: Endometriosis (ENDO) was induced in 8-14 week-old wild-type C57BL/6 or Balb/C mice by intraperitoneal injection of syngeneic donor endometrium (15-40mg) from either the proestrus (high estrogen) or estrus (low estrogen) phase of the estrous cycle. Toll-like receptor 4 knock-out (*TLR4*$^{-/-}$) mice were also induced with endometriosis using only the permutation of 40mg proestrus donor tissue. After three weeks development, the spinal cord was removed from recipient mice and dissected into segments spanning T13-S1. Spinal sections were then processed via fluorescent immunohistochemistry for glial fibrillary acidic protein (GFAP; astrocytes) and CD11b (microglia), and images analyzed using Image J software. Results: Endometriosis-like lesions were successfully established; the extent and location of disease dependent on mouse strain, estrogen status and amount of donor material injected. Preliminary analysis of animals from robust disease conditions (proestrus 40mg tissue) indicates an increased variance in the area occupied by GFAP-immunoreactivity in the T13-L5 dorsal horn of C57BL/6 ENDO mice compared to saline-injected controls (n = 5 per group). In contrast, GFAP-immunoreactivity in Balb/C ENDO mice with endometriosis remains unchanged at this stage (n = 2 per group). Immunostaining results for CD11b in wild-type animals and all spinal glia in *TLR4*$^{-/-}$ mice will additionally be discussed. Discussion: The model described here has successfully recapitulated endometriosis-like disease in the mouse, and allowed for analysis of glial reactivity in the spinal cord. Our early data suggests that there are strain differences in the development of disease. In

addition, the altered glial reactivity observed in C57BL/6 ENDO mice may play an important role in facilitating pain development attributed to endometriotic disease.

Disclosures: K. Dodds: None. E.A.H. Beckett: None. S.F. Evans: None. M.R. Hutchinson: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.18/C4

Topic: B.12. Glial Mechanisms

Support: National Science Foundation Grant 0924372.

Title: Measurement of H+ fluxes from culture rat and mouse astrocytes using self-referencing H+ selective microelectrodes

Authors: *J.-I. CHOI[1], C. GOEKE[2], M. GUIZZETTI[2], R. P. MALCHOW[3];
[1]Univ. of Illinois At Chicago, Chicago, IL; [2]Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: pH regulation in the brain is important and even small changes in intracellular or extracellular pH influence the functions of numerous enzymes and ion channels. Activities such as extracellular glutamate removal process done by glial cells are associated with change in extracellular levels of H+, which can by itself have a potent modulatory effect on neuronal excitability and synaptic transmitter release. In the present study, we have used self-referencing H+-selective microelectrodes to examine standing levels of extracellular H+ from quiescent cortical astrocytes cultured from rats and mice and have also examined changes in the level of extracellular H+ that occur upon addition of the different neurotransmitters. Cultured astrocytes recorded in a 24 mM bicarbonate-based saline solution or1 mM HEPES buffered solution exhibit a standing acidic flux. The standing flux observed in the 1 mM HEPES condition remained when all of the extracellular sodium was replaced with choline. Application of glutamate induced a transient extracellular alkalinization, consistent with its transport into the glial cells. Application of adenosine triphosphate (ATP) induced a pronounced extracellular acidification. These results are the first to show extracellular H+ levels adjacent to isolated glial cells using self-referencing electrodes and suggest the possibility that changes in extracellular H+ by glial cells in response to the release of different neurotransmitters may play a role in modulation of activity within the nervous system. This study was funded in part by National Science Foundation Grant 0924372.

Disclosures: J. Choi: None. C. Goeke: None. M. Guizzetti: None. R.P. Malchow: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.19/C5

Topic: A.01. Neurogenesis and Gliogenesis

Support: BFU2013-48807-R

Title: Glial cell lineages in the olfactory bulb.

Authors: A. BRIBIAN[1], R. SANCHEZ-GONZALEZ[1], M. FIGUERES-OÑATE[2], *L. M. LOPEZ-MASCARAQUE[1];
[1]Inst. Cajal-CSIC, 28002 Madrid, Spain; [2]Inst. Cajal-CSIC, MADRID, Spain

Abstract: The clonal architecture of the brain is critical to understand the mechanisms involved in the specification of neural cell types as well as to understand which aspects of the brain function emanate from its organization. We have recently showed the clonal relationship of astrocytes location in different layers of the olfactory bulb and within the rostral migratory stream (RMS), concluding that a laminar specificity of astrocyte origin and morphology may underlie different functional demands (García-Marques and López-Mascaraque, 2016). We have also revealed the clonal organization of NG2 cell progeny in the olfactory bulb derived from pallial progenitors targeted by *in utero* electroporation at E14 (García-Marques et al., 2014). However, there is some controversy related to the origin and dispersion of the olfactory bulb oligodendrocytes and NG2-glia (also named OPCs), because of their spatial and temporal progenitor locations. To unravel the origin, fate and diversity of those glial populations we performed a clonal cell analysis from single progenitors. To this end, we used the StarTrack method, based on the combinatorial expression of 12 fluorescent reporters under regulation of specific promoters (NG2, GFAP and ubiquitous) to target olfactory bulb embryonic progenitors located into the more rostral part of the ventricular surface at different developmental time-points. This method produces inheritable marks that enable the long-term *in vivo* tracing of glial progenitor lineages and their progeny. Next, we will perform a detailed clonal analysis to elaborate a map of the laminar distribution and heterogeneity of adult glial cells in the olfactory bulb, based on their ontogenic origin.

Disclosures: A. Bribian: None. R. Sanchez-gonzalez: None. M. Figueres-oñate: None. L.M. Lopez-Mascaraque: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.20/C6

Topic: B.12. Glial Mechanisms

Title: Calcium response of satellite glial cells to axonal injury

Authors: *B. POLAT[1], E. N. EKMEKCIOGLU[1], T. AKGUL CAGLAR[1], K. BAYAT[2], G. OZTURK[1];
[1]Regenerative and Restorative Med. Res. Ctr. (REMER), Istanbul Medipol Univ., Istanbul, Turkey; [2]Electrical and Electronics Engin. Dept., Marmara Univ., Istanbul, Turkey

Abstract: Glial cells are known to play key physiological and pharmacological roles in maintenance of the nervous system and the characteristic response of its peripheric and central counterparts to injury. The main type of glia in sensory ganglia is the satellite glial cells (SGCs). SGCs surround individual neurons and are coupled by gap junctions. They show morphological and physiological difference from other glial types but very little research is done on this particular type of cells. Satellite glial cells are known to sense and response to nerve injury and contribute to survival and plasticity of the ganglia neurons. In this study, we observed that the SGCs respond to axonal injury of nearby neurons in terms of elevated Calcium levels.
For this purpose, dorsal root ganglia (DRGs) were dissociated and cultured on laminin coated surfaces. At 48 hours in vitro, just as the SGCs begin to migrate away from neurons, the cell population was loaded with high affinity calcium indicator and imaging was performed under physiological conditions (488nm) We utilized, a precise femtosecond laser induced single cell axotomy injury model. The experiments were repeated in presence of a chemical gap junction blocker and in absence of Calcium. Neurons with and without neighbouring SGCs were chosen for axotomy.
As expected, neurons showed increased fluorescence intensity when axotomised. Unexpectedly, SGCs responded to the injury of neigbouring neuron in terms of a burst of elevated calcium levels. We observed that the neurons with SGCs shown less Calcium increase and this increase dissipated sooner than the standalone neurons. In presence gap junction blocker, aforementioned calcium increase in satellite glial cells was decreased. Same experimental procedure was conducted in a Ca free environment and all calcium activity was absent.
These results briefly show that neighboring SGCs respond to neural injury by means of elevated calcium levels and this interrelation may be regulated via gap-junction coupling. The external calcium is used for this buffering system. We argue that this pattern of Calcium elevation in SGCs may be crucial for maintaining in-ganglia homoestasis as part of an injury response mechanism.

Disclosures: B. Polat: None. E.N. Ekmekcioglu: None. T. Akgul Caglar: None. K. Bayat: None. G. Ozturk: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.21/C7

Topic: B.12. Glial Mechanisms

Title: The effect of induced hyperglycemia on the dorsal root ganglia and sensory nerves in zebrafish

Authors: *H. ENNERFELT, J. CLARK;
Biol. Dept., Salisbury Univ., Salisbury, MD

Abstract: Diabetic peripheral neuropathy (DPN) is characterized by disrupted sensory signaling, the cost of which is rising sharply as the prevalence of diabetes mellitus increases exponentially. Patients with DPN often present with sensory system abnormalities that range from pain to loss of sensation. Although prevalent in diabetic humans, the molecular mechanisms behind DPN are unclear due to experimental limitations. Zebrafish are well suited for vertebrate research due to their similar physiology to humans, fecundity, and transparency that allows for direct visualization of fluorescent genes. Zebrafish have been used to study diabetic conditions such as retinopathy, nephropathy, and delayed wound healing. However, less is known about the health of peripheral nerves of hyperglycemic zebrafish. Therefore, in this study zebrafish are being used to study the effects of hyperglycemia on peripheral nerves hypothesized to be associated with DPN. To assure us that hyperglycemia is indeed being induced in the zebrafish, expression of the gene encoding insulin (*ins*) is examined using rt-qPCR. Using an established protocol to induce the zebrafish into a hyperglycemic state, we then examine the sensory neurons in the dorsal root ganglia (DRG). Following the induction of hyperglycemia in the zebrafish, our results suggest a number of sensory neurons migrate away from the DRG. In addition, there is apparent axonal defasciculation and blebbing in response to this metabolic change. Characterizing the response of peripheral nerve components in a hyperglycemic state may allow us to extend our work to determine the molecular mechanisms responsible for the breakdown of this important structure.

Disclosures: H. Ennerfelt: None. **J. Clark:** A. Employment/Salary (full or part-time): Salisbury University.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.22/C8

Topic: B.12. Glial Mechanisms

Support: Fundación Pandea

Conacyt-Mexico fellowship 472739

Title: Glutamate dependent translational control in cultured bergman glia cells

Authors: R. TIBURCIO-FELIX[1], S. ZINKER-RUZAL[2], M. FLORES-MÉNDEZ[2], *E. SUAREZ[3], A. ORTEGA[4];
[1]Genet., Cinvestav, Ciudad de Mexico, Mexico; [2]Genet., [4]Toxicology, [3]Cinvestav, Ciudad DE Mexico, Mexico

Abstract: GLUTAMATE-DEPENDENT TRANSLATIONAL CONTROL IN CULTURED BERGMAN GLIA CELLS
The amino acid L-glutamate (Glu) is considered to be the major mediator of excitatory signals in the Central Nervous System and is probably involved in most aspects of normal brain function including cognition, memory and learning. It also plays a major role in the development, including synapse induction and elimination, cell migration, differentiation and death. Most neurons, and even glial cells express glutamate receptors in their plasma membrane. Previously, we showed that Glu decreases [^{35}S]-Met incorporation into newly synthetized polypeptides in chick cerebellum cultured Bergmann glia cells (BGC) treated with 1 mM Glu for 15 min. After 30 min, [^{35}S]-Met incorporation has a slow recovery and after 2 h reaches normal levels. Furthermore, Glu responses are mediated by AMPA receptor activation, causing MAPK and mTOR signaling pathways activation. Accodingly, the rpS6 (ribosomal protein S6) is phosphorylated causing an increasing in translation. One important enzyme for Glu recycling is Glutamine (Gln) synthetase (GS) due to its ability to transform Glu to Glutamine as part of the so called *Glu/Gln shuttle*. In order to clarify the transient Glu effect on protein synthesis, we decided to isolate ribosomal fractions from control or Glu treated cells. To this end, a ribosomal profile was prepared by spinning the cell extracts through a continuous 15-50% sucrose gradient. The 40S, 60S, 80S and the polysomal fractions were isolated and the protein of each fraction was analyzed *via* western blots with anti rpS6 and anti p-rpS6 abs. An increase in rpS6 phosphorylation was detected in the 80S and polysomal fraction of Glu-treated cells for 15 and 30 min. We also detected a 15 min decrease of GS mRNA of GS in Glu treated cells but an increase after 30 min Glu.
These results favor the notion of a critical involvement of Glu signaling in the regulation of the

glial protein *repertoire* through the selective translation of specific mRNAs. A role of rpS6 phosphorylation is also suggested.

Disclosures: R. Tiburcio-Felix: None. S. Zinker-Ruzal: None. M. Flores-Méndez: None. E. Suarez: None. A. Ortega: None.

Poster

209. Astrocytes Development and Metabolism

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 209.23/C9

Topic: A.01. Neurogenesis and Gliogenesis

Support: TÜBİTAK 114R078

Selcuk University BAP

Title: Breast milk induced microchimerism in the brain

Authors: *M. S. AYDIN[1], E. EKMEKCIOGLU[2], E. VATANDASLAR[2], G. OZTURK[3], E. ERDOGAN[4], T. MUDOK[3];
[1]Fac. of Medicine, Dept. Histology and Embryology, Bezmialem Vakif Univ., Istanbul, Turkey; [2]Grad. Sch. of Hlth. Sci., [3]Intl. Sch. of Med., Istanbul Medipol Univ., Istanbul, Turkey; [4]Sch. of Med., Selcuk Univ., Konya, Turkey

Abstract: Microchimerism is the presence of cells of an individual in another genetically different host without inducing an immune response. It most commonly occurs between the mother and the offspring during pregnancy. Fetal cells cause fetal cell microchimerism by entering the maternal circulation through the placenta during the pregnancy while the passage of maternal cells to offspring is called maternal microchimerism. Transfer of maternal cells to the offspring through breast milk is also possible and this is defined as breast milk induced maternal microchimerism.
In this study, we aim to identify the cells that pass from mother to the central nervous system of the offspring during breastfeeding and characterize phenotypic features and the differentiation patterns of these.
The basic experimental paradigm of the study was to let green fluorescent protein expressing (GFP+) transgenic female mice breastfeed wildtype pups and to track GFP+ cells mainly in their nervous tissue. Immunohistochemistry, flow cytometry and PCR techniques were used to this end.
We detected GFP+ chimeric cells of the foster mother in the brain as well as in many other

organs of the pups at postnatal 7th day. We also demonstrated that many of these cells differentiate into glial cells.
The maternal microchimerism through breastmilk is a novel concept and it may have significant implications in the normal functioning and pathological processes of the nervous system.
This project was supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey, Project No: 114R078) and Selcuk University BAP (Scientific Research Projects).

Disclosures: M.S. Aydin: None. E. Ekmekcioglu: None. E. Vatandaslar: None. G. Ozturk: None. E. Erdogan: None. T. Mudok: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.01/C10

Topic: A.04. Transplantation and Regeneration

Title: Gene expression changes during brain regeneration in adult zebrafish

Authors: *K. PALSAMY, J. PARENT;
Neurology, Univ. of Michigan Med. Ctr., Ann Arbor, MI

Abstract: In the mammalian brain, neural stem cells and neurogenesis persist into adulthood mainly in the subependymal zone-olfactory bulb pathway and hippocampal formation. Adult neurogenesis is proposed to play an important role in learning and memory, but it also offers the potential for replacing injured or diseased brain tissues through regeneration by neural stem cells. Despite the persistence of neurogenesis, mammals have limited brain regenerative capacity. Zebrafish, however, display robust regeneration in many different brain regions and can make mature, functional neurons after injury. We use adult zebrafish to explore the molecular networks induced by traumatic brain injury and quinolinic acid (QA)-induced neurotoxicity. We performed RNA sequencing on telencephalic tissue collected 24 or 48 hours after brain injury, with or without QA lesioning, and identified many interesting candidate genes involved in various biological processes. Our gene ontology and pathway analysis study confirms upregulation of immune response, tissue regeneration, wound healing, cell migration, angiogenesis processes, and Jak-Stat, interleukins, inflammatory-induced chemokines and cytokines, notch, and B cell and T cell activation pathways respectively. Based upon expression level changes and their known roles in neuroprotection or stem cell function, we selected a few candidate genes to explore for their potential involvement in brain regeneration. We first confirmed the RNAseq expression changes of the candidates after injury using real-time RT-PCR. Work is ongoing to examine cellular expression changes by in situ hybridization, and to

generate loss-of-function models to examine how alterations in the candidate genes influence brain injury and the regenerative process. We are also using the QA excitotoxicity model in mouse striatal lesioning with the goal of comparing gene expression changes with the zebrafish model. Our eventual aim is to shed light on mechanisms underlying the limited regenerative capacity of the mouse brain with the goal of improving brain repair.

Disclosures: K. Palsamy: None. J. Parent: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.02/C11

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant NS082446

NIH DE022000

University of missouri spinal cord injuries research program

The hope center for neurological disorders just in time awars

Title: Hdac5 upregulation in retinal ganglion cells promotes optic nerve regeneration

Authors: *D. W. PITATHOMAS[1], Y. OH[2], V. CAVALLI[2];
[1]Neurosci., Washington university, Saint Louis, MO; [2]Washington university, St louis, MO

Abstract: Injuries in the optic nerve caused by ischemic optic neuropathy or glaucoma are followed by retinal ganglion cell (RGC) axon degeneration and cell death, and result in permanent visual function loss. In contrast, sensory neurons in dorsal root ganglion (DRG) are able to regenerate their axon following injury. This is partly due to their ability to activate an intrinsic pro-regenerative response. We have previously demonstrated a role for histone deacetylase 5 (HDAC5) in DRG axon regeneration. After sciatic nerve injury, HDAC5 leaves the nucleus to allow for the expression of pro-regenerative genes, and accumulates at the axon tip, to deacetylate tubulin. Both steps are important to stimulate axon regeneration. Here, we studied the role HDAC5 in RGC response to injury. We found that, in contrast to DRG neurons, HDAC5 is localized exclusively in the RGC cytoplasm and is insensitive to the canonical signaling pathways that regulate its nuclear localization in DRG neurons and other cell types. This rules out a major epigenetic role for HDAC5 in RGCs and rather suggests that HDAC5 plays a role in the cytoplasm. Similar to what we observed in injured sciatic nerve, optic nerve

injury led to a decrease in acetylated tubulin levels at the injury site. However, HDAC5 did not accumulate in injured optic nerve. We then tested whether increasing the levels of HDAC5 in RGC axons would improve axon regeneration. We expressed a cytoplasmic HDAC5 mutant, which localized to RGC axons, and observed that the number of regenerating axons beyond the optic nerve injury site doubled compared to control. We are currently testing whether HDAC5 has specific interacting partners and deacetylation targets in the axon that contribute to stimulate axon regeneration. These results suggest that increasing HDAC5 levels in the optic nerve can be exploited to promote axon regeneration

Disclosures: **D.W. Pitathomas:** A. Employment/Salary (full or part-time): Washington university. **Y. Oh:** None. **V. Cavalli:** None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.03/C12

Topic: A.04. Transplantation and Regeneration

Support: NYSTEM C028108

NYSTEM C026413

National MS Society RG 5505-A-2

Title: Muscarinic M_3 receptor signaling inhibits differentiation and myelin repair by mouse and human oligodendrocyte progenitors.

Authors: ***J. J. POLANCO**, R. R. WELLIVER, Z. M. KHAKU, M. A. O'BARA, F. J. SIM; Pharmacol. & Toxicology, Univ. At Buffalo, Buffalo, NY

Abstract: Recent studies have identified anti-muscarinic drugs as potent modulators of oligodendrocyte differentiation and myelination, however the precise muscarinic receptor subtype signaling have not determined. Based on the high relative expression of muscarinic acetylcholine receptor subtype 3 (M_3R) in human oligodendrocyte progenitor cells (OPCs) and functional pharmacological analysis in rodent OPCs, we hypothesized that M_3R signaling acts to inhibit OPC differentiation. In this study, we investigated the role of M_3R in both rodent and human OPCs. In human primary PDGFRα/CD140a$^+$ OPCs, lentiviral shRNAi knockdown of M_3R increased O4$^+$ oligodendrocyte differentiation and myelin protein expression (MBP) mRNA *in-vitro*, relative to scrambled controls. Importantly, M_3R-knockdown in human OPCs increased oligodendrocyte differentiation and MBP$^+$ myelin following transplantation into hypomyelinated

shiverer/*rag2* mice at 12 weeks after engraftment. Next, to study the role of M_3R in myelin repair, we conditionally deleted M_3R in $NG2^+$ OPC using Ng2-CreER:$M3R^{fl/fl}$ mice and induced demyelination in adult mouse spinal cord by injection of lysolecithin. M_3R deletion in adult OPCs resulted in significantly increased oligodendrocyte density as determined by both *Plp1 in-situ* hybridization and CC1 immunostaining. The rate and number of recruited OPCs was similar in tamoxifen and oil-injected control animals, indicating that the effect of M_3R deletion was primarily on induced differentiation. These results indicate that M_3R signaling is a species-conserved inhibitor of OPC and oligodendrocyte differentiation that may be specifically targeted to improve both endogenous and transplant-mediated myelin repair.

Disclosures: J.J. Polanco: None. R.R. Welliver: None. Z.M. Khaku: None. M.A. O'Bara: None. F.J. Sim: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.04/C13

Topic: A.04. Transplantation and Regeneration

Support: CONICET Grant 0441

UBA Grant 0526 BA

Title: Shh signaling mediates complete retina regeneration through the activation of stem/progenitor cells located in the ciliary margin.

Authors: J. DI NAPOLI[1], C. OLMOS CARREÑO[1], L. FIORE[1], M. MEDORI[1], L. R. TERUEL[1], V. SANCHEZ[1], K. DEL RIO-TSONIS[2], *G. E. SCICOLONE[1];
[1]Inst. of Cell Biol. and Neuroscience. Sch. of Medicine, UBA, Buenos Aires, Argentina; [2]Dept. of Biol., Miami Univ., Oxford, OH

Abstract: Retina regeneration in the embryonic chick can occur through two distinct pathways: 1) by retinal pigmented epithelium (RPE) transdifferentiation or 2) by the activation of stem/progenitor cells located in the ciliary margin (CM).
In chicks, retina regeneration is only possible during the fourth day of development (E4) and when it is stimulated by growth factors. Fibroblast growth factor 2 (FGF2) stimulates transdifferentiation and activation of stem/progenitor cells from the CM, whereas sonic hedgehog (Shh) induces activation of stem/progenitor cells from the CM.
RPE transdifferentiation produces an inverted retina without RPE, whereas regeneration from the

CM produces a normally oriented and laminated retina. Obtaining this last form of regeneration is the goal of any regenerative strategy.
The central aim of this work was to evaluate which treatment presented the highest efficacy to produce a complete retina along all its tangential extension. The Shh pathway and its analog activator SAG were evaluated for their potential to induce complete retina regeneration from the stem/progenitor cells of the CM.
After retinal removal at E4, we tested different treatments to evaluate retinal regeneration: FGF2 and RCAS-Shh or SAG alone or in combination with FGF2 were added to induce retinal regeneration.
We showed that the activation of the canonical Shh pathway induced by SAG produced the highest efficacy of complete retinal regeneration through the activation of stem/progenitor cells of the CM (100% of embryos) *in vivo.* We also showed that the retinal ganglion cells from this regenerated retina have positional information, as they expressed the Eph/ephrin system in a gradient similar to the retinas during development.
These results increase the probability of obtaining an efferent system that establishes a topographic order of connections in the tectum.
Retina stem/progenitor cells exist in other species, including humans. Thus, our findings provide insights on how retinal stem cells can be activated for possible regenerative therapies.

Disclosures: J. Di Napoli: None. C. Olmos Carreño: None. L. Fiore: None. M. Medori: None. L.R. Teruel: None. V. Sanchez: None. K. Del Rio-Tsonis: None. G.E. Scicolone: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.05/C14

Topic: A.04. Transplantation and Regeneration

Support: Bryon Riesch Paralysis Foundation

NIH R21NS093278

Title: Combinatorial transcription factor treatments to promote axon outgrowth in CNS neurons

Authors: *I. VENKATESH, M. SIMPSON, B. CALLIF, Z. WANG, M. BLACKMORE; Dept. of Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Developing neurons and peripheral neurons show a robust ability to regenerate axons following nerve injury. This is in part due to their intrinsic capacity to successfully re-activate

transcriptional networks supportive of re-growth. In contrast, injured mature CNS neurons fail to induce appropriate regeneration-associated genes (RAGs) resulting in abortive growth. We and others have shown that forced expression of transcription factors (TFs) can promote axon outgrowth in response to CNS injury, highlighting a promising strategy for therapeutic intervention. To build on this success, there is a need to identify molecular manipulations to enhance the efficacy of pro-regenerative TFs. An emerging idea in regeneration research is that regeneration depends on networks of interacting transcription factors, and that co-expression of multiple factors may be necessary to fully restore regenerative potential. We are taking a two-pronged strategy to test this idea. First, because AP-1 factors have been widely implicated in axon growth and are known to work interactively, we systematically over-expressed combinations of AP1 factors JUN, ATF3 and FOS in assays of neurite outgrowth in postnatal CNS neurons. Consistent with previous results, overexpression of JUN reliably increased neurite lengths. Interestingly, unlike recent results in peripheral neurons, we observed no synergistic effects of AP1 factor co-expression on neurite outgrowth. Immunohistochemistry indicated that even as cortical neurons extend neurites, basal levels of AP1 factors including JUN and ATF3 are highly variable and undetectable in many cells. Consistent with this, CRISPR-based knockdown of JUN minimally affected neurite outgrowth. These data hint at differences in the transcriptional regulation of axon growth in CNS neurons compared to DRG neurons. In ongoing work, we are taking a systems biology based approach to map the transcriptional landscape as CNS neurons age, to identify genome-wide changes in transcriptional networks that could underlie the age-related decline in CNS neuronal regenerative capacity. Future experiments will validate predicted networks through combinatorial overexpression and knockdown of hub TFs in assays of neurite outgrowth. Overall this research is expected to identify novel transcriptional regulatory codes that can be manipulated to increase axon regeneration following CNS injury.

Disclosures: I. Venkatesh: None. M. Simpson: None. B. Callif: None. Z. Wang: None. M. Blackmore: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.06/C15

Topic: A.04. Transplantation and Regeneration

Support: UK MS Society

Jean Shanks Foundation

MedImmune

Title: Remyelination and the Gut Microbiome

Authors: *C. MCMURRAN[1], O. ZIDON[1], Y. DOMBROWSKI[2], D. C. FITZGERALD[2], R. J. M. FRANKLIN[1];
[1]Wellcome Trust-MRC Cambridge Stem Cell Inst., Cambridge, United Kingdom; [2]Sch. of Medicine, Dent. and Biomed. Sci., Belfast, United Kingdom

Abstract: Across a range of tissues and species, regenerative processes are intimately linked to the innate immune system, which is in turn influenced by microbes in the gut.
After demyelination, the restoration of myelin sheaths depends upon infiltrating macrophages and their CNS-resident counterparts, microglia, which phagocytose debris and secrete growth factors. The normal functions of these innate immune cells depend on a healthy microbiome. Our hypothesis ties these two concepts together, and asks whether the gut microbiome can influence the efficiency of remyelination.
We have used a model in which mice are treated with high-dose antibiotics to deplete gut microbes, before stereotactic injection of lysolecithin to cause demyelination. Antibiotic treatment causes a depletion in oligodendrocyte progenitor cell differentiation in response to this. Future work will make use of germ-free mice to investigate whether this effect is mediated by the gut microbiome.

Disclosures: C. McMurran: None. O. Zidon: None. Y. Dombrowski: None. D.C. Fitzgerald: None. R.J.M. Franklin: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.07/C16

Topic: A.04. Transplantation and Regeneration

Support: DoA Grant W911NF-13-1-0164

Title: Neural anatomical and synaptic changes observed following CNS injury that underlie functional plasticity displayed in a regenerating annelid model system.

Authors: *V. G. MARTINEZ ACOSTA;
Biol., Univ. of the Incarnate Word, San Antonio, TX

Abstract: Recent studies in the regenerating model system, *Lumbriculus variegatus*, suggest functional recovery following nerve injury may be mediated by the release of inhibition on synaptic connections that are already in existence along the body axis and that increasing

neuronal activity further stabilizes the dominant connection in an anterior-posterior specific manner (Lybrand and Zoran, 2012). To further develop this hypothesis, this study characterizes changes in neural architecture occurring at the synaptic level that would allow for rapid adjustments in motor function during regeneration. Transmission electron micrographs of regenerating and non-regenerating worm fragments are compared. Similar to architecture identified in other oligochaete worms (Jamieson, 1981; Zoran et al., 1988), giant fiber collaterals protrude through the myelin-like sheathing of the giant axons. Collaterals of the medial giant fiber in both anterior and posterior body segments contained clusters of small translucent synaptic vesicles, which were localized to the lateral edges of the protruding processes. These synaptic vesicle clusters likely represent interneuronal synaptic outputs to escape motor reflex pathways (Drewes, 1984). Intermediate giant fibers (IGFs), which are unmyelinated, are located in bundles just below the larger giant fiber axons within neuropile. Extensive chemical synaptic contacts were observed terminating onto the IGFs. These synaptic terminals were associated with plasma membrane thickenings, reminiscent of pre- and postsynaptic densities, and contained predominately small translucent vesicles, along with some dense core vesicles. Confocal analysis of regenerating and non-regenerating worms also revealed the presence of extensive serotonergic fibers within the neuropile. Peripheral neurons, which extend out into the musculature around the segment are also positively labeled with antibodies for serotonin. Using both TEM and confocal micrographs of wound blastemal and regenerating tissue, we will construct a more detailed map of the neuroanatomical changes that occur along the sensory-interneuronal and interneuronal to motor boundaries.

Disclosures: V.G. Martinez Acosta: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.08/C17

Topic: A.04. Transplantation and Regeneration

Support: PRESTO (JST)

JSPS Postdoctoral Fellowships for Research Abroad

KANAE Foundation for the Promotion of Medical Science

NINDS NS093002

New Jersey Commission on Spinal Cord Research

Title: Semaphorins limit axon regeneration after spinal cord injury

Authors: *Y. UENO, M. UENO, J. NIEHAUS, Q. R. LU, Y. YOSHIDA;
Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Axon regeneration is limited in the central nervous system, which hinders reconstruction of functional circuit and recovery after spinal cord injury (SCI). Although multiple extrinsic molecules have been identified to inhibit axon regeneration, roles of semaphorin
family, one of major classes of repulsive axon guidance molecule, have not been thoroughly explored yet. We first found that expressions of a series of semaphorins (Sema3d, 4c, 4d, 5a, and 6d) were increased in $Olig2^{+}$ cells after SCI. Genetic deletion of
semaphorin ligands (Sema5a, 6d) or their receptors (Neuropilin1, PlexinA1) enhanced regeneration or reduced dieback of descending corticospinal and raphespinal axons. Chromatin immunoprecipitation sequencing (ChIP-seq) revealed that the basic-helix-loop-helix (bHLH) transcription factor Olig2 and Brg1, an ATP dependent chromatin remodeling enzyme, bound to enhancer regions of these semaphorin genes, suggesting that they are the key transcription regulators creating inhibitory environment. Our results highlight a novel molecular system inhibiting axon regeneration.

Disclosures: Y. Ueno: None. M. Ueno: None. J. Niehaus: None. Q.R. Lu: None. Y. Yoshida: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.09/C18

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01 MH091676

NIH Grant R21 MH099799

NIH Grant R01 MH067880

Glaucoma Research Foundation

Title: Quantifying changes in protein synthesis and transport in optic neuropathies.

Authors: *S. H. SHAH[1], L. M. SCHIAPPARELLI[2], S. SATURDAY[3], D. MCCLATCHY[4], Y. MA[4], J. YATES, III[4], H. CLINE[3], J. GOLDBERG[5];
[1]Neurosci., UCSD, La Jolla, CA; [2]Cell Biol., [3]Mol. and Cell. Neurosci., [4]Chem. Physiol., The Scripps Res. Inst., La Jolla, CA; [5]Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: Identifying molecular regulators of optic nerve degeneration and regeneration in response to injury and disease remains a critical step in preventing loss of vision and stimulating regeneration. In glaucoma, the leading cause of irreversible blindness worldwide, global proteome changes have been observed but not quantified with high temporal resolution. Here we undertook to understand how RGCs respond to axon injuries such as those that occur after optic nerve crush (ONC) or in response to elevated intraocular pressure, specifically examining protein synthesis and transport down their axons in the optic nerve. In particular, we hypothesize that identifying proteins that are newly translated in the retina after injury will allow us to dissect the proteomic response to pathologies. We used intravitreal injections of the non-canonical amino acid azidohomoalanine (AHA), which is incorporated in place of methionine during protein synthesis, and NHS-biotin, which binds to available lysine side-groups, combined with a novel quantitative mass spectrometry pipeline based on the DiDBiT protocol to detect biotinylated proteins (Schiapparelli et al, 2014, McClatchy et al, 2015) after optic nerve crush (ONC) in adult rats. We identified ~300 proteins from RGC axons whose transport down the optic nerve decreased after ONC, and ~30 whose transport increased, out of 500 RGC-tagged proteins detected in the optic nerve 12 hours following axon injury. We identified ~30 retinal proteins whose synthesis significantly increased within 27 hours after ONC out of ~400 quantifiable retinal proteins synthesized in the 27 hour window overall. Extending this methodology to glaucomatous injury will allow us to identify and quantify differentially translated and transported proteins in RGCs in the context of specific disease models, a key step in developing mechanistically-guided approaches to novel treatments.

Disclosures: S.H. Shah: None. L.M. Schiapparelli: None. S. Saturday: None. D. McClatchy: None. Y. Ma: None. J. Yates: None. H. Cline: None. J. Goldberg: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.10/C19

Topic: A.04. Transplantation and Regeneration

Support: National research foundation of korea government (NRF-2015R1A2A1A01003410

Title: Influence of altered DNA methylation on axon growth capacity following preconditioning peripheral nerve injury

Authors: *H. SHIN[1], K. KIM[2], M. KWON[3], B. KIM[1];
[1]Ajou University, Med. Sch., Suwon, Korea, Republic of; [2]Samsung Advanced Inst. for Hlth. Sci. and Technol., Seoul, Korea, Republic of; [3]Yale university, New haven, CT

Abstract: Although mature CNS neurons do not spontaneously regenerate injured axons, conditioning injury (CI) to the peripheral branches of DRG sensory neurons can robustly upregulate regeneration-associated genes (RAGs) and thereby enhance central axon regeneration after lesions in the spinal cord. The mechanism by which CI regulates transcriptional activation of RAGs has not been fully understood. The present study sought to determine potential influence of DNA methylation changes on the transcriptional activation of RAGs in the CI-induced regeneration model. Methylated DNA immunoprecipiation (MeDIP) was performed using DNA samples from L4-6 DRGs followed by next generation sequencing. The MeDIP-seq revealed that approximately 2000 genes were hypermethylated and a similar number of genes were demethylated after CI. However, the extent of changes in the level of DNA methylation was not correlated with gene expression levels measured by RNA-seq in most genes, including the RAGs. Interestingly, pharmacological inhibition (5-aza-2'-deoxycytidine, 5-aza) or activation (S-Adenosyl methionine, SAM) of DNA methylation significantly decreased neurite outgrowth potentials in preconditioned DRG sensory neurons. To examine effects of pharmacological perturbation of DNA methylation on RAG expression, we performed a PCR array customized for 44 RAGs reported in literature. We found that both 5-aza and SAM treatment led to robust upregulation of Socs3 and Serpine1 genes accompanied by downregulation of well-known growth promoting genes such as Gap-43 and Sprr1a. These findings suggest that alteration of global DNA methylation does have influence on enhanced axon growth potentials by preconditioning injury. However, these effects do not seem to be mediated by direct regulation of transcriptional activity of RAGs.

Disclosures: H. Shin: None. K. Kim: None. M. Kwon: None. B. Kim: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.11/C20

Topic: A.04. Transplantation and Regeneration

Support: NINDS-NS083942

the Ray W. Poppleton Endowment

NINDS-NS093002

PRESTO (JST)

JSPS Postdoctoral Fellowships for Research Abroad

KANAE Foundation for the Promotion of Medical Science

NIH grant P40RR018604

Title: Rewiring of sympathetic circuitry and immune suppression after spinal cord injury

Authors: ***M. UENO**[1,2], Y. UENO-NAKAMURA[1], J. NIEHAUS[1,2], P. G. POPOVICH[3], Y. YOSHIDA[1];
[1]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Japan Sci. and Technol. Agency (JST), Kawaguchi, Japan; [3]The Ohio State Univ. Med. Ctr., Columbus, OH

Abstract: Spinal cord injury (SCI) at high spinal levels often causes systemic immune suppression, which renders patients more susceptible to infection. However, the underlying mechanism remains unknown. Previous study showed that exaggerated activation of autonomic reflexes suppresses immune function, which concomitantly occurs with autonomic dysreflexia. Here we investigate the neuronal substrates responsible for immune suppression. We first examine the autonomic circuits connecting to the spleen, a major immune organ, using a trans-synaptic retrograde tracer. We find that abnormal sympathetic circuits develop below the lesion, which increase excitatory spinal interneurons involved in the circuit. Chemogenetic silencing of these spinal interneurons blocks immune suppression. These data highlight a novel neurogenic mechanism of immune suppression, and provide potential therapeutic methods to treat immune deficiency frequently seen in SCI patients.

Disclosures: M. Ueno: None. Y. Ueno-Nakamura: None. J. Niehaus: None. P.G. Popovich: None. Y. Yoshida: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.12/C21

Topic: A.04. Transplantation and Regeneration

Support: National Science Foundation Graduate Research Fellowship

Rackham Merit Fellowship

Rackham Graduate Student Research Grant

Title: Regeneration after zebrafish traumatic brain injury is dependent upon microglia

Authors: *J. Y. CHEN[1,2], K. SKAGGS[4], Y. QADEER[1], D. GOLDMAN[3], J. M. PARENT[1,2]; [1]Neurol., [2]Neurosci. Grad. Program, [3]Biol. Chem., Univ. of Michigan, Ann Arbor, MI; [4]Biol., Univ. of Findlay, Findlay, OH

Abstract: Unlike mammals, adult zebrafish are capable of virtually full restoration of damaged brain tissues after injury. This repair is characterized by the lack of glial scar formation and the ability of adult-born neurons to establish long-distance projections to the contralateral hemisphere. The remarkable regenerative capacity of zebrafish allows for exploration of the cell types and molecular pathways necessary for effective regeneration. Brain injury induces a widespread neuroinflammatory response that recruits activated microglia to damaged regions. This is followed by proliferation of telencephalic ventricular zone (VZ) radial glia, whose neural progenitor cell progeny migrate to areas of injury and give rise to new neurons - a process that is complete in roughly 21 days post lesion (dpl). We generated a right telencephalic stab lesion in adult zebrafish using a 30-gauge Hamilton syringe. To determine the role of microglia in the injury response, we ablated these cells by injecting liposomal clodronate at the time of stab lesioning. EdU pulse labeling and immunohistochemistry staining showed that clodronate significantly decreases post-injury microglia and cellular proliferation in the parenchyma, but has no effect on proliferation in the VZ where glutamine synthetase positive radial glia reside. Remarkably, we found that the loss of microglia after injury was associated with a significant decrease in regenerative potential; clodronate-treated animals displayed persistent tissue damage even at 90 dpl when compared to controls, suggesting that microglial signaling plays a key role in the regenerative process. In separate experiments, we performed RNAseq to examine a brain injury model, quinolinic acid (QA)-induced excitotoxic lesioning, with even more robust regeneration than stab wound alone. Comparing injured, with and without QA, and uninjured brains allowed us to identify stab lesion-induced altered expression of many genes, a subset of which were further upregulated in response to QA injury. We have chosen candidates known to be secreted by microglia for further analysis. These findings highlight a microglial-dependent repair mechanism in the zebrafish brain that may provide strategies for promoting mammalian brain repair.

Disclosures: J.Y. Chen: None. K. Skaggs: None. Y. Qadeer: None. D. Goldman: None. J.M. Parent: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.13/C22

Topic: A.04. Transplantation and Regeneration

Support: Hertie Foundation for financial support

Wings for Life

DFG

Title: Metabolic regenerative inhibitory signaling after nerve and spinal injury

Authors: *G. KONG[1], L. ZHOU[1], I. PALMISANO[2], E. MCLACHLAN[2], R. PUTTAGUNTA[1], S. DI GIOVANNI[2];
[1]Neuroregeneration and Repair, Hertie Inst. For Clin. Brain Res., Tuebingen, Germany; [2]Med., Imperial Col. London, London, United Kingdom

Abstract: While axonal regeneration and partial functional recovery in the injured peripheral nervous system (PNS) occur, axonal regeneration fails in the central nervous system (CNS) such as after a spinal cord injury (SCI), strongly contributing to unsuccessful functional recovery. Lack of regeneration in the spinal cord can be partially enhanced by an injury to the peripheral branch (conditioning lesion) or by overexpression in DRG neurons of selected regeneration-associated genes. We hypothesize that key retrograde signaling following peripheral but not central axonal injury regulates pathways that control the regenerative phenotype. Therefore, we believe that the combined investigation of protein as well as gene expression changes in the "DRG-axonal signaling unit" after central versus peripheral nerve injury is critical to identify crucial regenerative pathways. We performed combined RNAseq from DRG and proteomics from sciatic axoplasm in mice following an equidistant sciatic or spinal cord axotomy to investigate differential molecular responses in the "DRG-axonal signaling unit". Integrated bioinformatics analysis of the RNAseq and proteomics data followed by axonal injury experimental approaches identified key regulatory metabolic mechanisms that are currently investigated to enhance axonal regeneration and recovery after spinal injury.

Disclosures: G. Kong: None. L. Zhou: None. I. Palmisano: None. E. Mclachlan: None. R. Puttagunta: None. S. Di Giovanni: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.14/C23

Topic: A.04. Transplantation and Regeneration

Support: CONACYT 160614

CONACYT 299847

Title: Epigenetic and miRNA-mediated silencing of Oct4 prevents Müller glia injury-induced dedifferentiation in mammals.

Authors: *B. ESTRADA LEYVA, L. REYES-AGUIRRE, M. LAMAS;
Farmacobiología, Ctr. De Investigación Y De Estudios Avanzados De, Ciudad DE Mexico, Mexico

Abstract: Müller glia (MG), a unique glial type located in the vertebrate retina, responds to damage by becoming reactive and starting a gliosis process, or by dedifferentiating and replacing dead neurons. This regenerative capability is restricted to teleost fish and chick embryo but is notoriously absent in mammals. We have previously reported that MG *in vitro* exhibits a partial dedifferentiation after treatments with subtoxic concentrations of glutamate, but ultimately fails to fully acquire a progenitor phenotype. We have also shown that excytotoxic injury induces a transient expression of Oct4, usually found in pluripotent cells. The rapid silencing of this gene, which becomes undetectable at 24 hours post injury (hpi) suggest the presence of kinetic barriers restricting MG to its glial phenotype. These barriers are still poorly understood, but might be comprised of epigenetic mechanisms, like DNA methylation, or miRNAs-mediated translational repression. In this study, we evaluated miRNA levels in both intact and injured mice retina, and identified a significant increase in miR-145 at 24 hpi localised in MG after magnetic associated cell sorting (MACS). We also characterised the methylation profile of Oct4 and the expression levels of DNA methyltransferases (DNMTs), finding a significant increase of Dnmt3b at 24 hpi. Oct4 silencing was reverted by the injection of the DNMT-inhibitor SGI-1027, as well as by transfection of a miR-145 antagomir. These findings prove that Oct4 is silenced by a recurrent mechanism, which locks MG into its glial phenotype, preventing a successful regeneration.

Disclosures: B. Estrada Leyva: None. L. Reyes-Aguirre: None. M. Lamas: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.15/C24

Topic: A.04. Transplantation and Regeneration

Support: NIH EY024481

NIH NS066019

NIH MH104318

NIH P50 HD018655

DoD CDMRP DM102446

Adelson Medical Research Foundation

Baby Alex Foundation

Title: Retinal Zn^{2+} elevation after optic nerve injury suppresses retinal ganglion cell survival and axon regeneration: upstream mechanisms involve GLT-1 mediated glutamate efflux, NMDA receptor activation, and NO generation

Authors: *Y. LI[1,2], L. ANDEREGGEN[1], K. YUKI[1], P. A. ROSENBERG[1], L. I. BENOWITZ[1]; [1]Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [2]State Key Lab. of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen Univ., Guangzhou, China

Abstract: Zinc is essential for many cellular functions but also contributes to neuronal death in hypoxic-ischemic injury and other disorders. Shortly after injury to the optic nerve (nerve crush, NC), free zinc (Zn^{2+}) increases rapidly in amacrine cell terminals within the inner plexiform layer (IPL) of the retina, whereas levels within RGCs increase more slowly. Chelating Zn^{2+} leads to enduring survival of many RGCs and extensive axon regeneration [Li et al., *SfN Abstracts* 399.19 (2014); 568.02 (2015)]. Here we explored the mechanisms underlying presynaptic Zn^{2+} accumulation. Zn^{2+} can be liberated from metallothioneins (MTs) by nitric oxide (NO), and deletion of *nos1* (which encodes NO synthase-1, NOS1) prevented both the early Zn^{2+} signal in the IPL and the delayed signal in RGCs post-NC. Zn^{2+} accumulation was likewise suppressed by intraocular injection of the NO scavenger PTIO or the NOS1 inhibitor L-NPA. NOS1 is a Ca^{2+}/calmodulin dependent enzyme, and one route through which Ca^{2+} can enter cells to activate NOS1 is via NMDA receptors. The NMDA receptor antagonist MK801 fully prevented Zn^{2+} elevation in the IPL. In the spinal cord, nerve injury causes axon depolarization and reversal of glutamate transport (Li, Stys, *Neuroscience* 107: 675-83, 2001), resulting in non-vesicular

glutamate efflux. Supporting a role for glutamate transporter-mediated extracellular glutamate accumulation, both TBOA (1 mM), a general inhibitor of glutamate transporters, and dihydrokainate (DHK, 1 mM), a selective inhibitor of GLT-1, suppressed Zn^{2+} elevation in the IPL. The NO donor DETA-NONOate restored Zn^{2+} elevation in the presence of either MK801 or TBOA, confirming that glutamate transporter reversal and NMDA receptor activation are upstream of NO-induced Zn^{2+} accumulation. Together with our previous findings, these results support a model in which optic nerve injury leads to glutamate efflux by reversal of GLT-1 mediated transport, NMDA receptor activation, NO generation via NOS1, Zn^{2+} liberation from MTs, ZnT-3-dependent loading of Zn^{2+} into synaptic vesicles of amacrine cells, and delayed accumulation of Zn^{2+} in RGCs, where it critically suppresses RGC survival and optic nerve regeneration.

Disclosures: Y. Li: None. L. Andereggen: None. K. Yuki: None. P.A. Rosenberg: None. L.I. Benowitz: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.16/C25

Topic: A.04. Transplantation and Regeneration

Support: National Institute for Health Research (NIHR) Imperial Biomedical Research Centre

Hertie Foundation

Wings for Life

DFG

Start up funds-Division of Brain Sciences, Imperial College London

Title: HDAC3 signalling controls nerve regeneration

Authors: *L. ZHOU[1], A. HERVERA[2], I. PALMISANO[2], E. MCLACHLAN[2], G. KONG[1], T. HUTSON[2], M. DANZI[3], F. DE VIRGILIS[2], M. WOOD[4], R. PUTTAGUNTA[1], S. DI GIOVANNI[2];
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Abstract: Clarification of the gene regulatory mechanisms that contribute to regenerative failure after nerve injury remains critical for the design of molecules that promote regeneration and recovery after nerve and spinal damage. While we are beginning to learn how epigenetic mechanisms control the regenerative phenotype, here, after a pharmacological screening, we identified HDAC3 as a novel central epigenetic brake to axonal regeneration. HDAC3 activity is elevated when axonal regeneration is restricted but it is reduced when regeneration occurs. Accordingly, pharmacological and genetic HDAC3 inhibition promotes axonal regeneration both in the peripheral and central nervous system in the spinal cord. Mechanistically, only regenerative peripheral injury and calcium-dependent activation of PP4 dephosphorylate HDAC3 impairing its activity, acetylating histones and the regenerative gene expression programme, as shown by ChIP and RNA-seq. Together, we propose HDAC3-dependent signalling as a brake to axonal regeneration, whose inhibition represents a novel option to foster nerve regeneration and neurological recovery.

Disclosures: L. Zhou: None. A. Hervera: None. I. Palmisano: None. E. Mclachlan: None. G. Kong: None. T. Hutson: None. M. Danzi: None. F. De Virgilis: None. M. Wood: None. R. Puttagunta: None. S. Di Giovanni: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.17/C26

Topic: A.04. Transplantation and Regeneration

Title: *In vivo* study of the dopaminergic neuron regeneration in amphibian model of Parkinson's disease

Authors: *J. ZAREBA-PASLAWSKA, A. SIMON, M. KIRKHAM;
Dept. of Cell and Mol. Biol., Karolinska Inst., Stockholm, Sweden

Abstract: The tremendous development in human health care and increase in quality of life has almost duplicated the human life expectancy, compare to the turn of 19th and 20th century. It has a great influence on the increased number of people suffering from age related diseases, where the most common motor neurodegenerative disease is Parkinson’s disease (PD). The preferential loss of dopaminergic (DAergic) neurons in *substantia nigra* is a key hallmark of PD. Unfortunately, despite extensive studies, PD remains an incurable disorder with unknown origin. Both, motor and non–motor symptoms are debilitating for PD patients and contribute to the decrease of their life quality and expectancy.
The successful clinical experiments using human fetal tissue grafting for PD patients has proven

the concept of applying the cell replacement therapy in PD. However, mainly ethical and practical considerations hampered usage and development of this strategy. Nevertheless, the number of studies demonstrated that the cell replacement therapy based on stem cells provides the most promising results to obtain a fully functional repair of nigrostriatal DAergic neurons. By taking step forward and recruiting brain endogenous (resident) neural stem and progenitor cells (NSPCs) into regeneration process we might significantly improve, or to some extend even replace, current stem cells therapies. The key translational gap for endogenous NSPCs strategies is our scarce knowledge about naturally occurring process of DAergic neurons regeneration.
In our studies we aim to identify molecular and/or chemical markers characteristic for DAergic neurons regeneration *in vivo*. We take the advantages of the regeneration features of anuran amphibians (frogs), which possesses remarkable ability to regenerate neuronal structures as tadpoles (larval stage), which diminishes after metamorphosis in adulthood. This phenomenon is giving us the opportunity to compare regenerative and non-regenerative phase in one organism what greatly facilitate entire analysis process.
Our validation plan includes studying process of DAergic neurons regeneration in anuran amphibians (frogs) after DAergic neurons ablation. The experiment includes monitoring of proliferation response through BrdU incorporation and immuno-labeling for the cell cycle markers. Furthermore, glial cells support requirement, in principle microglia are also analyzed. We believe that by studying naturally occurring DAergic neurons regeneration we will identify key factors involved in driving and/or inhibiting this process which can be further translate to mammalians model of PD and ultimately to patients as a treatment strategy.

Disclosures: J. Zareba-Paslawska: None. A. Simon: None. M. Kirkham: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.18/C27

Topic: A.04. Transplantation and Regeneration

Support: NMINBRE Pilot award 8P20GM103451-12

NSF grant 1353123

Title: Identification of regenerative processes in spinal cord injury using transcriptomic data in a developmental study

Authors: *B. J. WHEATON[1], P. UMALE[2], J. SENA[2], A. SUNDARARAJAN[2], F. SCHILKEY[2], R. D. MILLER[1];
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Abstract: Spinal cord injury (SCI) typically results in little axon regrowth and permanent loss of function after injury in adult mammals. However, spontaneous axon regeneration is possible in some species (such as the lamprey) and at certain stages of development in mammals. The biology underlying such responses is poorly understood but, given the lack of success attained by single factor treatments to date, it is likely that it involves complex interactions of genes and cells-types.

To study this phenomenon, we applied an RNA-sequencing approach to identify the gene expression profile of regenerating and non-regenerating mammalian spinal cords using the marsupial Grey short-tailed opossum (*Monodelphis domestica*). In this model predictable axon regeneration and functional recovery follows injuries made very early in development, but not following injuries made later.

Spinal cords of regenerating and non-regenerating opossums were severed, allowed to recover for 1–7 days and collected for RNA studies or histology. RNA-seq (Illumina Hi-Seq) reads were mapped to the opossum genome (MonDom5), quantified before differential expression (DE) analysis was performed. RNA sequencing allows for identification of multiple factors involved in the response to injury. However, in whole cord homogenates such as this, determining patterns in the identified genes can be challenging. Here we present our analyses using gene ontology (GO) analysis, association of DE genes with single cell-type transcriptomic datasets, and histology.

GO analysis revealed over-expression of genes involved with immune function following injuries at both ages. This involved enrichment of macrophage activation and blood coagulation genes in regenerating cords, but a much wider variety of gene classes in non-regenerating cords, including complement activation, cell adhesion and cell communication.

When the DE genes were associated with cell type data (from published single cell datasets) SCI transcriptomes suggest a complex cellular response. Regenerating animals demonstrate a rapid microglial response (eg. *C1qA*, *IL1b*, *CSF1*) accompanied by a quickly resolving astrocytic response (*Herc6*, *Tagln*, *Aqp1*) and a delayed oligodendrocyte response (*Ermn*, *Mag*, *Mog*). This is in contrast with non-regenerating animals where a rapid oligodendrocyte response and a sustained astroglial response predominate, suggesting that temporal differences in injury progression exist.

The opossum represents a unique mammalian model for the study of axon regeneration in the absence of any external manipulations and could therefore lead to the identification of intrinsic factors underlying regeneration.

Disclosures: B.J. Wheaton: None. P. Umale: None. J. Sena: None. A. Sundararajan: None. F. Schilkey: None. R.D. Miller: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.19/C28

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01NS085176

NIH Grant R01GM111514

Craig H. Neilsen Foundation

Johns Hopkins Catalyst Award

Title: Regulation of mammalian axon regeneration by Lin28/let-7 pathway

Authors: *X. WANG[1,2], P. A. HALL[1,2], C. D. KATCHIS[1,2], C. LIU[3], F. ZHOU[1,2];
[1]Neurosci., [2]Orthopedic Surgery, Johns Hopkins Univ., Baltimore, MD; [3]Inst. of Zoology, Chinese Acad. of Sci., Beijing, China

Abstract: Recent studies have revealed that the diminished intrinsic axon growth ability of neurons in the mature mammalian CNS, controlled by coordinated regulation of gene expression, is the major reason that they do not regenerate their axons after the injury. Epigenetic regulation is emerging to be a key cellular mechanism to control coordinated expression of gene networks, making it a potential mechanism for controlling the intrinsic axon growth ability of mature mammalian neurons. The highly conserved RNA-binding proteins Lin28a/b have been shown to be involved in variant biological processes including development, reprogramming, carcinogenesis, metabolism and tissue repair. Its role in axon regeneration, however, has never been studied. Here we report that the Lin28/let-7 axis plays an important role in regulation of axon regeneration. Specifically, we observed significant changes in expression levels of Lin28a/b and let-7a/b in mouse DRGs following sciatic nerve injury. Functionally, we found that manipulation of Lin28a/b or let-7s in DRG neurons significantly affected sensory axon regeneration both *in vitro* and *in vivo*. Our results demonstrated for the first time that Lin28 and let-7 pathways are essential for mammalian axon regeneration. We are currently investigating if manipulation of Lin28a/b and/or let-7s can promote axon regeneration in the central nervous system, and the molecular mechanisms by which these molecules regulate axon regeneration.

Disclosures: X. Wang: None. P.A. Hall: None. C.D. Katchis: None. C. Liu: None. F. Zhou: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.20/C29

Topic: A.04. Transplantation and Regeneration

Support: NIH/NEI, Glaucoma Research Foundation, Pew Biomedical Program, Ziegler Foundation

Title: Examining astrocytes' roles and mechanisms in regulating retinal axon regeneration and pathfinding

Authors: *M. RIBEIRO, B. J. YUNGHER, E. R. BRAY, K. K. PARK;
Dept. of Neurolog. Surgery, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Optic nerve injury is a hallmark feature of optic neuropathies including glaucoma, and is characterized by axon degeneration and retinal ganglion cell (RGC) death, resulting in visual impairment. Although no effective treatments are available, experimental manipulations of cellular signaling pathways by intravitreal delivery of trophic factors, such as brain-derived neurotrophic factor or ciliary neurotrophic factor and/or by targeted-deletion of certain genes, such as phosphatase and tensin homolog (*Pten*), have been shown to protect RGCs and promote axon regeneration in animal models subjected to acute axonal trauma through procedures such as optic nerve crush. Glial cells including astrocytes have biological properties that can influence neural connectivity, both structurally and functionally. Although the exact functions of astrocytes in the CNS have been controversial, some of the beneficial roles fulfilled by astrocytes include providing neurotrophic and mechanical support for the injured (and regenerating) axons. Using optic nerve crush injury, we characterize astrocytes' dual roles in i) releasing trophic factors, leading to activation of distinct signaling pathways, promoting neuronal survival and axon elongation, and ii) expressing adhesion proteins that provide physical support for the axons to extend from the optic nerve to the brain. Experimentally, we investigate the cellular sources of trophic factors in the injured optic nerve using fluorescence in situ hybridization and assess RGC axon regeneration in conditional knockout mice that delete *Stat3* or *N-cadherin* specifically in astrocytes. We report and discuss findings from these studies that are aimed at characterizing the roles of astrocytes and distinct genes in affecting RGC axon regeneration and navigation.

Disclosures: M. Ribeiro: None. B.J. Yungher: None. E.R. Bray: None. K.K. Park: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.21/C30

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant F30EY025527

Title: Transcriptional profiling to discern differential retinal axon growth capacity

Authors: *E. BRAY, B. YUNGHER, K. THAKOR, M. RANDOLPH, M. DANZI, J. BIXBY, V. LEMMON, K. PARK;
Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Optic neuropathies damage retinal ganglion cell (RGC) axons of the optic nerve, resulting in permanent visual deficits. Substantial progress has been made in our ability to promote axon regeneration following axonal injury, but regeneration remains limited to a small number of RGCs. RGCs are a heterogeneous population, with more than 30 described subtypes. Through the use of transgenic mouse lines it has been determined that an RGC's subtype may predict its regenerative capacity. The molecular mechanisms that regulate subtype dependent differences in regenerative capacity have just started to be identified. In this study we report RGCs with distinct subtype specific growth capacity. Using RNAseq we identified the transcriptional networks of regeneration competent and incompetent RGCs. These genes will serve as novel therapeutic targets to enhance the regeneration of all RGCs, a critical step towards restoring lost vision.

Disclosures: E. Bray: None. B. Yungher: None. K. Thakor: None. M. Randolph: None. M. Danzi: None. J. Bixby: None. V. Lemmon: None. K. Park: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.22/C31

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01MH091676

NIH Grant R21MH099799

NIH Grant R01 MH067880

Glaucoma Research Foundation

Title: Novel strategies for detection and identification of the axonal "transportome" and pre-synaptome" in the rodent visual system.

Authors: *L. M. SCHIAPPARELLI[1], S. SHAH[3], D. MCCLATCHY[2], H. HE[1], J. LI[1], Y. MA[2], S. SATURDAY[1], J. GOLDBERG[4], J. YATES, III[2], H. CLINE[1];
[1]Dept. of Mol. and Cell. Neurosci., [2]Dept. of Chem. Physiol., The Scripps Res. Inst., La Jolla, CA; [3]Neurosci. Grad. Program, UCSD, La Jolla, CA; [4]Dept. of Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: The brain processes information, makes decisions, and mediates cognitive and motor outputs through the function of different types of connected neurons organized in complex arrangements. In addition, neurons in brain circuits are often intermingled with non-neuronal cells that provide physiological support. Dissecting the biochemical processes taking place in different individual cellular components of neuronal circuits in the intact animals is crucial for the understanding the biology the brain cells. Given the complex structure of most neuronal circuits, identification and characterization of cellular processes and molecular components of each cell type is extremely difficult with current methodologies. Our interests focus on identification and characterization of translation and transport of protein subpopulations in specific neuronal components from brain circuits. To reliably identify and analyze protein subpopulations according to their source (for instance, brain region, cell type and/or target area), and to obtain meaningful information about cellular processes in different components of neuronal circuits, we have developed and combined new strategies for *in-vivo* labeling, circuit tracing, and ultrastructural visualization of biotin-labeled proteins with a novel methodology for accurate identification of biotin-labeled proteins by mass spectrometry called DiDBiT (Schiapparelli et al 2014). Using these combined strategies, we were able to identify ~1000 proteins that were newly synthesized over a period of 24 hours in healthy rat retinas. In addition, we identified what we call the retina “Transportome”, specifically proteins from retinal ganglion cells (RGCs) that are transported to their major targets in brain visual areas. In detail, we detected more 500 axonally transported proteins in the optic nerve (ON) and ~250 proteins that reached either the lateral geniculate nucleus (LGN) or the superior colliculus (SC) in adult rats. The protein subpopulations detected in either LGN or SC represent in part the “Pre-synaptome” of this specific long range projection. These populations of proteins can also be visualized in axons and presynaptic terminals in ON, LGN and SC by light and electron microscopy. These experiments will allow us to compare populations of proteins transported from the retina to presynaptic terminals in different visual targets, for example the LGN and SC, and to characterize molecular components present in these different types of neuronal projections with a specificity never reported before.

Disclosures: L.M. Schiapparelli: None. S. Shah: None. D. McClatchy: None. H. He: None. J. Li: None. Y. Ma: None. S. Saturday: None. J. Goldberg: None. J. Yates: None. H. Cline: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.23/C32

Topic: A.04. Transplantation and Regeneration

Support: NSF GRFP 1122374

NIH R01GM080639

NIH T32GM007287

HHMI Investigator Award to PWR

Title: Neoblast specialization during regeneration of the planarian S. mediterranea nervous system.

Authors: *K. KRAVARIK, P. REDDIEN;
Biol. Department, HHMI, Whitehead Inst. for Biomed. Research, MIT, Cambridge, MA

Abstract: Planarians are well known for their ability to regenerate entire animals from small tissue fragments. Planarian regeneration requires a population of dividing cells called neoblasts that are distributed throughout the body. Historically, neoblasts have largely been considered a homogeneous population of stem cells capable of differentiating into all cell types. Most studies, however, involved analysis of neoblasts at the population rather than the single cell level, making it difficult to determine how heterogeneous this population is. Here, we use the planarian nervous system, comprised of hundreds of different cell types, to study how neoblasts specialize into specific cell fates. We recently showed that 41 transcription factors expressed in distinct regions of the nervous system are also enriched in subsets of neoblasts during regeneration, marking putative progenitor cell types. Together with previously known transcription factors required for additional neuronal cell types, we infer that these genes specify a diversity of neuronal lineages within neoblasts, and we hypothesize that there may be one or more multipotent neural progenitor populations within neobasts, from which known neural sub-types are specified.
To test this hypothesis, we isolated single cells in the G2/M phase from intact head-pieces and sequenced the mRNA of 188 individual cells. Analysis of these data indicate that there are several unexplored populations of neoblasts that express transcription factors and other genes associated with neural development, and mark distinct populations of differentiated neurons in

uninjured animals. We are currently exploring the requirements of these transcription factors during regeneration.

Disclosures: K. Kravarik: None. P. Reddien: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.24/C33

Topic: A.04. Transplantation and Regeneration

Support: NIH NS 079631

Title: Activating neuron-intrinsic growth pathways to promote dorsal root regeneration

Authors: ***M. MANIRE**[1,2], H. KIM[1,2], J. ZHAI[1,2], G. M. SMITH[1,2], J. ZHONG[3,4], Y.-J. SON[1,2]; [1]Shriners Hosp. Pediatric Res. Ctr., [2]Anat. and Cell Biol., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA; [3]Burke Med. Res. Inst., Weill Cornell Med. Col. of Cornell Univ., White Plains, NY; [4]Brain and Mind Res. Inst., Weill Cornell Med. Col. of Cornell Univ., New York, NY

Abstract: Primary sensory axons injured by dorsal root (DR) injuries fail to regenerate into the spinal cord, leading to permanent sensory loss. Re-entry is prevented at the dorsal root entry zone (DREZ), the CNS-PNS interface. There is no curative therapy for patients, and present approaches for overcoming this regeneration failure have had only limited success. We have previously shown that induced expression of constitutively active B-RAF (kaBRAF, V600E) enhanced the regeneration competence of injured DRG neurons of adult mice (O'Donovan et al., 2014). In the present study, we tested whether robust regeneration of DR axons across the DREZ can be achieved by activating B-RAF alone or in combination with deletion of myelin-associated inhibitors or neuron-intrinsic growth suppressors (PTEN or SOCS3). To this end, we used *LSL-kaBRAF: brn3a-CreER^{T2}* mice in which kaBRAF can be induced selectively in sensory neurons. We have also bred *LSL-kaBRAF: brn3a-CreER^{T2}* mice with tKO mice lacking Nogo, Mag and OMgp or mouse lines carrying floxed alleles of *PTEN* or *SOCS3*. Single, double, and triple conditional mice were subjected to either cervical (C5-T1) or lumbar (L4-L5) DR crush, and AAV2-GFP vectors were used to selectively label regenerating axons of large-diameter neurons. We compared the extent of regeneration at 3 weeks or 2 months after DR injury using conventional anatomical, functional and behavioral analyses. We found that although induction of kaBRAF alone enabled many axons to penetrate the DREZ, these axons did not continue to regenerate deep into the spinal cord. Supplementary deletion of myelin-inhibitors only modestly

increased kaBRAF-induced regeneration. Deletion of PTEN or SOCS3 alone or together did not promote DR regeneration. In marked contrast, simultaneous deletion of PTEN, but not of SOCS3, dramatically enhanced kaBRAF-induced regeneration enabling many more axons to penetrate the DREZ and to grow more deeply into the spinal cord. Currently, we are testing whether such synergistic activity of BRAF activation and PTEN inactivation prolongs intraspinal regeneration, resulting in robust and long-range functional regeneration after DR injury. Our study has the potential to provide novel insights into how to promote far more robust DR regeneration than has been obtained before and to determine whether such vigorous regeneration will lead to functional recovery of sensory function after spinal root injury.

Disclosures: M. Manire: None. H. Kim: None. J. Zhai: None. G.M. Smith: None. J. Zhong: None. Y. Son: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.25/C34

Topic: A.04. Transplantation and Regeneration

Support: UCLA Stein Eye Institute EyeSTAR Program

Title: Activity-dependent molecular programs for optic nerve regeneration

Authors: *Q. WANG[1,2], E. NIE[1], S. T. CARMICHAEL[1];
[1]Neurol., [2]Ophthalmology, UCLA, Los Angeles, CA

Abstract: In the mammalian visual system, retinal ganglion cells (RGCs) are unable to regenerate their axonal projections to targets in the brain following traumatic or ischemic damage or in degenerative diseases such as glaucoma. Cumulative work by many in the field have shown that these RGC axons can be coaxed into regrowing following optic nerve axotomy by manipulating both extrinsic (e.g. oncomodulin) and intrinsic molecular pathways (e.g. PTEN, SOCS3); however, despite these efforts, RGC axons are still inefficient at growing past the optic chiasm and innervating their visual targets. The optic nerve retinal ganglion system provides an excellent model for the study of candidate molecular systems in axonal sprouting.
Neuronal activity and activity-dependent cues play a critical role in driving correct circuit formation during development. In other CNS injury models, such as stroke, increasing neuronal activity through forced limb overuse has been shown to promote the formation of new circuits associated with improved motor recovery. Whether activity-dependent mechanisms can similarly promote reformation of retinofugal pathways following injury remains unclear. Our lab has

previously generated an RNA-Seq dataset of genes differentially regulated in novel neuronal connections formed in the limb overuse stroke model (Nie E., SfN 2015, Poster 307.22/F4). Using an AAV-mediated approach for *in vivo* overexpression and knockout (via CRISPR/Cas9) of candidate genes in RGCs, we are determining whether manipulation of these genes can also promote retinal axon regeneration in a unilateral optic nerve crush model and whether these effects are comparable to or can be further enhanced by visual stimulation of regenerating RGCs. Analysis of this data is ongoing.

Disclosures: Q. Wang: None. E. Nie: None. S.T. Carmichael: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.26/D1

Topic: A.04. Transplantation and Regeneration

Support: NINDS Grant 5K99NS088211-02

NIH Grant 2R37NS040929

HHMI

Title: The mechanosensitive ion channel piezo regulates axon regeneration

Authors: *Y. SONG[1,2], L. Y. JAN[2], Y. JAN[2];
[1]Dept. of Pathology and Lab. Med., Univ. of Pennsylvania and CHOP, Philadelphia, PA;
[2]Departments of Physiol. and Biochem., Univ. of California San Francisco, San Francisco, CA

Abstract: Differentiated neurons exhibit a limited ability of repair. Given the demonstrated effect of mechanical forces on neuronal outgrowth *in vitro*, it is important to identify mechanosensitive (MS) ion channels that regulate axon regeneration. Here we show that DmPiezo, a Ca^{2+}-permeable non-selective cation channel, functions as an intrinsic inhibitor to hamper axon regeneration in *Drosophila*. Axonal injury activates the Ca^{2+}-permeable DmPiezo channels, leading to activation of nitric oxide synthase and the downstream cGMP kinase Foraging/PKG to restrict axon regrowth. Loss of DmPiezo function enhances axon regeneration of class III dendritic arborization (da) sensory neurons *in vivo*. This mutant phenotype can be rescued by expression of mammalian Piezo channels, but not by a mutant deficient in channel activity, suggesting that the ability of Piezo to regulate axon regeneration is evolutionarily conserved. These findings implicate Piezo channels as an integrator in neuronal maintenance and a potential therapeutic target for treating nervous system trauma.

Disclosures: Y. Song: None. L.Y. Jan: None. Y. Jan: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.27/D2

Topic: A.04. Transplantation and Regeneration

Support: NSF-DBI-1126118

Thomas F. and Kate Miller Jeffress Memorial Trust J-1026

Title: The role of myelin and other factors involved in optic nerve regeneration in xenopus laevis

Authors: *D. M. ROSENTHAL, M. BRYSON, M. GERBO, B. MISHAGHI, A. WATSON, F. L. WATSON;
Washington and Lee Univ., Lexington, VA

Abstract: The South African-Clawed frog, *Xenopus laevis* retains the ability to regenerate its injured optic nerve into adulthood, whereas mammals lose this ability prior to birth. Here we explore whether the difference in the low number of myelinated retinal ganglion cell (RGC) axons in *Xenopus* (6-10%) compared to that in mammals (100%) contributes towards the regenerative capacity of amphibian optic nerves. We crush the optic nerves of post-metamorphic *Xenopus* using forceps and harvest their optic nerves at various time points post-surgery (up to 210 days). To measure axonal regrowth, we use frogs expressing GFP under regulatory control of an RGC-specific promoter as a marker to visualize RGCs as they extend into the optic tectum. The time course of fluorescence recovery shows complete loss of fluorescence by post-injury day 7, partial recovery can be seen by day 35 but full recovery does not occur until day 210. To determine whether the presence of myelin debris poses a barrier to regeneration, we immunostain longitudinal nerve sections with myelin basic protein, a marker for live, intact myelin. Preliminary results show differences in the rate of myelin removal between nerve segments distal and proximal to the crush site. Specifically, myelin expression is absent in distal longitudinal optic nerve sections by post-injury day 7 but not absent in the proximal nerve segment until post-injury day 11. No myelin is detectable in the optic nerve by post-injury day 210 indicating that regrowth of RGC axons occurs in unmyelinated axons and that subsequent remyelination occurs at a rate that extends past post-injury day 210. In addition, we assess the expression patterns of γ-synuclein, a protein preferentially expressed in large caliber RGCs and previously implicated in neurodegenerative diseases. Results from our RGC-specific RNASeq injury model screen and subsequent immunostaining of retinal sections shows γ-synuclein decreases unilaterally in the

crushed eye during the period of recovery from injury. Interestingly, analysis of longitudinal optic nerve sections shows protein expression of γ-synuclein decreases in RGC axons in both the operated nerve and the unoperated contralateral nerve, a staining pattern that suggests a more global response to injury and local axonal regulation.

Disclosures: D.M. Rosenthal: None. M. Bryson: None. M. Gerbo: None. B. Mishaghi: None. A. Watson: None. F.L. Watson: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.28/D3

Topic: A.04. Transplantation and Regeneration

Support: NIH T32 NS07641

NIH T32 EY13934

NIH P30 EY07003

NIH RO1 EY07060

Title: The matrix metalloproteinase (Mmp9) functions in Muller glia stem cells and photoreceptor progenitors during injury-induced photoreceptor regeneration in the zebrafish.

Authors: *N. J. SILVA[1], J. LI[3], D. HYDE[3], P. HITCHCOCK[2];
[1]Neurosci., [2]Ophthalmology and Visual Sci., Univ. of Michigan, Ann Arbor, MI; [3]Biol. Sci., Univ. of Notre Dame, Notre Dame, IN

Abstract: Injury and disease in the central nervous system (CNS) activates a multifaceted network of signaling molecules from dying neurons, surviving neurons, and non-neuronal cells. One such molecule belongs to the family of zinc-dependent endopeptidases known as matrix metalloproteinase 9 (Mmp9). Mmp9 is an inflammatory proteinase, which is modulated by various cytokines, such as Tnf-α, Il-1, and Il-8. Previous studies by Nelson et al., (2013) demonstrate that Tnf-α is produced by dying photoreceptors and required for Müller glia proliferation. We show that selective injury and death of photoreceptors induces the expression of genes encoding the inflammatory molecules, *tnf-α, tnf-β, nfκβ1, nfκβ2, mmp9*, and *il-8.* The expression of *mmp9* is predominately in Müller glia and neuronal progenitors. Importantly, Mmp9 is catalytically active during the proliferation and migration of the photoreceptor progenitors. Intravitreal injections of the pro-inflammatory cytokine, TNF-α is sufficient to

stimulate the expression of *mmp9* in uninjured retinas. Interestingly, anti-inflammatory treatment by dexamethasone decreases the expression of *tnf-α, tnf-β,* and *mmp9* and suppresses cell proliferation and regeneration of rod photoreceptors. Our results demonstrate a critical role for neuroinflammation and places *mmp9* with Tnf-α signaling during photoreceptor regeneration in the adult zebrafish retina.

Disclosures: N.J. Silva: None. J. Li: None. D. Hyde: None. P. Hitchcock: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.29/D4

Topic: A.04. Transplantation and Regeneration

Support: Wellcome Trust RTF

Title: Imaging remyelination in the central nervous system.

Authors: *M. F. E. HILL[1,2], J. R. GUY[5,3], K. M. BRINDLE[4], R. J. M. FRANKLIN[2];
[1]Clin. Neurosci., [2]Wellcome Trust-MRC Stem Cell Inst., [3]Dept. of Biochem., [4]Cancer Res. UK, Univ. of Cambridge, Cambridge, United Kingdom; [5]Translational Neuroradiology Unit, Natl. Institue of Neurologic Disorders and Stroke, Bethesda, MD

Abstract: Demyelination (the loss of myelin) is seen in many common neurological diseases including multiple sclerosis. Although usually robust, where the regenerative response (remyelination) fails, chronic denudation of the axon leads to axonal loss and neurological impairment. There is therefore a need for therapies that enhance remyelination, and outcome measures to assess their efficacy. Currently there are no non-invasive methods for specifically assessing remyelination.
Here we present evidence of a novel non-invasive technique for imaging remyelination in the central nervous system using T1 weighted Magnetic Resonance Imaging and Magnetic Resonance Microscopy. Using a lentiviral system to infect progenitor cells, we are able to demonstrate T1 weighted contrast enhancement of cells expressing an organic anionic transporter channel (OATP), which takes up gadolinium contrast agent. We are able to control the expression of this transporter under myelination specific promoters, thus making contrast agent uptake specific to myelin synthesis by OPCs which have differentiated into myelinating oligodendrocytes. We demonstrate OATP expression in vitro, in ex vivo slice cultures and in vivo; showing expression in both endogenously infected cells, and in transplanted cells. By applying this technique to models of demyelination we aim to develop a non-invasive method for

imaging remyelination, applicable as an outcome measure, for both the development of transplant, and pharmacological therapies.

Disclosures: M.F.E. Hill: None. J.R. Guy: None. K.M. Brindle: None. R.J.M. Franklin: None.

Poster

210. Injury-Induced Regeneration and Remyelination

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 210.30/D5

Topic: A.04. Transplantation and Regeneration

Title: Adult leech CNS regeneration at the single cell level: effects of electrical stimulation

Authors: *S. COHEN, O. SHEFI;
Bar Ilan Univ., Ramat Gan, Israel

Abstract: Central nervous systems (CNS) of adult mammals fail to regenerate following an injury. Invertebrates' CNS, on the other hand, can undergo substantial regeneration with significant functional recovery. Moreover, many invertebrates provide the opportunity to work with identified neurons based on their size, location and characteristic electrical activity pattern within the tissue. Thus, invertebrates serve as a useful model for exploring cell strategies and mechanisms underlying successful CNS regeneration at the single cell level. Electrical stimulation (ES) has been previously reported as a promoting factor of axonal regeneration in sensory and motor neurons. The effects have been demonstrated mostly in the PNS but there are also experimental evidence for such occurrence in the CNS. For example, low-frequency ES at 20 Hz for a short period was found to be as effective as continuous stimulation over 2 weeks. In this research we aim to study the influence of different ESs on the neuronal growth. We have examined the effects of brief ES (20Hz, 30min) on the regeneration strategy of the leech CNS at the single cell level over a period of 72 hours. We used an ex-vivo model of the leech ganglia chain. We followed the regeneration process of single cells and compared the regeneration strategy with and without the electrical stimulation. Our preliminary results demonstrated that the dynamics of regeneration and growth rate were affected by the ES. Moreover, regenerated neurons under ES tended to change their dendritic tree orientation, as compared to control neurons. The results suggest that ES may (1) contribute for the direction of the spatial orientation of the regenerative axons and (2) influence the normal regeneration time course by interfering in specific time points.

Disclosures: S. Cohen: None. O. Shefi: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.01/D6

Topic: B.12. Glial Mechanisms

Support: RO1MH067121

Title: Ablation of astrocytic ephrin-B1 impairs synaptogenesis, synapse function, and animal behavior

Authors: *A. Q. NGUYEN[1], J. KOEPPEN[1], M. GARCIA[1], S. HANNA[1], A. OBENAUS[2], I. ETHELL[1];
[1]Univ. of California, Riverside, Riverside, CA; [2]Loma Linda Univ., Loma Linda, CA

Abstract: Astrocytes interact with and ensheath neuronal synapses through fine processes which allow for regulation of synaptic function and aiding in the stabilization and maintenance of synapses. Contact of astrocytes to synapses can be facilitated by astrocytic ephrin-B1 to neuronal EphB receptors; ephrin-B1/EphB signaling pathways participate in cell-cell interactions which regulate various developmental processes, such as axon guidance, cell migration and proliferation, synaptogenesis, and dendritic spine formation. In our study, we show that during adulthood, astrocytic ephrin-B1 continues to play a role in synapse formation, function, and maintenance. Using a Cre/LoxP system; inducible loss of astrocytic ephrin-B1 was achieved by crossing homozygous floxed ephrin-B1 female mice with Cre-GFAP-ERT2 male mice and providing experimental mice with tamoxifen to induce ablation of astrocytic ephrin-B1 in adulthood. Morphologically, we found a significant increase in the number of excitatory synapses in the SR region of the CA1 hippocampus of knock-out astrocytic ephrin-B1 mice through immunostaining of presynaptic terminals with vGlut1 and through DiI labeling of dendritic spines. Functionally, however, we see reduced population spike responses as well as attenuation of long-term potentiation in the CA1 hippocampus of knockout animals following stimulation of Schaffer collaterals. Behaviorally, knockout animals displayed impaired social memory in a three-chamber social interactions test but improved contextual fear learning, indicated by increased freezing, in contextual fear conditioning test. Our studies suggest astrocytic ephrin-B1 may play a role in maintenance of synapses; ablation of astrocytic ephrin-B1 triggers synaptogenesis of non-functional synapses, resulting in attenuation of functional responses, but may allow increased contextual learning when provided enough training. Future studies will determine its role during various developmental stages of synapse development. *This work was supported by the NIH grant RO1MH067121.*

Disclosures: A.Q. Nguyen: None. J. Koeppen: None. M. Garcia: None. S. Hanna: None. A. Obenaus: None. I. Ethell: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.02/D7

Topic: B.12. Glial Mechanisms

Support: NIH grant RO1MH067121

Title: Astrocytic ephrin-B1 and its role in injury-induced synapse remodeling in the adult hippocampus

Authors: *J. KOEPPEN[1], A. NGUYEN[1], M. GARCIA[1], S. HANNA[1], A. OBENAUS[2], I. ETHELL[1];
[1]Univ. of California Riverside, Riverside, CA; [2]Loma Linda Univ., Loma Linda, CA

Abstract: Astrocytes can facilitate brain repair after injury by protecting neurons from glutamate excitotoxicity and regulating the blood-brain barrier; however, the role of astrocytes in rewiring neuronal networks is not well understood. Several astrocyte-derived factors that are implicated in synapse formation and maintenance may also regulate synapse remodeling following brain injury. Our studies suggest a new role of astrocytic ephrin-B1 in synapse remodeling following traumatic brain injury (TBI). Trans-synaptic interactions between EphB receptor and its membrane-bound ligand ephrin-B play an important role in synapse formation and their disruption has been implicated in neurodegenerative diseases. We observed an increase in astrocytic ephrin-B1 a controlled cortical impact (CCI) model of TBI. The observed up-regulation coincides with a decrease in excitatory synapses within the Stratum Radiatum (SR) of the CA1 hippocampus. Ablation of astrocytic ephrin-B1 resulted in the recovery in the number of excitatory synapses by 7dpi not seen in WT mice. We propose that the up-regulation of ephrin-B1 levels in astrocytes surrounding the synapse may disrupt the trans-synaptic EphB/ephrin-B interactions resulting in synapse collapse. In addition to the effect of ephrin-B1 after TBI *in* vivo we also investigated how ephrin-B1 reverse signaling affects synapse elimination *in vitro*. Indeed we observed an increased association of excitatory synapses with astrocytes overexpressing ephrin-B1 in primary astrocyte-neuron co-cultures. Utilizing ephrin-B1 signal transduction null mutants we have examined how ephrin-B1 signaling affects synaptoneurosome engulfment in primary astrocytes. To determine the role of ephrin-B1 reverse signaling in astrocytes we investigated STAT3 activation. STAT3 activation has been observed in reactive astrocytes after injury and is implicated in reactive astrogliosis. We have observed an up-regulation in phosphorylated levels of STAT3 within the hippocampus at 3 dpi, which was suppressed by astrocyte-specific ablation of ephrin-B1 *in vivo*. Furthermore, we observed an increase in STAT3 activation *in vitro* following activation of ephrin-B1 in astrocytes. Our studies suggest that astrocytic ephrin-B1 may play an active role in injury-induced synapse remodeling

after injury through the activation of STAT3-mediated signaling in astrocytes. *This work was supported by the NIH grant RO1MH067121*.

Disclosures: J. Koeppen: None. A. Nguyen: None. M. Garcia: None. S. Hanna: None. A. Obenaus: None. I. Ethell: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.03/D8

Topic: B.12. Glial Mechanisms

Support: BBSRC UK grant BB/K009192/1

Title: Vesicular and non-vesicular gliotransmission: synergy and role in synaptic plasticity in the neocortex

Authors: *Y. PANKRATOV;
Univ. of Warwick, Coventry, United Kingdom

Abstract: Communication between neuronal and glial cells is thought to be very important for many brain functions. Astroglial cells possess the SNARE-like Ca^{2+}-dependent machinery for vesicular release of "gliotransmitters" like D-serine, glutamate, and ATP. Acting via release of gliotransmitters, astrocytes can modulate synaptic strength. The mechanisms of gliotransmission remain uncertain with exocytosis being the most intriguing and debated pathway. Previously, we showed that SNARE-dependent exocytosis of gliotransnitters can be triggered by elevation of astroglial Ca^{2+} via direct UV-uncaging or via glia- specific receptors (PAR-1, CB1 or α1-adrenoreceptors). Our recent studies revealed three types of responses of pyramidal neurons to activation of astrocytes in the neocortical slices of wild-type, dn-SNARE and Best1 KO mice: (1) burst of spurious fast NMDAR-mediated currents (sFICs, decay time about 25-40 ms) which were sensitive to ifenprodil, in contrast to baseline synaptic mEPSCs; this burst of glia-driven GluN2B-mediated currents was eliminated by perfusion of astrocytes with light chain of Tetanus Toxin; also it was inhibited in the dn-SNARE mice; (2) TREK-1 channel-dependent GluN2B-mediated slow inward currents (SICs, decay time 100-1000 ms); and (3) very slow (decay >3-5 sec) Best1-dependent currents. The glia-induced sFICs and SICs made approximately equal contributions to overall glutamate release. Importantly, both sFICs and SICs were dramatically decreased in the Best1KO mice but were rescued by application of exogenous D-Serine suggesting that major role of astroglial Best1 channels in Ca^{2+}-dependent release of D-Serine. Combined, our results show that cortical astrocytes can release glutamatergic gliotransmitters by

combination of Ca^{2+} and SNARE-dependent exocytosis and non-vesicular mechanisms dependent on TREK-1 and Best1 channels. Our data show that synergetic action of astrocyte-derived ATP and glutamatergic gliotransmitters is essential for synaptic plasticity. In particular, LTP was impaired in the neocortex of dnSNARE mice but could be rescued by application of exogenous D-Serine or non-hydrolysable ATP analogs. Moreover, we observed the deficit in working memory of dn-SNARE mice; this is a first evidence of physiological relevance of glial exocytosis in vivo. Interestingly, environmental enrichment rescued the LTP and working memory in dnSNARE mice, most likely via up-regulation of non-vesicular Ca^{2+}-dependent release of d-Serine via Best1 channels. To conclude, our data strongly support the importance of synergy between vesicular and non-vesicular gliotransmission.

Disclosures: Y. Pankratov: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.04/D9

Topic: B.12. Glial Mechanisms

Support: Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers Supported by The MEXT

Grant-in-Aid for Scientific Research (A)

Grant-in-Aid for Young Scientists (A)

Human High Performance Project Supported by The MEXT

Title: Dopaminergic activity-dependent astrocytic glycogenolysis in exercising rat hippocampus

Authors: *T. MATSUI[1], I. TORRES-ALEMÁN[2], H. SOYA[1];
[1]Fac. of Hlth. and Sport Sci., Univ. of Tsukuba, Ibaraki, Japan; [2]Cajal Institute, CSIC, Madrid, Spain

Abstract: Hippocampal lactate produced by astrocytic glycogenolysis plays a critical role in memory functions as a neuronal energy source and/or a neuromodulator. Although noradrenaline and serotonin are mediators for astrocytic glycogenolysis in the cortex, it is still unknown whether astrocytic glycogenolysis depends on neuronal activity and what mediates it in the hippocampus. Since running exercise elicits speed-dependent neuronal activation corresponding with energy demand, particularly in the hippocampus, we investigated this issue using a

treadmill running model for rats. First, we exercised rats on a treadmill for 30 min at different speeds (mild: 10 m/min, moderate: 20 m/min, and hard: 30 m/min) and sacrificed the rats using high-power (10 kW) microwave irradiation to detect glycogen, lactate, and monoamine levels in the brain (the hippocampus, cortex, cerebellum, brainstem, and hypothalamus), immediately after exercise. Glycogen in the brain, except in the hypothalamus, decreased in a speed-dependent manner associated with increased brain lactate levels. In the hippocampus, decreased glycogen and increased lactate were associated with running-speed-dependent dopaminergic activation, but not noradrenergic or serotonergic activities. Next, in vivo microdialysis during speed-incremental exercise (0, 10, 20, and 30 m/min) revealed that levels of hippocampal extracellular dopamine, but not noradrenaline or serotonin, increased with running speed. Furthermore, in primary cultured astrocytes of the rat cortex and hippocampus, the challenges of, respectively, dopamine and dopamine D2 receptor agonist (not D1) induced dose-dependent glycogen decrease. Finally, a local injection of dopamine D2 receptor antagonist (not D1) prevented hippocampal glycogen decrease during the mild running exercise. Our results provide the first evidence for dopaminergic activity-dependent astrocytic glycogenolysis in the exercising hippocampus, suggesting a novel role of dopamine in exercise-enhanced memory functions.

Disclosures: T. Matsui: None. I. Torres-Alemán: None. H. Soya: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.05/D10

Topic: B.12. Glial Mechanisms

Title: Astrocytic PKC- and PKA-dependent regulation of astrocyte glutamate uptake and effects on neuronal excitability

Authors: *I. M. HOLMAN[1], Y. KIM[2], J. TRUONG[2], L. YOUNG[2], T. A. FIACCO[2];
[1]Neurosci., [2]Cell Biol. and Neurosci., UC Riverside, Riverside, CA

Abstract: Uptake of synaptically released glutamate by astrocytes is essential for maintaining a healthy level of excitatory activity in the brain and for regulating spillover to adjacent synapses. It is known that during neuronal activity, astrocytic metabotropic glutamate receptors (mGluRs) and other G protein-coupled receptors (GPCRs) are activated. Previously, our lab demonstrated that astrocytic G_q GPCRs rapidly potentiate glutamate uptake in a protein kinase C (PKC) - dependent manner (Devaraju et al., J. Neurophysiol., 2013). Here, we extend these findings with new data demonstrating that the PKC activator (-)-Indolactam V dialyzed into single astrocytes in stratum radiatum of acute mouse hippocampal slices rapidly potentiates astrocyte glutamate

uptake. Whole-cell synaptically-evoked transporter currents double in amplitude by 30 minutes compared to control recordings using the inactive enantiomer (+)-Indolactam V. Furthermore, inhibition of intracellular astrocytic protein kinase A (PKA) suggests that PKA normally functions to diminish astrocyte glutamate uptake over a similar time course. Collectively, our findings suggest that astrocytic PKC is both necessary and sufficient for rapid potentiation of astrocyte glutamate uptake, while PKA acutely depresses astrocyte glutamate uptake. Experiments are now underway to determine the effects of this bidirectional modulation of astrocyte glutamate uptake on neuronal excitability. Specifically, we are focusing on how changes in astrocyte glutamate uptake affect activation of extrasynaptic NMDA receptors, which are located in close proximity to astrocyte processes and are therefore likely targets for changes in astrocyte glutamate uptake compared to synaptic receptors. Preliminary data suggest that rapid potentiation of astrocyte glutamate uptake diminishes the NMDA receptor-dependent tonic current recorded in CA1 pyramidal neurons. Experiments are now in progress to examine the effects of changes in astrocyte glutamate uptake on other measures of extrasynaptic NMDA receptor activity.

Disclosures: I.M. Holman: None. Y. Kim: None. J. Truong: None. L. Young: None. T.A. Fiacco: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.06/D11

Topic: B.12. Glial Mechanisms

Support: NIH Grant HD081037

Rett Syndrome Research Trust

Title: Astrocytic modulation of neuronal signaling in Rett syndrome

Authors: *B. RAKELA[1], P. BREHM[2], G. MANDEL[3];
[1]Neurosci., Vollum Institute/ Oregon Hlth. and Sci. Univer, Portland, OR; [2]Vollum Institute/ Oregon Hlth. and Sci. Univ., Portland, OR; [3]Vollum Institute/ Oregon Hlth. and Sci. Univ., portland, OR

Abstract: Astrocyte-mediated events are important for maintaining neuronal homeostasis and proper synaptic transmission. Recently, astrocytes were implicated in the autism-like spectrum disorder Rett syndrome (RTT), where global mutations in the X-linked methyl DNA-binding

protein MeCP2 result in dysregulation of neuronal function. Using brain slices prepared from wildtype (WT) mouse barrel cortex (p7-p12), paired recordings between SR101-positive astrocytes and neurons show that astrocyte stimulation (depolarization or agonist application) leads to a potent activation of synaptic signaling in neighboring pyramidal neurons. These synaptic currents are GABAergic and excitatory, as determined by their gabazine sensitivity (20μM) and reversal potential identified by gramicidin perforated patch recordings. Interestingly, when these stimulations were repeated in male MeCP2 null mice, a mouse model for RTT, astrocyte-mediated activation of neuronal GABAergic signaling was completely abolished. To determine whether the defective astrocyte-mediated signaling in the MeCP2 null cortex was due to the neuron, the astrocyte, or both, I exploited female RTT mice bearing a MeCP2-GFP fusion gene, allowing discrimination between WT (GFP+) and Mecp2 null (GFP-) cells, this mosaicism allowed me to record from every combination of WT and mutant cells. I found that loss of GABAergic signaling in neurons occurred whenever a MeCP2-deficient astrocyte was stimulated, regardless of the status of MeCP2 expression in the neurons. These data suggest that in WT mice, astrocytes are capable of modulating neuronal signaling by contributing to the GABAergic tone in the cortex; this contribution is absent in Rett syndrome, which further implicates astrocytes in the dysregulation of neuronal processes seen in this disorder.

Disclosures: B. Rakela: None. P. Brehm: None. G. Mandel: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.07/D12

Topic: B.12. Glial Mechanisms

Title: Studying neuronal ATP dynamics *In vivo*

Authors: *L. HOESLI[1,2], A. S. SAAB[1,2], J. HIRRLINGER[3,4], B. WEBER[1,2];
[1]Pharmacol. and Toxicology, Univ. Zürich, Zuerich, Switzerland; [2]Neurosci. Ctr. Zurich, Univ. and ETH Zurich, Zurich, Switzerland; [3]Neurogenetics, Max-Planck Inst. for Exptl. Med., Göttingen, Germany; [4]Fac. of Med., Carl-Ludwig-Institute for Physiology, Universtiy of Leipzig, Leipzig, Germany

Abstract: Neuronal energy requirements depend to a large extent on electrical activity and the ATP demand of Na^+/K^+-ATPases. Astrocytes and oligodendrocytes are suggested to fuel neuronal ATP demands by providing lactate to neuronal compartments such as dendrites and axons. One important source of lactate may be glycogen, which has been described to be relevant for cognitive functions such as learning and memory. However, little is known about activity-

dependent neuronal ATP dynamics *in vivo* and how glycogen levels or lactate distribution impact neuronal ATP homeostasis. Here, we used a transgenic mouse model that expresses an ATP biosensor (genetically encoded FRET sensor) in neurons, which allows us to study cortical ATP levels *in vivo* using two-photon microscopy. To simultaneously monitor neuronal activity, the calcium sensor RCaMP was introduced by viral delivery. Interestingly, spontaneous neuronal activity was not accompanied by any ATP level changes. Even sensory stimulations like whisker or hind limb, which evoke cortical activity measured by calcium transients, did not affect neuronal ATP levels. However, local cortical stimulations with a microelectrode induced significant ATP level drops that were dependent on stimulus strength and intensity. Hence, neuronal ATP levels are quite stable and only drop upon high energy consuming stimulation paradigms. Whether and how the glial metabolic support machinery such as glycogen levels or lactate distribution may be critical in regulating the neuronal ATP homeostasis is currently under investigation.

Disclosures: L. Hoesli: None. A.S. Saab: None. J. Hirrlinger: None. B. Weber: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.08/D13

Topic: B.12. Glial Mechanisms

Title: Optical activation of hippocampal astrocytes boost synaptic transmission in CA1 pyramidal neurons

Authors: *S. MEDEROS, A. HERNANDEZ-VIVANCO, G. PEREA;
Inst. Cajal, CSIC, Madrid, Spain

Abstract: Astrocytes play critical roles in homeostatic functions. In the last years, studies focused on neuron-astrocyte signaling have revealed crucial roles of astrocytes in neuronal activity and synaptic physiology (Araque, et al. 2014). To extend our knowledge of the properties and consequences of astrocyte signaling in brain function, new tools and experimental approaches need to be developed. Here, taking advantage of optogenetic tools we manipulated astrocyte activity and evaluated their consequences on neuronal physiology. The ectopic expression of channelrhodopsin-2 (ChR2, a light-activated ion channel protein) was targeted specifically to hippocampal astrocytes by viral transfection (Perea et al., 2014). By immunohistochemically analysis we found a restricted expression of ChR2 to astrocytes. Using electrophysiological techniques in hippocampal slices, we found that optical activation of astrocytes enhanced local excitatory synaptic transmission in CA1 hippocampal pyramidal

neurons. ChR2-stimulated astrocytes induced an increase of NMDA-mediated slow inward currents in pyramidal cells, indicating the ability of ChR2 to stimulate the release of glutamate from transfected hippocampal astrocytes. The pharmacological analysis indicated that astrocyte induced modulation of synaptic transmission was mediated by activation of glutamatergic receptors at neuronal membranes. Additionally, stimulation of ChR2-transfected astrocytes in IP3R2 knockout mice, which show down-regulated evoked astrocytic intracellular calcium signaling, revealed similar potentiation of synaptic transmission to wildtype mice. Neuronal activity to light stimulation was recorded in vector-transfected slices and no significant changes were observed. Then, optical activation of astrocytes stimulates glutamate release that controls the synaptic strength of pyramidal neurons influencing the operation of hippocampal circuits. References: Araque A. *et al.* Gliotransmitters travel in time and space. Neuron. 2014;81(4):728-39. doi:10.1016/j.neuron.2014.02.007. Perea G, Yang A, Boyden ES, Sur M. Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. Nat Commun. 2014;5:3262. doi: 10.1038/ncomms4262. Supported by MINECO: CSD2010-00045; BFU2013-47265R.

Disclosures: S. Mederos: None. A. Hernandez-Vivanco: None. G. Perea: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.09/D14

Topic: B.12. Glial Mechanisms

Support: NIH Grant NS078886

NIH Grant 5R01HD081037

Title: An imaging screen to identify astrocyte factors controlling neuronal maturation and synaptogenesis

Authors: *J. MCGANN, G. MANDEL;
Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: While it is well established that neurons and astrocytes are in intimate contact during nervous system development, only recently have studies revealed that proper neuronal maturation and synaptogenesis is dependent on astrocyte-specific factors. Several factors have been identified that regulate this dependence, but thus far they are limited primarily to secreted molecules. Little is therefore known about contact-mediated mechanisms of signaling or the

factors involved in the coordinated regulation of these processes. To shed light on this critical area of neurobiology, we have designed and undertaken a high-throughput image-based shRNAi screen to identify genes required in astrocytes to support normal neuronal development. Our screen utilizes P0 mouse cortical astrocytes grown in culture in 96-well format and subjected to lentiviral shRNAi knockdown. Subsequently, untreated P0 neurons prepared from a mouse expressing TdTomato globally are introduced and allowed to mature. We then image dendritic and axonal outgrowth followed by fixation and staining for the synaptic proteins Homer and Synaptophysin, as well as a marker of astrocyte reactivity, Glial Fibrillary Acidic Protein. Finally, we analyze the images computationally, identifying numerous morphological and cellular phenotypes, including total neurite length, branch number and position, synaptic density, and synaptic maturity. Thus far, we have screened for consequences of knock down of two functional gene families, encoding cell adhesion molecules and cytokine/chemokine receptors. Our screen has identified dozens of genes with consistent and significant effects on both astrocyte and neuronal biology. Two representative candidates that have been confirmed in three independent analyses are Neurotensin receptor 2 (Ntsr2), mutants of which have abnormal gait and a decreased startle response, and Progestin and AdipoQ Receptor family member 8 (Paqr8), mutations of which are implicated in human epilepsy. The identification of contact-dependent non-cell-autonomous effects with this methodology is robust and is broadly applicable to studying other functional gene families, as well as neurons and astrocytes from different sources, including models of neurological disease.

Disclosures: J. McGann: None. G. Mandel: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.10/D15

Topic: B.12. Glial Mechanisms

Support: The research leading to these results has received partial funding from the European Union Seventh Framework Programme (FP7) under grant agreement no 604102 (HBP).

Title: Astrocyte-neuron interactions in long-term plasticity: a computational study

Authors: T. MANNINEN[1], A. SAUDARGIENE[2], R. HAVELA[1], *M.-L. LINNE[1];
[1]Tampere Univ. Technol., Tampere, Finland; [2]Vytautas Magnus Univ., Kaunas, Lithuania

Abstract: One significant challenge in neuroscience is to identify the key cellular and molecular mechanisms involved in modulation of synaptic long-term plasticity (Hellgren Kotaleski and

Blackwell, Nat Rev Neurosci 2010). In addition to neuronal mechanisms, astrocytic calcium signaling pathways and gliotransmission have increasingly been implicated as key elements in modulation of short- and long-term plasticity, in a variety of cortical areas in vitro (see, e.g., Perea and Araque, Science 2007; De Pitta and Brunel, Neural Plasticity 2016). In this study, we sought to study the role of several experimentally established modulatory mechanisms of plasticity by computational means. Computational data driven modeling is one promising tool to study complex interactions and time courses of multiple events that cannot easily be assessed simultaneously in a wet-lab experiment. First, altogether more than 60 previously published models of astrocyte-neuron interactions were evaluated in detail (Manninen et al. 2016, in press). Second, distinct models for three cellular compartments were developed and validated: models of the pre- and postsynaptic terminals of the cortical neurons and a model for an adjacent cortical astrocyte process. Third, the full model was integrated and validated against cortical long-term plasticity data. In short, the model includes the well-established biophysical mechanisms for neuronal sodium and neuronal and astrocytic calcium excitability, as well as the signaling pathways putatively important in plasticity. Extensive simulations were then run using classical stimulation protocols for long-term plasticity as synaptic input. Our simulations show that changes in the temporal patterns of synaptic input can select for specific neuronal signaling pathways, such as the endocannabinoid signaling. This raises calcium levels in the astrocyte process, which has the potential to cause, in a concentration-dependent manner, release of the gliotransmitter glutamate from the astrocyte to further modulate the vesicle release in the presynaptic terminal. In summary, our simulations demonstrate that the interacting neuronal and astrocytic processes possess the molecular mechanisms to dynamically modulate the presynaptic terminal and, hence, the long-term plasticity. Our work provides one of the first models combining cellular excitability and physicochemical properties of astrocyte-neuron interactions for deciphering the dynamics and mechanisms involved in long-term plasticity. It can be used in future in silico and in vitro studies to address how neural networks learn to represent memories.

Disclosures: T. Manninen: None. A. Saudargiene: None. R. Havela: None. M. Linne: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.11/D16

Topic: B.12. Glial Mechanisms

Support: NIH Grant NS082007-01A1

NIH Grant AG051510-01

Title: Lrp4 in astrocytes modulates glutamatergic transmission

Authors: *X. SUN;
Augusta Univ., Augusta, GA

Abstract: Neurotransmission requires precise control of neurotransmitter release from axon terminals. This process is regulated by glial cells; however, underlying mechanisms are not fully understood. Here we report that glutamate release is impaired in mutant mice lacking low density lipoprotein receptor-related protein 4 (Lrp4), a protein critical for neuromuscular junction formation. Electrophysiological studies indicate compromised release probability in astrocyte-specific *Lrp4* knockout mice. *Lrp4* mutant astrocytes suppress glutamate transmission by enhancing the release of ATP, whose levels are elevated in the hippocampus of *Lrp4* mutant mice. Consequently, the mutant mice are impaired in locomotor activity and spatial memory and are resistant to seizure induction. These impairments could be ameliorated by adenosine A1 receptor antagonist. The results reveal a critical role of Lrp4, in response to agrin, in modulating astrocytic ATP release and synaptic transmission. Our study provides insight into the interaction between neurons and astrocytes for synaptic homeostasis and/or plasticity.

Disclosures: X. Sun: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.12/D17

Topic: B.12. Glial Mechanisms

Support: HFSP long-term fellowship #LT000010/2013 to TP

NIH/NIMH F31 Grant #MH106208 to JD

NIH R01 Grant NS037585

Title: Cholinergic transmission tunes astrocyte- dependent gating of the NMDA receptor co-agonist binding site

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Abstract: It is firmly established that D-serine is an endogenous agonist controlling the glycine-binding site of synaptic N-methyl D-aspartate receptors (NMDARs). However, conditions and

stimuli that govern D-serine release have remained unidentified.
Using molecular genetics, behavior, amperometry, *in vitro* electrophysiology and optogenetics we demonstrate that D-serine availability fluctuates across the sleep wake-cycle in mouse hippocampus, from low extracellular concentrations in the light (sleep)-phase to higher levels in the dark (active)-phase. Correspondingly, the saturation of the NMDAR co-agonist site oscillates during the 24h period at CA3-CA1 synapses, reaching full saturation in the dark (active)-phase, and this is sufficient to impact behavioral performance in a hippocampal-dependent learning task. We found that D-serine oscillations 1) are caused by daily fluctuations in astrocytic vesicular release and 2) are driven by a sleep homeostasis/activity-dependent mechanism wherein wakefulness promotes and maintains elevated extracellular D-serine concentrations.
Abundant evidence points to a modulatory function of endogenous acetylcholine (ACh) on synaptic plasticity and NMDAR function in cortical regions. Interestingly, cholinergic neurons from the medial septum and diagonal band of Broca (MS-DBB) represent the only source of ACh in the hippocampus and their activity co-varies with sleep stages such that ACh release is the highest during wakefulness. We found that optogenetic stimulation of MS-DBB cholinergic fibers causes an increase in D-serine levels at CA3-CA1 synapses and that pharmacological manipulation of the α7nAChR directly modulates D-serine availability. In addition, cell-specific knock-out of α7nAChR in astrocytes, but not neurons, is sufficient to abolish daily D-serine oscillations.
Additionally we determined that *in vivo* administration of an α7nAChR partial agonist (EVP-6124), evaluated in a Phase III clinical trials for the treatment of schizophrenia, elevates D-serine levels. Together our data demonstrate the existence of a new pathway, of clinical relevance, through which wakefulness-dependent release of ACh from the MS-DBB dictates hippocampal D-serine availability and NMDAR activity via astrocytic α7nAChRs.

Disclosures: **T. Papouin:** None. **J. Dunphy:** None. **K.T. Dineley:** None. **P.G. Haydon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaCure.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.13/D18

Topic: B.12. Glial Mechanisms

Support: CAPES

CNPq

FAPERJ

Ministério da Saúde

Title: Heterogeneity in the synaptogenic potential of cultured astrocytes from distinct brain regions

Authors: *A. S. BUOSI, I. C. P. MATIAS, A. B. ARAUJO, F. C. A. GOMES;
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Abstract: Astrocytes, the most abundant glial cells in the Central Nervous System (CNS), comprise a heterogeneous population of cells. It is well known that across brain regions, as well as within the same region, astrocytes differ in their morphology, developmental origin, gene expression profile, physiological properties, functions and response to injury and disease. Recently, they have been recognized as important regulators of synapse formation and maturation. However, it is not yet clear if there are differences in the synaptogenic potential of distinct subtypes of astrocytes. Objectives: to analyze the synaptogenic property of astrocytes from different regions of the CNS. Methods: cortical, hippocampal, midbrain and cerebellar neuronal cultures from Swiss mice between 14 to 16 embrionary ages (E14-16) were treated with astrocyte conditioned medium (ACM) prepared from astrocytes derived from newborn mice. Synapse formation was evaluated by immunocytochemistry for the synaptic proteins, Synaptophysin and PSD95. The expression profile of the synaptogenic molecules secreted by astrocytes from distinct brain regions was analyzed by q-PCR. Results: ACM from the four regions analyzed increased significantly the number of Synaptophysin/PSD95 puncta on neurons from the same and from different brain regions. Differences on astrocytic synaptogenic potential between the regions were observed according to the protein concentration in conditioned medium. Thus, cerebellar astrocytes have higher synaptogenic effects when ACM is less concentrated, suggesting that there is quantitative and qualitative differences in their protein content. Gene expression analysis of synaptogenic molecules classically secreted by astrocytes showed that glypicans 4 and 6, hevin and SPARC are molecules with notable variation of expression between astrocytes from different brain regions. Furthermore, the analysis of synaptogenic factors levels and its distribution on different astrocytes confirmed that variance. Conclusions: these findings highlight the heterogeneity of astrocytes and suggest that their synaptogenic potential may be different in each brain region, mainly due to distinct gene expression profiles. The protocol of this study was approved by the Committee for Animal Research of the Federal University of Rio de Janeiro.

Disclosures: A.S. Buosi: None. I.C.P. Matias: None. A.B. Araujo: None. F.C.A. Gomes: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.14/D19

Topic: B.12. Glial Mechanisms

Title: The flavonoid hesperidin modulates synapse formation and the synaptogenic potential of astrocytes

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Abstract: The Central Nervous System (CNS) is target for several neurodegenerative diseases characterized by neuronal death, synaptic and glial dysfunctions. Currently, new drugs have been studied as therapeutic alternatives for modulating cognitive processes in physiological and pathological conditions, and, among them, flavonoids have been noted for their remarkable pleiotropic actions. However, little is known about the mechanisms of these compounds on neuronal and glial physiology and their impact on learning and memory. Here we investigated the actions of the flavonoid hesperidin on synapse formation in vitro and in vivo, as well as the role of astrocytes as mediators of hesperidin's actions. **Methods:** Cortical neurons and astrocytes cultures were prepared from 14-15 embryonic days and 1-2 post-natal days Swiss mice, respectively. The neuronal cultures were treated with hesperidin (5 uM) for 24 hours or astrocyte conditioned medium (ACM) for 3 hours, and analyzed for synapses formation. Signaling and secretion of TGF-β1, a synaptogenic astrocyte derived-molecule (Diniz et al., 2012; Diniz et al., 2014), were analyzed in astrocyte cultures treated by hesperidin. Moreover, we evaluated if TGF-β1 secretion by astrocytes was involved in synapse formation induced by the ACM. **Results:** Hesperidin increased by 40% the number of synapses between cortical neurons. This event was followed by a 75% decrease in neuronal death and 85% increase in the pre-synaptic activity, as observed by immunocytochemistry and FM1-43 assay. ACM increased the number of synapses by 130%, whereas ACM from astrocytes treated by hesperidin increased by 250%, showing that hesperidin enhances the synaptogenic potential of astrocytes in vitro. Neutralization of TGF-β1 activity in the ACM significantly reduced its synaptogenic potential, suggesting that hesperidin's actions were dependent on TGF-β1 secretion by astrocytes. Preliminary in vivo results showed that hesperidin also modulated the number and levels of synaptic proteins in adult Swiss mice. **Conclusion:** Our data indicate a new function for hesperidin on synapse formation, and suggest a new mechanism of action of this compound in synaptogenesis, through the control of soluble factors production by astrocytes. The protocol of this study was approved by the

Committee for Animal Research of the Federal University of Rio de Janeiro. **Support:** CNPq, CAPES, FAPERJ, Ministério da Saúde.

Disclosures: I. Matias: None. L. Diniz: None. A. Buosi: None. G. Neves: None. J. Stipursky: None. F.C.A. Gomes: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.15/D20

Topic: B.12. Glial Mechanisms

Support: NIH Grant AA022239

Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation

Title: Impairment of astroglia calcium activation by ethanol and consequences for local circuit activity

Authors: L. YE, M. ORYNBAYEV, *M. PAUKERT;
Physiol., UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Among the most prominent acute neurological consequences of ethanol exposure are impaired motor coordination (ataxia) as well as impaired attention and memory. Noradrenergic signaling, originated by locus coeruleus neurons, plays important roles in attentional shifts, optimization of brain circuit activity to the behavioral context, and in gating synaptic plasticity. Ethanol can reduce locus coeruleus activity; however, the functional consequences at the cellular and circuit level are not well understood. To overcome this obstacle, we employed a linear treadmill in combination with in vivo two-photon calcium imaging on mice expressing the genetically encoded calcium indicator GCaMP3 in cerebellar Bergmann glia and cortical astrocytes. Our previous experiments have revealed that locomotion leads to norepinephrine-dependent simultaneous calcium activation of Bergmann glia in the cerebellum and astrocytes in primary visual cortex. Here, we found that locomotion-induced calcium elevations in Bergmann glia as well as primary visual cortex astrocytes are reversibly and dose-dependently inhibited by ethanol exposure. Acutely enhancing norepinephrine release was sufficient to completely reverse this effect of ethanol suggesting that suppression of norepinephrine release might be the underlying mechanism by which ethanol inhibits behavioral state-dependent astroglia calcium activation. We investigated the consequences of ethanol impairment of astroglia calcium activation for calcium dynamics in local neurons. We recorded locomotion-induced calcium

elevations in Purkinje cells, the principal neurons of the cerebellar cortex, using transgenic mice expressing a variant of the genetically encoded calcium indicator GCaMP6. Locomotion induced a slow calcium elevation in Purkinje cells with an onset ~2 s following the onset of locomotion and lasting for 5-10 s, similar to corresponding calcium elevations in Bergmann glia. Prolonged Purkinje cell calcium elevations were inhibited by ethanol exposure and could be recovered by acutely enhancing norepinephrine release, suggesting the recruitment of a similar signaling pathway, or a hierarchical relationship between astroglia and neurons. We were able to distinguish between these two possible mechanisms using inositol triphosphate receptor type 2 knockout mice, in which the locomotion-induced global calcium activation of Bergmann glia was absent. Our findings provide a cellular and circuit mechanism that may contribute to impairments of attention by acute ethanol exposure.

Disclosures: L. Ye: None. M. Orynbayev: None. M. Paukert: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.16/D21

Topic: B.12. Glial Mechanisms

Support: CNPq

CAPES

FAPERJ

Ministério da Saúde

Title: Amyloid-β Oligomers directly target astrocytes and impair their neuroprotective actions on synapses: prevention by astrocyte-derived tgf-β1

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Abstract: Alzheimer's disease (AD) is characterized by progressive decline of cognitive functions, mainly due to neuronal/synaptic dysfunction induced by amyloid-β peptide oligomers (AβOs). Although the effects of AβOs in neurons have been extensively studied, if and how

AβOs impact astrocytes remain largely unknown. Given the key role of astrocytes in synaptic formation and plasticity, we investigated the effect of AβOs on astrocytes and how it impacts synapse formation and function. Here, we show that murine hippocampal astrocytes are able to bind and internalize AβOs in vitro. Astrocyte conditioned medium (ACM) reduced AβOs binding to neurons, preventing AβO-induced synaptic loss. This "synaptic protection" ability of astrocytes was impaired by prior stimulation of astrocytes with AβOs. Also, protection provided by ACM was severely inhibited by blocking the signaling of TGF-β (Transforming growth factor-β), previously identified as a neuroprotective and synaptogenic factor secreted by astrocytes (Diniz et al., 2012; 2014). Intracerebroventricular injection of AβOs led to synaptic loss and memory deficit, followed by reduction in the levels of TGF-β1 in the hippocampus. Injection of TGF-β1 in the brain of these mice rescued synaptic/memory déficits caused by AβOs. Thus, we show that astrocytes and their soluble factors, particularly TGF-β1, can repress synaptic loss and AD progression. Further, we show that astrocytes are targets for AβOs, shedding light into a new mechanism underlying AβOs synaptotoxicity, indirect through glial cells. The protocol of this study was approved by the Committee for Animal Research of the Federal University of Rio de Janeiro.

Disclosures: L.P. Diniz: None. V. Toretelli: None. I. Matias: None. J. Morgado: None. A. Araújo: None. H. Melo: None. G. da Silva: None. S. Ferreira: None. F. de Felice: None. F.C.A. Gomes: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.17/D22

Topic: B.12. Glial Mechanisms

Title: Characterization of neurite growth using permissive and inhibitory astrocyte cell lines

Authors: S. CHRISTIAN, M. SMITH, A. NEWSOM, T. REGETZ, V. LUMBERT, L. KRAUSE, A. RENDON, *D. R. COOK-SNYDER;
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Abstract: Central nervous system (CNS) injuries, including stroke and traumatic brain injury, are leading causes of disability and death worldwide in part due to the limited axon regeneration capacity of the CNS. Improved *in vitro* models, particularly those that are fast growing and readily modifiable by genetic and pharmacologic manipulations, are critical to further our understanding of the mechanisms inhibiting axon regeneration following CNS injury, and to develop high throughput screens for novel therapeutics. To address this need, our studies

characterize co-cultures of N2a neuroblastoma cells, which can differentiate and extend neurites, with two complementary CNS astrocyte cell lines: A7 cells, which are axon growth permissive, and Neu7 cells, which are axon growth inhibitory. Thus, we hypothesized that differentiated N2a neuroblastoma cells would show greater neurite growth in A7 co-cultures than Neu7 co-cultures. Our results suggest that serum starvation induces N2a differentiation in a dose- and time-dependent manner, and that differentiated N2a cells extend more and longer neurites when co-cultured with A7 cells than with Neu7 cells. Moreover, A7 and Neu7 exposure to a variety of stimuli, including potassium chloride and acidified media, produces hypertrophy and proliferation consistent with reactive astrocytosis, although neither cell line demonstrates upregulation of glial fibrillary acid protein. Continued characterization of N2a-A7 and N2a-Neu7 co-cultures and their potential for reactive astrocytosis may provide efficient and complementary models for rapid identification of mechanisms underlying axon regeneration following CNS injury.

Disclosures: S. Christian: None. M. Smith: None. A. Newsom: None. T. Regetz: None. V. Lumbert: None. L. Krause: None. A. Rendon: None. D.R. Cook-Snyder: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.18/D23

Topic: B.12. Glial Mechanisms

Support: RIKEN special postdoctoral researcher

ANR

Title: Live STED microscopy reveals diversity of astrocytic coverage and Ca^{2+} signaling at individual tripartite synapses

Authors: *M. ARIZONO[1,2,3], A. PANATIER[4,1], J. ANGIBAUD[2,1], J. STOBART[5], L. BELLOCCHIO[4,1], G. MARSICANO[4,1], K. MIKOSHIBA[3], S. H. R. OLIET[4,1], B. WEBER[5], V. NÄGERL[2,1];
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Abstract: Astrocytes detect synaptic activity and regulate excitatory synaptic transmission through a variety of mechanisms including glutamate uptake and gliotransmitter release. The bidirectional communication between astrocytes and neurons takes place at the 'tripartite synapse', where astrocytic processes form intimate physical contacts with individual synapses. However, little is known about the influence of astrocytic coverage on individual synapses. A major obstacle to study these interactions has been the small size of the peri-synaptic astrocytic processes, which cannot be resolved by conventional fluorescence microscopy.
To overcome this problem, we combined live-cell STED microscopy of dendritic and astrocytic morphology with confocal Ca^{2+} imaging of astrocytes in living organotypic hippocampal slices. For this purpose, we used combinations of transgenic mouse lines (Ai6GFAP-ZsGreen for astrocytes, Thy1-YFP for neurons) and viral vectors (Sindbis-Citrine for neurons, AAV-GCaMP6s for astrocytes).
The super-resolution imaging approach revealed a reticular organization of astrocytic processes, which was characterized by bouton-like compartments strung together by a web of hyperfine connecting shafts. By co-labeling astrocytes and spines, we found that their physical association exhibited a wide spectrum of forms, from point-like contacts to nearly full coverage of the spine by the astrocyte. Notably, individual spines often were in contact with astrocytic compartments, suggesting that they form the glial element of the tripartite synapse. Confocal Ca^{2+} imaging showed that these astrocytic structures exhibited highly localized and uncorrelated Ca^{2+} transients during basal synaptic transmission, which often stayed restricted to the immediate proximity of a spine.
Together, our results reveal that tripartite synapses are highly individualistic structures, which exhibit unique spine-astrocyte morphological interactions and that astrocytic Ca^{2+} signals can be confined enough to affect only single synapses. These findings support the view that astrocytes can influence synaptic transmission with single spine specificity.

Disclosures: M. Arizono: None. A. Panatier: None. J. Angibaud: None. J. Stobart: None. L. Bellocchio: None. G. Marsicano: None. K. Mikoshiba: None. S.H.R. Oliet: None. B. Weber: None. V. Nägerl: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.19/D24

Topic: A.01. Neurogenesis and Gliogenesis

Support: Australian NHMRC grant 456027

Australian NHMRC grant 1048849

Australian NHMRC grant 631552

NINDS R01 NS34661

Title: Astroglial-mediated remodeling of the interhemispheric midline is required for the formation of the corpus callosum

Authors: *I. GOBIUS[1], L. MORCOM[1], R. SUAREZ[1], J. BUNT[1], P. BUKSHPUN[2], W. REARDON[5], W. DOBYNS[6], J. L. R. RUBENSTEIN[3], J. BARKOVICH[4], E. SHERR[2], L. RICHARDS[1];
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Abstract: The corpus callosum is the major axon tract that connects and integrates neural activity between the two cerebral hemispheres. However, ~1:4000 children are born with developmental absence of this tract, and the primary etiology of this condition remains unknown. Here, we demonstrate that midline crossing of callosal axons is dependent upon the prior remodeling and degradation of the intervening interhemispheric fissure during development. In mice and humans, this remodeling event is initiated by astroglia either side of the interhemispheric fissure, which intercalate with one another and degrade the intervening leptomeninges. Callosal axons then preferentially extend over these specialized astroglial cells as they cross the midline. We demonstrate that a key regulatory step in interhemispheric remodeling is the differentiation of these astroglia from radial glia, which is initiated by Fgf signaling to downstream Nfi transcription factors. Crucially, our findings from human neuroimaging studies reveal that developmental defects in interhemispheric remodeling are likely to be a primary etiology leading to human callosal agenesis.

Disclosures: I. Gobius: None. L. Morcom: None. R. Suarez: None. J. Bunt: None. P. Bukshpun: None. W. Reardon: None. W. Dobyns: None. J.L.R. Rubenstein: None. J. Barkovich: None. E. Sherr: None. L. Richards: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.20/D25

Topic: B.12. Glial Mechanisms

Support: NSF-HBCU UP HRD-1401026

NIH COBRE Pilot 1P20GM103653-01A1

Title: Astrocytes are necessary for synchronized bursting behavior of neuronal networks in culture

Authors: K. R. SANCHEZ[1], F. PERRY[1], M. A. HARRINGTON[1], *M. TEMBURNI[2];
[1]Biol. Sci., [2]Biol., Delaware State Univ., Dover, DE

Abstract: Synchronous oscillations are thought to be necessary for establishing functional neuronal networks for normal vertebrate brain development - however, the mechanisms of synchronization are not fully understood. Existing models of synchronous activity assume that it is a process intrinsic to neurons. Recently astrocytes, have been shown to participate in neuronal communication by releasing “gliotransmitters” like glutamate, and ATP. We hypothesize that astrocyte-neuron interactions are crucial for the development of synchronous activity seen in the developing vertebrate brain. We test this hypothesis by establishing pure and mixed (astrocyte and neuronal) cultures from the developing chicken brain (optic tectum) and recording neuronal activity using the multi-electrode array system, MED64 and Axion. Pure neuronal cultures were obtained by treating cultures with the mitotic inhibitor 5-fluorodeoxyuridine (FUdR) which kills mitotically active astrocytes but spares post-mitotic neurons. Neurons were kept alive in the absence of astrocytes by supplementing the culture medium with 50% astrocyte conditioned medium. Typically mixed cultures of astrocytes and neurons show random spiking activity in one week and synchronous activity in two weeks. Our initial results indicate that pure neuron only cultures show random spiking activity without synchronization even after two weeks thus clearly establishing a role for astrocytes in the development of synchronous activity. To insure the integrity of the neuronal structure in the absence of astrocytes Immunofluorescence (IF) microscopy was done. To further dissect the molecular pathways involved we are targeting three pathways within astrocytes that have been demonstrated to be crucial for communication with neurons - metabotropic glutamate receptor (mGluR). Activation of these G-protein coupled receptor by their respective neurotransmitters mobilizes intracellular calcium release leading to exocytosis of either glutamate. We are expressing dominant negative peptides designed to disrupt downstream signaling from these receptors and thereby calcium mobilization and exocytosis of gliotransmitters. Intracellular Ca^{++} release in astrocytes is monitored using GCaMPs.

Disclosures: K.R. Sanchez: None. F. Perry: None. M.A. Harrington: None. M. Temburni: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.21/D26

Topic: B.12. Glial Mechanisms

Title: Dual-color calcium imaging of astrocytes and neurons in the ferret visual cortex

Authors: *V. KELLNER, J. SCHUMMERS;
Max Planck Florida Inst., Jupiter, FL

Abstract: The role of astrocytes as more than support cells in the brain has begun to emerge. Studies have shown that astrocytes respond to sensory stimuli in a manner that is not a mere reflection of neuronal activity. Specifically, astrocytes respond to visual stimuli in the ferret visual cortex with similar but sharper orientation tuning curves compared to nearby neurons. The interactions between astrocytes and neurons that shape network responses to physiological stimuli remain unclear. Few studies have measured astrocytic and neuronal activity simultaneously in the intact brain. Those that have, used bulk loading of calcium dye which labels the astrocyte cell body but not the thin processes. Recent work has determined that astrocyte processes can function independently of the cell body, which emphasizes the importance of measuring calcium activity in the processes as well. The recent development of genetically encoded calcium indicators (GECIs) has enabled the targeting of calcium in astrocytic processes and newly improved red-shifted GECIs enable the simultaneous imaging of astrocytes and neurons. Using this technology in the anesthetized ferret visual cortex, with its columnar functional organization, we are able to simultaneously measure visual responses in astrocytes and neurons. By varying parameters of the stimulus we are able to show that astrocytes integrate neuronal activity. We also show, using electroencephalography (EEG) to measure brain states, that astrocytes and neurons differ in their responses to visual stimuli during synchronized and desynchronized brain states. Imaging neuronal and astrocytic calcium signaling simultaneously will enable us to measure the transfer function between neurons and astrocytes on a trial-by-trial basis.

Disclosures: V. Kellner: None. J. Schummers: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.22/D27

Topic: B.12. Glial Mechanisms

Support: Natural Sciences and Engineering Research Counci

Canada Research Chair Program

Fonds de la Recherche du Québec - Santé

EMBO fellowship (ALTF 453-2014)

Title: Astrocytes regulate dendritic inhibition of pyramidal cells by somatostatin interneurons

Authors: *M. MATOS[1], I. RIEBE[1], C. REYNELL[1], J. VALLÉE[1], I. LAPLANTE[1], A. PANATIER[2], J.-C. LACAILLE[1], R. ROBITAILLE[1];
[1]Neurosciences, Univ. De Montréal, Montréal, QC, Canada; [2]Neurocentre Magendie, Univ. de Bordeaux, Bordeaux, France

Abstract: γ-aminobutyric acid (GABA) interneurons play crucial roles in the control of pyramidal neurons (PNs) and network activity. In addition, another level of regulation could be mediated by astrocytes, as they perform important roles in the regulation of excitatory synaptic networks. However, the interaction of astrocytes with GABAergic synaptic inhibition by interneurons remains ill defined. To address this question, we employed an optogenetic approach, with selective channelrhodopsin 2 (ChR2) expression in somatostatin-containing interneurons (SOM-INs), to specifically activate SOM-INs, while monitoring Ca^{2+} in astrocytes and recording inhibitory currents in PNs from CA1 hippocampal slices of SOM-ChR2/EYFP mice. Our data show that low-frequency (0.1 Hz) photostimulation of SOM-INs evokes inhibitory postsynaptic currents (eIPSCs) in PNs. They were potentiated by endogenous ATP-derived adenosine acting on adenosine A_1 receptors (A_1R), indicated by their decrease after bath-perfusion with A_1R selective antagonist DPCPX (100 nM) (decrease to 66.0 ± 3.1% of the baseline, $p< 0.01$). This was further mimicked by preventing ATP-to-adenosine catabolism with ectonucleotidase inhibitor AMP-CP (200 μM) (decrease to 50.0 ± 7.0% of the baseline, $p< 0.01$). To probe for a possible astrocytic calcium (Ca^{2+})-based mechanism for the adenosine acting on A_1R, as we previously showed (Serrano et al., 2006, J Neurosci. 26**:**5370), we examined the Ca^{2+} dynamics in astrocytes after stimulation of SOM-INs. Our data reveals that light-stimulation of SOM-INs induced Ca^{2+} transients (27.2 ± 2.6% mean ΔF/F, n=12) mainly mediated by astrocyte-specific GABA transporter type-3 (GAT-3) (54.0 % ± 6.0% ΔF, $p< 0.001$) as they were blocked by GAT-3 inhibitor (S)-SNAP-5114 (100 μM). Importantly, astrocyte Ca^{2+} chelation with

BAPTA (20 mM) led to a decrease on SOM-INs light-eIPSCS in PNs (decrease to 56.1 ± 6.2% of the baseline, $p< 0.001$), an effect occluded by previous treatment with (S)-SNAP-5114. This suggests that GAT-3 mediated a glial Ca^{2+}-dependent process leading to potentiation of eIPSCs on PNs. Finally, the inhibitory effect of A_1R antagonist DPCPX on eIPSCs was occluded by previous block of GAT-3. Overall, our results suggest that, in response to GABA released by SOM-INs, astrocytic GAT-3 triggered a Ca^{2+} signalling mechanism, leading to the release of ATP-derived adenosine which activated A_1Rs. This, in turn, potentiated SOM-INs synaptic inhibition in PNs. These data uncover a novel physiological interaction between a specific subpopulation of interneurons and astrocytes, involved in the regulation of dendritic inhibition of hippocampal pyramidal cells.

Disclosures: M. Matos: None. I. Riebe: None. C. Reynell: None. J. Vallée: None. I. Laplante: None. A. Panatier: None. J. Lacaille: None. R. Robitaille: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.23/D28

Topic: B.12. Glial Mechanisms

Support: grant from TRB Chemedica (Switzerland)

Title: Effects of GM1 on activation of signaling pathways in neurons and astrocytes, dependence of astrocyte-neuron interactions

Authors: *H. FIUMELLI[1], I. ALLAMAN[2], J.-L. MARTIN[3], P. J. MAGISTRETTI[1,2];
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Abstract: Preclinical and clinical studies have shown that treatment with the ganglioside GM1 is neuroprotective and restorative, relieving symptoms and slowing down disease progression in Parkinson's patients. Substantial evidence from *in vitro* experiments indicates that trophic actions of GM1 on various populations of central nervous system neurons resemble those of neurotrophic factors, suggesting that GM1 functions are mediated by similar molecular pathways. Testing this hypothesis on primary cultured cortical neurons, we did not detect changes in markers of the neurotrophin/tyrosine kinase receptor pathway following GM1 treatment (60 uM, up to 1h). However, in co-cultures of neurons and astrocytes (prepared on separate physical substrates and sharing the same culture media), we found that GM1 increased

TrkB and Erk1/2 phosphorylation levels and induced the expression of immediate early gene such as Arc, Egr1, and BDNF, suggesting that diffusible substances between astrocytes and neurons are important for GM1 actions. To gain insight into the long-term gene expression changes induced by GM1 in astrocytes and neurons, we analyzed the effects of GM1 treatment (60 uM, 24h) on the gene expression profile of co-cultured astrocytes and neurons, as well as of pure cultures of astrocytes and neurons, using whole transcriptome RNA-seq. Differential expression analysis, with a cutoff of fold change > 1.5 and FDR < 0.05, between control and GM1 treated cultures revealed that co-cultured cells display considerably higher numbers of differentially expressed genes than pure cultures. We identified 1717 and 801 genes modulated by GM1 in co-cultured neurons and astrocytes respectively, whereas only 78 and 291 genes were regulated in pure cultured neurons and astrocytes respectively. Interestingly, for each cell type, only few differentially expressed genes were in common between both culture configurations (astrocytes, 52; neurons, 42). We next subjected the differentially expressed gene sets to pathway and gene ontology analysis to highlight functional processes affected by GM1. We found that differentially expressed genes in co-cultured astrocytes were mapped to cell adhesion and recognition, immune response and cholesterol synthesis, while those in co-cultured neurons were associated with synaptic transmission, regulation of ionotropic signaling, behavior, cognition, extracellular matrix and inflammatory response. Altogether, these data indicate that the molecular and functional signals elicited by GM1 are highly dependent on interactions between astrocytes and neurons.

Disclosures: H. Fiumelli: None. I. Allaman: None. J. Martin: None. P.J. Magistretti: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.24/D29

Topic: B.12. Glial Mechanisms

Support: Korea Healthcare Technology R&D Project, Ministry of Health & Welfare HI15C1928

National Research Foundation of Korea (NRF) grant funded by the Korean government 2014R1A1A2056508

Title: Neuron-astroglia interactions following AAV1 transduction with Rheb(S16H) of hippocampal neurons: Construction of neuroprotective system in the hippocampus *In vivo*

Authors: *M.-T. JEON[1,2], H. JANG[1,2], M. CHOI[5], U. JUNG[8], K. JEONG[1,2], B. JIN[6,7], R. E. BURKE[9,10], C. MCLEAN[11,12], S.-G. LEE[5], S. KIM[1,2,3,4];
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Abstract: Here we report that transduction of rat hippocampal neurons with constitutively active form of ras homolog enriched in the brain [Rheb(S16H)] using adeno-associated virus serotype 1 [AAV1-Rheb(S16H)] significantly increases the levels of cilliary neurotrophic factor (CNTF) and CNTF receptor α (CNTFRα) in astrocytes and neurons, respectively, even though the AAV1 transduction is limited within neurons. Neuronal brain-derived neurotrophic factor (BDNF) increased by AAV1-Rheb(S16H) transduction stimulated the production of CNTF in astrocytes, and CNTF and CNTFRα neutralization attenuated the Rheb(S16H)-induced neuroprotection against thrombin-induced neurotoxicity in the hippocampus. Moreover, neutralization and inhibition of tropomyocin receptor kinase B (TrkB), which is a specific receptor for BDNF, attenuated the production of CNTF in astrocytes *in vivo* and *in vitro*, resulting in inhibition of neuroprotective effects in the AAV1-Rheb(S16H)-transduced hippocampus. In the hippocampus of patients with Alzheimer's disease (AD), the protein levels of TrkB and CNTFRα were significantly increased compared with age-matched controls, even though the levels of BDNF and CNTF expression were significantly decreased or not altered, respectively, in the hippocampus of patients with AD, suggesting that there might be an endogenous neuroprotective system, exerting to protect hippocampal neurons against neurodegeneration. Thus, we conclude that viral vector transduction of neurons with Rheb(S16H) intensifies a beneficial construction of neuroprotective system through the heterotypic interactions between neurons and astrocytes, and the construction of neuroprotective system in the hippocampus may have therapeutic values against neurodegenerative diseases such as AD.

Acknowledgements
This work was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI15C1928). This research was also supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (2014R1A1A2056508).

Disclosures: M. Jeon: None. H. Jang: None. M. Choi: None. U. Jung: None. K. Jeong: None. B. Jin: None. R.E. Burke: None. C. McLean: None. S. Lee: None. S. Kim: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.25/D30

Topic: B.12. Glial Mechanisms

Support: NIH Grant F31 MH106198

NIH Grant MH104506

NIH Grant MH078823

Taylor Family Institute for Innovative Psychiatric Research

Title: Endogenous lactate reserves may fuel synaptic transmission via oxidative phosphorylation in hippocampal primary neurons

Authors: *C. SOBIESKI, N. WARIKOO, S. MENNERICK;
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Abstract: Glucose is metabolized via glycolysis and oxidative phosphorylation to produce ATP required for neurotransmission. The contributions of glycolysis and oxidative phosphorylation in fueling synaptic function are unclear. In autaptic hippocampal primary cultures, we inhibited glycolysis with 2-deoxy-D-glucose (2DG) and oxidative phosphorylation with oligomycin (oligo). Although 2DG+oligo and oligo alone compromised sodium currents, indicating collapse of the somatic sodium gradient, only combined inhibition blocked evoked transmission, suggesting intact action potential propagation in the axons following inhibition of either glycolysis or oxidative phosphorylation alone. After combined inhibition, we were unable to evoke vesicle release by action potentials, direct depolarization of synaptic terminals (100 mM K^+), or by application of 0.5 mM sucrose, suggesting absence of docked, primed vesicles. Although 2DG or oligo did not affect basal synaptic activity, previous work suggests obstructing glycolysis or oxidative phosphorylation disrupts vesicle endocytosis during sustained stimulation. It remains unclear whether endocytosis is more sensitive to a loss of glycolysis or oxidative phosphorylation. Following inhibition of oxidative phosphorylation, PSCs failed to recover after vesicle depletion (30s of 90 mM K^+). PSCs recovered normally after blocking glycolysis. Sensitivity to oxidative phosphorylation may be consistent with the astrocyte-neuron lactate shuttle hypothesis, where local glutamate uptake by perisynaptic astrocytes fuels astrocytic glycolysis and lactate shuttling to neurons. To test astrocytes role in vesicle recovery, we employed microcultures containing (+) or lacking (-) astrocytes. Disrupting the lactate shuttle with either –astrocyte microcultures or acute application of alpha-cyano-4-hydroxycinnamic acid (4-CIN) did not affect EPSC recovery compared to +astrocyte controls. Furthermore, 16 hr

aglycemia failed to affect either +astrocyte or -astrocyte EPSC recovery following vesicle depletion. Trace lactate (0.31 mM) measured in aglycemic saline was likely important for sustaining transmission since blocking its transport via 4-CIN during aglycemia significantly increased neuronal death. Our results suggest that basal synaptic transmission is sustained by either glycolysis or by oxidative phosphorylation, but oxidative phosphorylation is required for vesicle recycling. Oxidative phosphorylation can be fueled by reserves derived from astrocytic glycolysis, but these results contradict the current view that local, immediately supplied lactate fuels synaptic transmission.

Disclosures: C. Sobieski: None. N. Warikoo: None. S. Mennerick: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.26/D31

Topic: B.12. Glial Mechanisms

Support: National Natural Science Foundation of China. Grant number: 81300766

Title: The mediating effect of Lycium Barbarum Polysaccharides on retinal glial cells in a mouse model of acute ocular hypertension

Authors: *X. MI;
The First Hosp., Guang Dong, China

Abstract: Purpose: Acute ocular hypertension (AOH) could induce retinal damage through ischemia/reperfusion and mechanical injuries. The neuroprotective effect of *Lycium Barbarum* polysaccharides (LBP) has been discussed in our preliminary study. The purpose of this study is to investigate the effect of LBP on mediating retinal glial cells and recovering visual function.
Methods: AOH mouse model was induced in unilateral eye for one hour by introducing 90 mmHg ocular pressure. The animal was fed with LBP solution (1mg/kg) or PBS vehicle daily from 7 days before the AOH insult till sacrifice at day 4 post insult. The effects of LBP on retinal glial cells were examined by the glial cell number counting (for astrocytes and microglia) as well as the associated biomarker changes using S-100, GFAP, GS and AQP-4 as well as Iba-1. To investigate the visual function of mice, optokinetic test (OKT) and electroretinography (ERG) were performed.
Results: The neuroprotective effect of LBP was confirmed by number counting of retinal ganglion cells, which was as same as our previous report. Under the treatment of LBP, the recovery of animal's visual function was detected from the OKT and ERG data. After AOH

insult, increased number of astrocytes and microglia was observed. Together with increased expression of GFAP, GS and AQP-4, the morphological change of retinal astrocytes was also observed. After treatment with LBP, there was less increased number of astrocytes (lowering 43.5% when compared to PBS vehicle, $p<0.01$) and microglia (lowering 25.9% when compared to PBS vehicle, $p<0.01$), together with decreased expression of GFAP, GS, AQP-4 and APP when compared with the PBS vehicle treated retina.
Conclusion: The present study suggests the neuroprotective role of LBP is related to regulate the reactivity of retinal glial cells.

Disclosures: X. Mi: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.27/D32

Topic: B.12. Glial Mechanisms

Support: DFG grant Hi1414/2-1

SFB-TR43 grant

SPP1757 grant

Title: Metabolic axon-glia interaction assessed by real-time imaging of ATP

Authors: *A. TREVISIOL[1], A. S. SAAB[2], U. WINKLER[3], J. HIRRLINGER[3], K. A. NAVE[1]; [1]Max Planck Inst. For Exptl. Med., Goettingen, Germany; [2]Inst. of Pharmacol. & Toxicology, Univ. of Zurich, Zurich, Switzerland; [3]Carl-Ludwig-Institute for Physiology, Fac. of Medicine, Univ. of Leipzig, Leipzig, Germany

Abstract: Myelin provides electrical insulation to axons in white matter but it also limits any possible access to extracellular metabolites to the Node of Ranvier. However, the hypothesis that oligodendrocytes and their associated myelin sheaths might function as a direct source and interface for metabolites delivery into the axons they engulf, has been supported by an increasing number of observations. We generated a mouse line that expresses in neurons a FRET-based sensor ($AT1.03^{YEMK}$; Imamura et al. 2009) for ATP, a central molecule in the cell's energy homeostasis. We developed a set-up that allowed us to control both axonal electrical activity in the optic nerve (ON, a CNS white matter tract) and the delivery of metabolites through a superfusion solution (aCSF) while imaging the ATP-sensor in axons by confocal microscopy. We challenged the ON by different combinations of high-frequency stimulation and low-

substrate condition to determine how axonal ATP fluctuations correlate with electrical activity measured as compound action potentials (CAP, classically used as neuronal energy-readout). Using this system we show that glia-derived monocarboxylates, i.e. lactate and pyruvate, are essential to maintain axonal energy balance. The temporal dynamics of ATP correlated well with the fast and reversible changes in CAP under conditions of sufficient glucose supply in the aCSF. However, when activity of monocarboxylate transporter (MCT) was thwarted in the myelin and in the glia compartment, we observed a larger decrease in axonal ATP, which surprisingly was not followed by a similar decrease in CAP, suggesting a more complex relationship of ATP and CAP than supposed previously.
We finally assessed the impact of a myelin impairment on the axonal energy status by crossbreeding our ATP-reporter mouse with *Plp1*-null mutants. The lack of Plp1, one of the most abundant protein within myelin, has been shown to cause axonal degeneration in adult mice, but the exact mechanism has never been clarified. We have found evidence that during high-frequency stimulation of *Plp1*-null ONs, axonal ATP metabolism is impaired in an early phase of the pathology. Our data suggests that impairment in the metabolic axon-glia interplay might be one of the first events during pathogenesis in white matter diseases.

Disclosures: A. Trevisiol: None. A.S. Saab: None. U. Winkler: None. J. Hirrlinger: None. K.A. Nave: None.

Poster

211. Astrocytes: Synaptogenesis, Synaptic Plasticity, and Neuron-Interaction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 211.28/D33

Topic: B.12. Glial Mechanisms

Support: Human Frontier Science Program

Department of Biotechnology, India

Department of Science and Technology, India

Title: Active dendritic conductances regulate the impact of gliotransmission on rat hippocampal pyramidal neurons

Authors: *S. ASHHAD, R. NARAYANAN;
Indian Inst. of Sci., Bangalore, India

Abstract: Glial cells in the brain actively communicate with neurons through release of transmitter molecules that result in neuronal voltage deflections, thereby playing vital roles in

neuronal information processing. An important manifestation of this is the N-methyl-D-aspartate receptor (NMDAR)-dependent slow inward currents in neurons. Although a significant proportion of information processing in neurons is performed in their dendritic arborization, the intra-neuronal spatial dynamics of these events or the role of active dendrites in regulating their amplitude and spatial spread have remained unexplored. Here, we employed somatic and/or dendritic recordings from rat hippocampal pyramidal neurons and demonstrate that a majority of NMDAR-dependent spontaneous slow excitatory potentials (SEP) originate at dendritic locations and are significantly attenuated through their propagation across the neuronal arbor. We substantiated the astrocytic origin of SEPs through paired neuron-astrocyte recordings, where we found that specific infusion of inositol trisphosphate ($InsP_3$) into either distal or proximal astrocytes enhanced the amplitude and frequency of neuronal SEPs. Importantly, SEPs recorded after $InsP_3$-infusion into distal astrocytes exhibited significantly slower kinetics compared to those recorded after proximal infusion. Furthermore, employing neuron-specific infusion of pharmacological agents and morphologically realistic conductance-based computational models, we demonstrate that dendritically expressed hyperpolarization-activated cyclic-nucleotide-gated and transient potassium channels play critical roles in regulating the strength, kinetics and compartmentalization of neuronal SEPs. Finally, through the application of subtype-specific receptor blockers during paired neuron-astrocyte recordings, we provide evidence that GluN2B- and GluN2D-containing NMDARs predominantly mediate perisomatic and dendritic SEPs, respectively. Furthermore, in replicating experimentally observed somatodendritic SEP amplitudes in morphologically realistic conductance-based models, we arrived at a testable prediction that the density of extrasynaptic NMDARs should increase with dendritic distance from the soma. Our results add a significantly complex dimension to neuron-glia interactions by unveiling an important role for active dendrites in regulating the impact of gliotransmission, and suggest astrocytes as a source of dendritic plateau potentials that have been implicated in localized plasticity and place cell formation.

Disclosures: S. Ashhad: None. R. Narayanan: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.01/D34

Topic: A.07. Developmental Disorders

Support: Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Specific Hypolipidemia caused by VLDL degradation in Children with ASD

Authors: *H. MATSUZAKI[1], K. IWATA[1], K. NAKAMURA[2], M. TSUJII[3], N. MORI[4]; [1]Univ. of Fukui, Eiheiji-Cho, Yoshida-Gun, Japan; [2]Neuropsychiatry, Hirosaki Univ. Sch. of Med., Hirosaki, Japan; [3]Contemporary Sociology, Chukyo Univ., Toyota, Japan; [4]United Grad. Sch. of Child Develop., Osaka Univ., Suita, Japan

Abstract: Background: The neurobiological basis for autism remains poorly understood, but evidence is mounting in support of lipid metabolism playing a role in autism spectrum disorder (ASD). We have already revealed that Very Low Density Lipoprotein (VLDL) triglyceride was significantly decreased in children with ASD than those of normal control subjects (US PATENT #8518659). To elucidate the mechanism of VLDL down-regulation, it was necessary to clarify whether VLDL was reduced due to activation of the degradation or inhibition of its synthesis.
Objectives: In order to clarify the mechanism of VLDL down-regulation in ASD, we carried out measurements of free metabolite in plasma of children with ASD and examined correlation between VLDL triglyceride and the metabolites detected.
Methods: This study enrolled 30 children (6-11yrs old) with ASD recruited from the Asperger Society Japan and 30 age-matched healthy control subjects recruited by advertisement. Fasting human blood samples were collected by venipuncture in a sitting position with a tourniquet from all participants for both groups who are Japanese and drug-naïve. LC/CE-TOFMS measurement of free metabolite in the plasma was carried out using an Agilent LC System and CE Capillary Electrophoresis System (Agilent Technologies, Waldbronn, Germany). The size distribution of plasma lipoprotein particles was evaluated by high sensitivity lipoprotein profiling system with high-performance liquid chromatography (Skylight Biotech, Inc., Akita, Japan).
Results: By TOFMS analysis, a total of 258 metabolites were detected in the plasma of all set. Of these, 83 metabolites showed significantly different relative areas between the ASD children and the controls. The present study identified deviated plasma metabolite levels associated with oxidative stress and mitochondrial dysfunction in children with ASD. More, we found significant correlation between VLDL triglyceride decrease and 20 metabolites change including 12 free fatty acids, 3 free acylcarnitines, alanine, loganin, 2-hydroxybutyric acid, 3-hydroxybutyric acid and O-acetylcarnitine (ALCAR) in the ASD participants. Of these 20 metabolites, Alanine and Loganin were lower but the others were higher in the ASD participants than controls.
Conclusions: These results suggested that VLDL degradation may cause VLDL-specific hypolipidemia in children with ASD. This finding might be surrogate marker implicated in oxidative stress and mitochondrial dysfunction of ASD.

Disclosures: H. Matsuzaki: None. K. Iwata: None. K. Nakamura: None. M. Tsujii: None. N. Mori: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.02/E1

Topic: A.07. Developmental Disorders

Support: NIMH R01 MH100173-01A1 (McPartland)

NIMH RO1 MH100173-02S1 (McPartland)

NIMH R21 MH091309 (McPartland)

Patterson Trust 13-002909 (McPartland)

Autism Speaks Translational Postdoctoral Fellowship (Naples)

Autism Science Foundation (Naples)

Slifka-Ritvo Innovation in Autism Research Award (Naples)

Title: Attention and neural response to simulated social interactions in ASD

Authors: *J. A. TRAPANI[1], A. J. NAPLES[2], M. J. ROLISON[2], J. H. FOSS-FEIG[2], J. C. MCPARTLAND[2];
[1]Child Study Ctr., Yale Univ., New Haven, CT; [2]Child Study Center, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Atypical gaze, exhibited by difficulties with eye-contact and joint attention, is often an early sign of autism spectrum disorder (ASD) and research measuring event-related potentials (ERPs) with electrophysiology reveals atypical structural encoding of faces in ASD. Behaviorally, social differences in ASD are most pronounced during interactions, yet most existing experimental investigations of gaze processing use paradigms in which participants passively observe faces.
Using gaze contingent ERP, this study aimed to investigate attention to dynamic faces as measured by eye movements and neural response to eye-contact in children with ASD and typical development (TD). We also explored relationships among attentional and neural markers and clinical characteristics.
Participants were school-aged children with ASD (n=61) and TD (n=40) matched on age and IQ. ERPs were recorded using 128-channel sensor nets while eye movements were recorded concurrently with a remote eye-tracking system. In the current experimental paradigm, faces appear on screen and respond to participant gaze by either sharing or averting gaze. ERPs were time-locked to face movement; occipital N170 and central P300 were extracted for analyses.

An interaction between Condition and Group indicated the individuals with ASD had larger P100s to averted gaze while TD individuals had larger P100s to direct gaze [$F(1,79) = 4.68$, $p = .03$]. Across conditions, individuals with ASD had larger P100s to gaze [$F(1,79) = 7.79$, $p = .007$]. A main effect of Condition indicated that N170s to direct gaze were more negative than to averted gaze [$F(1,79) = 4.98$, $p = .028$]. A main effect of Group revealed that individuals with ASD looked less to the eyes of the face following gaze change [$F(1,99) = 27.95$, $p < .001$], and more to the bridge of nose (between eyes) following gaze change [$F(1,99) = 6.871$, $p = .01$]. In an interactive paradigm, direct gaze elicited greater face specific neural activity (N170) than averted gaze, suggesting reciprocal eye contact recruits greater face-specific brain activity than gaze aversion. Typically developing individuals showed larger P100s to reciprocal eye contact, while individuals with ASD exhibited larger P100s to averted gaze. This finding suggests typically developing individuals may prioritize social aspects of gaze (e.g., mutual eye-contact), whereas individuals with ASD may prioritize more directly functional aspects of gaze, such as directing attention. Increased attention to the eyes of the face correlated with better social function, suggesting that the ability to maintain reciprocal eye-contact in an interactive context is highly correlated with symptom presentation.

Disclosures: J.A. Trapani: None. A.J. Naples: None. M.J. Rolison: None. J.H. Foss-Feig: None. J.C. McPartland: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.03/E2

Topic: A.07. Developmental Disorders

Support: MRC/Sackler studentship

Title: Developing a passive viewing phenotypic measure of autistic traits

Authors: *O. E. PARSONS[1], R. BETHLEHEM[2], J. FREYBERG[2], O. SLUIJTERS[2], B. STONIER[2], S. BARON-COHEN[2];
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Abstract: Background Autism spectrum conditions (ASC) are associated with atypical face processing, reduced accuracy in face discrimination tasks and difficulties with recognizing emotional expression. Autistic traits and features of the autism phenotype are also expressed to a lesser extent in the general population. One current limitation of research in ASC is that the focus is skewed towards higher-functioning, verbal individuals due to pragmatic issues in testing

those lower on the spectrum.
Objectives The aim of this project was to develop a passive viewing paradigm sensitive to aspects of the ASC phenotype. We wanted to establish whether individual differences in neural responses during a face oddball task were correlated with characteristics associated with ASC in a typical population.
Method We used a fast serial visual presentation (FSVP) task to expose participants to a sequence of images. Stimuli were presented at a frequency of 6.0Hz, with faces being presented within this sequence at 1.2Hz. We used the steady state evoked potential (SSVEP) power at the social stimuli frequency relative to the baseline frequency as a measure of neural activation in response to faces. We recruited 37 typical participants who were asked to complete 3 questionnaire measures (Autism Quotient, Empathy Quotient and Systemising Quotient) associated with behavioural phenotypes in ASC as well as a behavioural measure of emotional recognition, the Reading the Mind in the Eyes Task (RMET).
Results Bayesian correlations between SSVEP response and the 3 questionnaire measures were not indicative of evidence of an association in either hemisphere. However, neither did the results provide strong evidence that scores on the questionnaires were truly independent from neural responses to faces. There was good evidence to suggest a true correlation between scores on the RMET and activation in the left fusiform gyrus.
DiscussionThe results reported here are the first to find an association between a behavioural measure and neural responses to faces using a FSVP paradigm. This approach has several advantages, particularly for working with a clinical population: It is a passive task, it only takes a few minutes to complete and the SSVEP provides a high signal-to-noise ratio. These results suggest a potential overlapping neural mechanism involved in both emotional processing and automatic face categorization. The implications of these results are discussed with reference to their potential application in ASC research.

Disclosures: O.E. Parsons: None. R. Bethlehem: None. J. Freyberg: None. O. Sluijters: None. B. Stonier: None. S. Baron-Cohen: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.04/E3

Topic: A.07. Developmental Disorders

Support: Department of Defense (AR140105)

State of Arizona (Arizona Alzheimer's Consortium)

Dr. Baxter is supported in part by the Arizona Alzheimer's Disease Core Center (NIA P30 AG19610-03).

Title: Cognitive and brain aging in autism spectrum disorder: executive functioning and frontal and temporal lobe differences

Authors: *B. B. BRADEN[1], C. J. SMITH[2], A. THOMPSON[1], T. K. GLASPY[1], E. WOOD[1], D. VATSA[1], L. C. BAXTER[1];
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Southwest Autism Res. & Resource Ctr., Phoenix, AZ

Abstract: Introduction: The effects of aging in adults with autism spectrum disorder (ASD) are understudied, but of increasing importance in order to anticipate unique needs of this growing group of individuals. Executive functioning is particularly vulnerable in both high-functioning ASD and in normal aging. The current study combined neuroimaging and cognitive tasks to examine the relationship between cognition and brain measures in a late middle-aged ASD cohort and age-matched typically developing (TD) men. **Methods:** We evaluated 16 ASD and 17 age-matched TD individuals from ages 40 to 64. Participants completed a battery of neuropsychological tests and a functional MRI task assessing working memory (N-Back Task). Functionally connected network activity during the N-Back Task was assessed via independent component analysis. Structural connectivity was assessed via diffusion tensor imaging (fractional isotropy; FA) and gray matter volumes were assessed via T1-weighted images. **Results:** The participants were well matched according to age, IQ (83-131), and education (9-20 years). The ASD participants made more errors on an executive function task (Wisconsin Card Sorting Test; WCST) but performed similar to TD on tests of verbal learning and memory (Rey Auditory Verbal Learning Test), vocabulary (Wechsler Adult Intelligence Scale-III), and visual search (Group Embedded Figures Task). Functional connectivity analysis of the N-Back task showed decreased engagement of a network including the left inferior frontal lobe, bilateral hippocampi, amygdala, and striatum, and the thalamus in ASD participants compared to TD. Structurally, the ASD group had decreased white matter integrity (FA) bilaterally in the fimbria of the hippocampus and the genu of the corpus callosum (CC) and smaller bilateral hippocampi and right amygdala volumes. Furthermore, decreased CC integrity predicted increased executive function errors on the WCST in the ASD group. **Conclusion:** Results showed that older adults with ASD had weaknesses in executive functioning, less engagement of a working memory neural network, decreased fronto-temporal white matter integrity, and smaller limbic structures including the hippocampus. Reduced executive function performance correlated with differences in structural connectivity of the frontal lobe. The present cognitive and brain differences in older adults with ASD are concerning and overlap with changes that also occur during degenerative aging processes.

Disclosures: B.B. Braden: None. C.J. Smith: None. A. Thompson: None. T.K. Glaspy: None. E. Wood: None. D. Vatsa: None. L.C. Baxter: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.05/E4

Topic: A.07. Developmental Disorders

Support: State of Arizona (Arizona Alzheimer's Consortium)

Department of Defense (AR140105)

Dr. Baxter is supported in part by the Arizona Alzheimer's Disease Core Center (NIA P30 AG19610-03).

Title: Cerebellar differences associated with fine motor dysfunction in aging autism cohort

Authors: *B. R. DEATHERAGE[1], B. BRADEN[1], C. J. SMITH[2], M. K. MCBEATH[3], T. K. GLASPY[1], L. C. BAXTER[1];
[1]Neuroimaging, Barrow Neurolog. Inst., Phoenix, AZ; [2]Southwest Autism Resource & Res. Ctr., Phoenix, AZ; [3]Psychology, Arizona State Univ., Tempe, AZ

Abstract: Background: Gait disturbance, clumsiness, and other mild movement problems are often observed in autism spectrum disorder (ASD; Rinehart *et al.,* 2006). This study focused on brain changes that may indicate the neural basis for these motor symptoms that are common although not ubiquitous among ASD individuals. As the brain ages, these ASD-related symptoms may be exacerbated. Using magnetic resonance imaging (MRI), we examined cerebellar volumes and white matter integrity in a cross-sectional study comparing middle-aged adults with ASD and age-matched typically developing (TD) controls (n=33). We hypothesized that older adults with ASD would exhibit smaller cerebellar volumes along with decreased white matter integrity that would be related to fine motor dysfunction, as compared to their TD counterparts.
Methods: All images were collected using a Phillips 3T scanner. 3D T1 and diffusion tensor images were obtained to measure gray and white matter volume and white matter integrity. Freesurfer, a volumetric measurement software, was used to determine group cerebellar volume differences. In order to determine white-matter integrity with automated segmentation, we used Voxel-Based Morphometry. A finger oscillation (Finger Tapping) test was administered to determine if cerebellar differences predict fine motor performance.
Results: 16 ASD and 17 TD participants were matched according to age and similar for IQ and level of education. Smaller white matter volume and reduced integrity was found in the ASD group within the bilateral cortico-ponto-cerebellar white matter tracts as compared to TD. ASD individuals' finger tapping speed exhibited a trend of being slower compared to TDs. Cerebellar white matter predicted finger tap scores in the ASD participants. There were no differences

between ASD and TD participants for cerebellar cortical volume (gray matter).
Conclusion: A measure of cerebellar white matter correlated with reduced fine motor function in ASD subjects. Cerebellar atrophy, specifically in the white matter, may account for the prominence of fine motor dysfunction in older adults with ASD. Previous studies have found anatomical differences in younger ASD subjects (Courchesne *et al.,* 2011). This cross-sectional study extends findings to aging adults, with novel results that correlate cerebellar white matter with fine motor speed, suggesting cerebellar changes, especially in the white matter, are related to decreased motor functioning. Longitudinal assessments (every two years) are planned to determine whether older individuals with ASD show exacerbation in atrophy and fine motor change over time.

Disclosures: B.R. Deatherage: None. B. Braden: None. C.J. Smith: None. M.K. McBeath: None. T.K. Glaspy: None. L.C. Baxter: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.06/E5

Topic: A.07. Developmental Disorders

Support: NIH 1R01 MH089626-01

UCDMC MIND Institue Autism Phenome Project Funds

Swiss National Science Foundation Grant 2P2LAP_164911

Title: Electrophysiological response to sounds of different loudness in children on the autistic spectrum and typically developing children: an ERP study.

Authors: *R. DE MEO[1,2], T. G. FISHER[3,4], Y. TAKARAE[6], S. M. RIVERA[1,7,5], C. D. SARON[1,7];
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Abstract: Atypical responses to the sensory environment have long been known as a common feature of the Autism Spectrum Disorder ("ASD") phenotype and are part of DSM-5 criteria. For example, individuals with ASD judged auditory stimuli as uncomfortable at lower intensities as

compared to typically developing (TD) controls. The present study, part of a larger multi-disciplinary project to identify autism subphenotypes (The Autism Phenome Project) recorded 61-channel EEG auditory event-related potentials (ERPs) elicited by 50ms complex tones randomly presented at 50, 60, 70 or 80 dB to TD toddlers (n=24) and those diagnosed with ASD (n=36). The ASD diagnosis was confirmed based on ADOS, ADI-R, and expert clinical opinions using DSM-IV criteria. All children (age 2.5-4 years) were judged to have clinically normal hearing. Participants passively listen to ~1000 stimuli presented with a random inter-stimulus interval of 1 to 2 s while watching a quiet video of their choice. Group-averaged ERPs were computed at each intensity based on individual brain responses. Global Field Power (GFP), an estimate of electrocortical response strength, was calculated for the four intensities over the 600 ms post-stimulus epoch and compared with a 2 x 4 ANOVA with group as a between subjects factor and loudness a within subjects factor at each time point. Effects were considered significant if they remained at $p < .05$ for at least consecutive 11 time-points (here, 11 ms). Two time-windows showed a main effect of group (185-240ms and 530-555ms post-stimulus onset) with ASD children eliciting a higher averaged response in both time-windows. A main effect of loudness was found in two time-windows (60-110 ms and 136-542 ms post-stimulus onset) with 50 dB < 60 dB < 70 dB < 80 dB in both time-windows. A Group x Loudness interaction was found in two time-windows (45-60 ms and 240-315 ms post-stimulus). Over the 45-60 ms period, the TD group showed a linear increase of response strength with increased intensity, whereas children with ASD showed differences in response strength between soft (50 and 60dB) vs. loud (70 and 80dB) sounds. Over the 240-315 ms period, TD children again showed different response intensities depending on loudness whereas children with ASD showed similar response strength for all intensities. These data suggest that unusual responses to auditory stimuli shown in ASD children may result from atypical cortical processing at both early (< 150 ms) and late (> 150 ms) latencies. Next steps include exploration of the heterogeneity of these response differences within both groups in relation to demonstrated phenotypic differences.

Disclosures: R. De Meo: None. T.G. Fisher: None. Y. Takarae: None. S.M. Rivera: None. C.D. Saron: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.07/E6

Topic: A.07. Developmental Disorders

Support: NIH Grant R01-MH081023

NIH Grant K01-MH097972

Title: Many (but not all) DTI findings for ASD disappear with stringent motion matching

Authors: *S. K. SOLDERS[1], R. A. CARPER[2], R.-A. MÜLLER[2];
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Abstract: Background: Common findings in diffusion tensor imaging (DTI) studies of children and adolescents with autism spectrum disorder (ASD) include reduced fractional anisotropy (FA), and increased mean diffusivity (MD) and radial diffusivity (RD) of white matter tracts. But, recent findings suggest that group differences in head motion can produce similar effects (Koldewyn et al., 2014; Yendiki et al., 2013). Since few ASD studies control for such differences, previous results must be considered with caution. **Objective:** Determine whether group level motion matching alters DTI findings when comparing ASD and typically developing (TD) groups. **Method:** DTI was gathered from 57 ASD and 50 TD participants (7-18yrs). We compared 3 subsets at increasing levels of motion matching stringency: [A] full set; [B] after excluding scans with moderate image noise, slice-wise signal dropout, or shifts of head placement as identified visually; [C] highest stringency, after quantitative assessment to match groups on average inter-volume translation, rotation, proportion of slices with signal dropout, and signal dropout severity (Yendiki et al., 2013). At each stringency level, groups were compared on FA, MD, RD, and AD (axial diffusivity) using two analytic approaches: Tract-Based Spatial Statistics (TBSS) and probabilistic tractography (FSL) of bilateral association and commissural tracts. **Results:** TBSS: In set A, FA was significantly reduced in ASD compared to TD throughout much of the right hemisphere. In set B, this effect remained, but was less extensive. In the most stringent set C, no significant differences were found for FA, but effect sizes for some clusters (e.g., in right inferior fronto-occipital fasciculus [rIFOF]) *increased* with quality control and matching stringency. No significant group differences for MD, AD, or RD were seen in any set. Tractography: In set A, the ASD group had significantly reduced FA in bilateral inferior longitudinal fasciculi (bILF). In set B, this effect disappeared, but the ASD group had higher RD in bILF than the TD group. This effect remained in set C, where ASD participants also had higher MD than TD participants. **Conclusion:** Consistent with Koldewyn et al. (2014), our findings suggest that many previously reported DTI findings for ASD may have been artifacts of group differences in head motion. However, findings of effect sizes that increased with motion matching indicate that tract-specific anomalies (e.g., in bILF and rIFOF) probably exist in ASD. Overall, our results highlight the need for careful quality control and motion matching in comparisons between clinical and TD groups.

Disclosures: S.K. Solders: None. R.A. Carper: None. R. Müller: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.08/E7

Topic: A.07. Developmental Disorders

Support: Perelman School of Medicine's Institute for Translational Medicine and Therapeutics' (ITMAT) Transdisciplinary Program in Translational Medicine and Therapeutics (maturational human biology pilot grant program)

NIH (R01DC008871-TR)

National Center For Research Resources UL1RR024134

the Nancy Lurie Marks Family Foundation (NLMFF-TR)

pre-doctoral fellowship from the Autism Science Foundation (ASF- RGP)

IDDRC grant to CHOP (U54 HD08694)

Oberkircher Family Chair in Pediatric Radiology at CHOP

Title: The relationship of cortical GABA to corresponding gamma-band activity in autism: clinical and preclinical studies

Authors: *R. G. PORT[1], C. GAJEWSKI[2], S. J. SIEGEL[3], T. P. L. ROBERTS[4];
[1]Neurosci. Grad. Group, [3]Dept. of Psychiatry, [2]Univ. Of Pennsylvania, Philadelphia, PA; [4]Dept. of Radiology, Lurie Family Foundations MEG Imaging Ctr., Children's Hospital of Philadelphia, PA

Abstract: While there has been great progress researching Autism Spectrum Disorder (ASD), there is currently "no smoking gun" with regards to it's pathogenic origin. Increasingly it appears that multiple apparently separate, though ultimately converging, aberrant mechanisms lead to the constellation of behavioral phenotypes defining ASD. A leading hypothesis for one biological basis of ASD is neuronal imbalance in excitatory and inhibitory systems (E/I imbalance). A large body of research supports such imbalance, which span from bench to bedside (e.g. from ex-vivo immunohistochemistry to clinical neuroimaging-based spectroscopy). Additionally, the functional outcome of such E/I imbalance has been suggested by recent studies that observed significant coupling between regional GABAergic tone and local circuit electrophysiological functioning in typically developing (TD) adults. Such a relationship has not been demonstrated in the auditory system or in children, and it's relevance to ASD is undetermined. First, a clinical study (involving both MEGA-PRESS H^1 edited spectroscopy and magnetoencephalography) of

57 individuals (ranging from 6-42yrs old), suggested that while TD individuals demonstrate an association between Superior Temporal Gyrus GABA+/Cr and auditory-related cortical gamma-band coherence in childhood, individuals with ASD do not. Such improper development may result in the reduced auditory gamma-band coherence observed in the sub-population of adult with ASD. Second, a translational study tried to establish if such E/I imbalance was recapitulated in a murine mode relevant to ASD. Mice heterozygous for *Pcdh10* (and their WT littermates) underwent in-vivo electroencephalography as well as ex-vivo neurochemical measurement (using high performance liquid chromatography). The *Pcdh10* heterozygous mice demonstrated a specific reduction in gamma-band auditory steady state responses versus than WT littermates. Moreover, WT mice demonstrated a positive association of gamma-band activity and gamma-band coherence, which *Pcdh10* heterozygous mice failed to exhibit.

Disclosures: R.G. Port: None. **C. Gajewski:** None. **S.J. Siegel:** 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; SJ Siegel reports grant support from Astellas, and Merck. F. Consulting Fees (e.g., advisory boards); SJ Siegel reports consulting payments from Astellas, and Zynerba. **T.P.L. Roberts:** F. Consulting Fees (e.g., advisory boards); Dr. Roberts discloses consulting arrangements with Prism Clinical Imaging, Siemens Medical Solutions, Elekta Oy, Guerbet and Johnson and Johnson (Janssen division).

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.09/E8

Topic: A.07. Developmental Disorders

Support: NIMH K-23

NIH P50 HD055784

UCLA Davis Geffen School of Medicine Dean's Office support

Title: Alterations in resting state electroencephalogram in young children with autism spectrum disorder as a result of behavioral interventions

Authors: *X. A. TRAN[1], S. S. JESTE[2];
[2]Univ. of California, Los Angeles: Ctr. for Autism Res. and Treatment, [1]UCLA, Los Angeles, CA

Abstract: In typically developing (TD) young children, gamma power measured at baseline correlates with later language ability (Benasich 2008). In children with autism spectrum disorders (ASD), theta power measured at baseline inversely correlates with nonverbal IQ (Jeste *in press*). We probed if behavioral intervention would cause a change in EEG power at theta and gamma frequencies. We asked if baseline EEG power at theta and gamma frequencies predicts improvement in language ability in children with ASD who underwent behavioral intervention. We hypothesized that higher gamma power and lower theta power measured prior to intervention would predict improvement in verbal IQ (VIQ) in children with ASD after intensive behavioral intervention.

Participants included 44 children with ASD (38.6% female; mean age 61.25 $\pm$ 9.01 months) and 16 TD controls (50% female; mean age 61.51 $\pm$ 9.78). The ASD cohort was recruited from an intensive, 10-week intervention program at UCLA. Baseline EEG was recorded while each subject watched a video of soap bubbles; data were processed per prior protocols (McEvoy 2015). For the ASD group, EEG was recorded prior to intervention (visit 1) and after intervention (visit 2). TD data were collected at same time points without intervention. Relative spectral power for theta (4-7 Hz) and gamma (31- 48 Hz) frequency bands was calculated using Welch's method, in 9 regions of interest (ROIs) across the scalp. We performed repeated measures ANOVA with 2 within-group comparisons (visit, ROI). Post hoc analysis using paired samples t-tests were performed for any significant main effects. We used linear regression to examine the relationship of theta and gamma power with VIQ.

There was a significant main effect of visit for the theta frequency band. Significant main effect of ROI was found at theta and gamma frequencies. In the ASD group, there was significant increase in relative theta power at left frontal, right central and right posterior ROIs. There was no change in relative gamma power between the 2 visits in the ASD cohort. Visit 1 relative theta and gamma power did not significantly predict change in VIQ.

After intervention, children with ASD showed an increase in theta power, which may reflect increased vigilance and attention while at rest. These results may be confounded by the medication taken by some children with ASD at the time of recording. Baseline theta and gamma power were not predictors of VIQ improvement in children with ASD, which conflicts with past findings in TD children. This may be explained by different patterns of theta and gamma activity between ASD and TD cohorts. In future analysis, we will compare baseline differences between ASD and TD children.

Disclosures: X.A. Tran: None. S.S. Jeste: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.10/E9

Topic: A.07. Developmental Disorders

Support: NIH P50-- HD055784

NIH Grant T32MH073526

Title: Resting electroencephalogram signal complexity identifies patterns of atypical brain development

Authors: *J. FROHLICH, S. S. JESTE;
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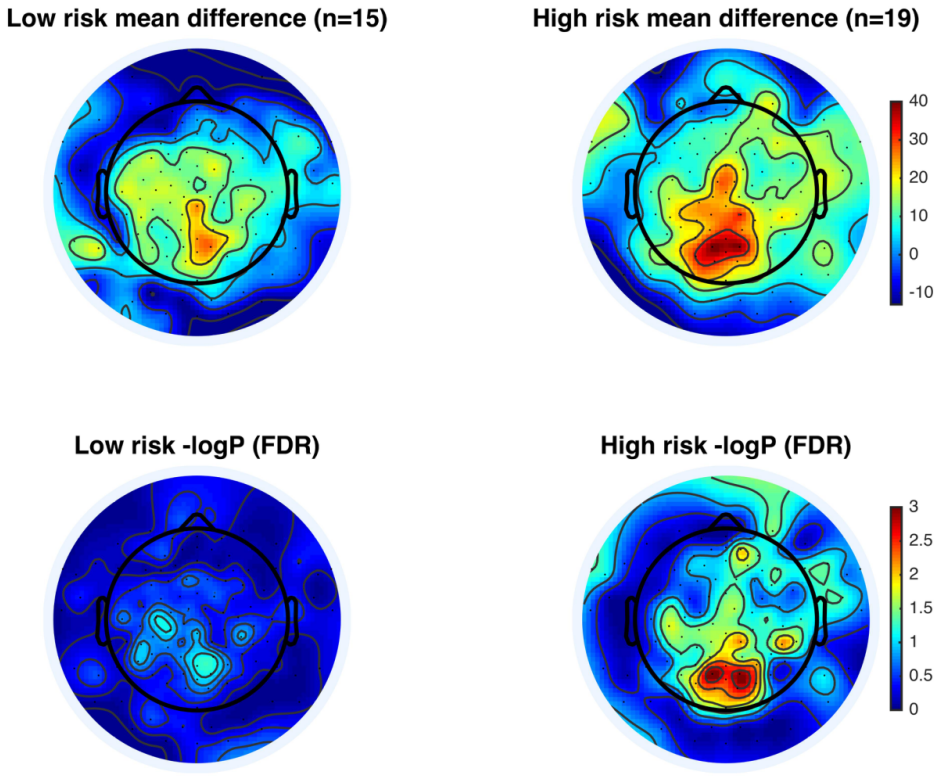
Abstract: Biomarkers of atypical brain development may predict neurodevelopmental disorders such as autism spectrum disorder (ASD) in high-risk infants. Resting electroencephalogram (EEG) signal complexity has previously been used to classify infants 6 to 24 months of age according to ASD risk with high accuracy (Bosl et al, 2011) using multiscale entropy (MSE). However, developmental changes in EEG complexity from age 3 months remain uncharacterized, and it is unknown whether other measures of signal complexity are also useful for identifying neural patterns of ASD risk. Frequency variance (FV) is a measure of complexity that is sensitive to phase reset events and correlates with age in typically developing preschool age children (Frohlich et al, 2015). We examined changes in FV in high-risk infants with ≥ 1 sibling with ASD (n = 19) and low-risk infants with no siblings with ASD (n = 15) using a longitudinal study design at ages 3 and 9 months. Using methods described in Frohlich et al (2015), we calculated FV from beta-gamma (12-48 Hz) filtered resting EEG. Physiological artifacts were removed with independent component analysis (ICA) (Delorme and Makeig, 2004). Paired samples t-tests were performed between distributions of FV values from each of 124 EEG electrodes in high-density geodesic EEG nets (Electrical Geodesics, Inc) with multiple testing corrected with Benjamini-Hochberg False Discovery Rates (FDR). 44 electrodes--mostly occipital--in the high-risk group and no electrodes in the low-risk group showed significant decreases in FV from month 3 to month 9. Low-risk infants showed a trend of decreased FV less pronounced than high-risk infants. No electrodes in either group showed significant increases from month 3 to month 9. Signal complexity decreases in early infancy as a result of increased cortical synchronization. High-risk infants may show accelerated development to compensate for genetic alterations that confer ASD risk. Future work will relate trajectories of signal complexity to clinical outcomes in children at high risk for ASD.



Disclosures: J. Frohlich: None. S.S. Jeste: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.11/E10

Topic: A.07. Developmental Disorders

Support: NIMH (K23)

Autism Speaks (Meixner Postdoctoral Fellowship)

NIH P50 HD055784

Title: EEG measures of high frequency neural activity suggest variation in the balance of neural excitation and inhibition across autism spectrum disorder

Authors: *A. DICKINSON, I. M.REZAZADEH, C. DISTEFANO, S. SPURLING JESTE; UCLA, Los Angeles, CA

Abstract: The balance of neural excitation and inhibition (E/I) is often hypothesized to be disrupted in autism spectrum disorder (ASD). However, there are conflicting reports as to how this balance is altered. For instance, decreased levels of the excitatory neurotransmitter, glutamate (Robertson et al., 2015), and decreased levels of the inhibitory neurotransmitter, GABA (Rojas et al., 2014), have both been reported in ASD. Variability in E/I balance may inform the considerable clinical and behavioral heterogeneity seen in individuals with ASD. Here we investigate whether electroencephalography (EEG) measures of E/I balance, and their variability, may be used to discriminate clinically relevant sub-groups in a large sample of children with ASD.

Previous research has suggested that high frequency neural activity in both the beta (13-30Hz) and gamma (>30Hz) range can be used to indirectly study E/I balance. For instance, both higher peak gamma frequency (Edden et al., 2009), and higher peak beta power (Gaetz et al., 2011) have been reported to be associated with higher levels of the inhibitory neurotransmitter, GABA. In a clinically heterogeneous group of children with ASD (N=70) studied in the UCLA Center of Autism Research and Treatment, with IQ ranging from 16 - 132 and ages ranging from 12 months to 12 years, we measured both peak power and frequency in the beta and gamma frequency bands using EEG. High density EEG recordings were obtained during an eyes-open resting-state condition. Due to the inherent difficulty of measuring high frequency neural activity separately from the many non-neural artifacts occurring in the same frequency bands, EEG data were cleaned using independent component analysis. Sources of both beta and gamma activity were successfully identified in the signal following source localization (implemented through DIPFIT). Peak power and frequency in both the beta and gamma bands were extracted. Substantial variability was evident in all four metrics across the sample which will facilitate the use of methods such as the similarity network fusion algorithm and graph theory to construct networks of participants based on the EEG data. This will provide a comprehensive approach to explore physiologically meaningful sub-groups based on these metrics.

This work highlights that the contrasting reports of E/I balance in the ASD literature may be due to a focus on identifying a universal E/I imbalance at a group level. We put forward the suggestion that identifying heterogeneity through assays of E/I balance is crucial, as differences in E/I balance could potentially be used a biomarker to stratify individuals with ASD into clinically relevant subgroups.

Disclosures: A. Dickinson: None. I. M.Rezazadeh: None. C. DiStefano: None. S. Spurling Jeste: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.12/E11

Topic: A.07. Developmental Disorders

Support: NIH P50 HD055784

Title: Electrophysiological signatures of visual statistical learning in three-month old infants at risk for autism spectrum disorder

Authors: *A. T. MARIN[1], T. HUTMAN[1], M. DAPRETTO[1], C. PONTING[1], S. JOHNSON[2], S. JESTE[1];
[1]Semel Inst. for Neurosci. and Human Behavior, [2]Psychology, UCLA, Los Angeles, CA

Abstract: Visual statistical learning (VSL) refers to the detection of regularities within the visual environment (Bulf et al., 2011), and may be a precursor to later cognition and social communication (Romberg & Saffran, 2010). Studies have demonstrated reduced evidence of VSL in pre-school aged children with Autism Spectrum Disorder (ASD) using a novel electrophysiological (EEG) paradigm (Jeste et al., 2015). No studies have examined VSL in infants at risk for ASD.
We asked whether EEG signatures of VSL could be quantified at 3-months of age, and whether VSL distinguished high- and low-risk (HR, LR) infants (HR infants have an older sibling with ASD), and whether there was an association between VSL at 3-months and later cognitive function.
3-month-old infants (n=25, HR: n=12, LR: n=13) were exposed to a continuous stream of shapes based on a modified version of the Kirkham et al. (2002) VSL task. High density EEG was recorded and the event-related potential (ERP) of interest was the frontal Positive Slow Wave (PSW). A general linear model evaluated within-subject effects of region and condition and between-subject effects of group with respect to PSW mean amplitude. Learning was operationalized as differentiation between conditions. Whole group correlations between ERP markers of learning and cognitive skills at 6-months were also performed.
There was a significant group by condition interaction and a significant main effect of region. Post-hoc tests revealed greater mean amplitude within the middle region as compared to the right and left. Post-hoc tests revealed that HR infants significantly differentiated conditions, while LR infants did not. The absolute value PSW difference amplitudes correlated with 6-month visual reception and fine motor scores.
EEG correlates of VSL seem to differentiate HR from LR infants as early as 3-months of age, with HR infants displaying evidence of VSL. The relationship between VSL and non-verbal cognitive skill suggests that pattern learning is domain specific. Group differences in VSL

suggest that infants at risk for ASD may demonstrate strengths in processing visual patterns—a strength that may occur at the expense of later social communication skills.

Disclosures: A.T. Marin: None. T. Hutman: None. M. Dapretto: None. C. Ponting: None. S. Johnson: None. S. Jeste: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.13/E12

Topic: A.07. Developmental Disorders

Support: Dup15q Alliance Grant 20144605

NIH Grant U54HD087101-01

Title: Stability of beta power in resting state EEG of children with Duplication 15q syndrome

Authors: *S. HUBERTY, J. FROHLICH, S. JESTE;
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Abstract: Duplication 15q11.2-q13.1 syndrome (Dup15q) is a rare genetic disorder featuring copy number variation of several genes on the q arm of chromosome 15. This region contains genes critical to brain development, including three GABAA receptor genes and ubiquitin ligase E3A (Ube3a). The disorder is characterized by global delay, hypotonia, intellectual disability (Battaglia, Parrini, & Tancredi, 2010), and an increased risk for epilepsy (Conant et al., 2014). Autism Spectrum Disorder (ASD) is highly comorbid with Dup15q syndrome, however children with Dup15q syndrome have more impaired motor skills and adaptive behavior compared with children with nonsyndromic ASD (Distefano et al., 2016). Recent cross sectional analyses in our lab indicate that children with Dup15q syndrome (*n=11)* show increased resting state beta1 (12 – 20 Hz) and beta2 (20 – 30Hz) power compared with an age and IQ matched nonsyndromic ASD cohort and an age matched typically developing cohort. However, given that beta power can be modulated by the cognitive and psychological state of the child, we asked whether this biomarker was stable across time. Stability of this signal would support the contention that this measure relates to underlying genetic mechanisms.
We quantified beta power in high spatial density, resting-state EEG recordings from four children with Dup15q syndrome at two visits, ranging 2-4 months apart. Genetic reports confirmed isodicentric duplications of chromosome 15q in all participants, three male (29, 44, and 56 months old), and one female (22 months old). EEGlab was used to bandpass filter the

EEG data from 1-50 Hz and to remove physiological artifacts using independent component analysis (ICA). We computed relative beta1 and beta2 power across 9 scalp regions of interest (ROIs) at each visit. A general linear model evaluated within-subjects effects of region and time on relative beta power.
Resting-state beta1 and beta2 power did not significantly differ between visits 1 and 2 in any of the 9 ROIs. We did not find a significant region by time interaction on beta1 or beta2 power. Beta power in children with Dup15q syndrome appears to be stable across multiple measurements. The stability of beta power over time reinforces the hypothesis it relates to the underlying genetic mechanisms of Dup15q syndrome and may be a biomarker for the syndrome. Over the next six months, we will continue to collect resting-state EEG at multiple time points on a larger cohort of children with Dup15q syndrome to confirm the stability of this biomarker.

Disclosures: S. Huberty: None. J. Frohlich: None. S. Jeste: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.14/E13

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH100186

Harvard Catalyst | The Harvard Clinical and Translational Science Center (NCRR and the NCATS NIH) Grant UL1 RR025758

NSERC Postdoctoral Fellowship 454617

Title: Assessment of cortical plasticity with continuous theta-burst stimulation in adults with autism spectrum disorders and neurotypical controls

Authors: *A. JANNATI[1], G. BLOCK[1], L. M. OBERMAN[2], A. ROTENBERG[3], A. PASCUAL-LEONE[1];
[1]BIDMC, Harvard Med. Sch., Boston, MA; [2]E.P. Bradley Hospital, Warren Alpert Med. School, Brown Univ., East Providence, RI; [3]Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Objective: To evaluate the modulation of corticospinal excitability with continuous theta-burst stimulation (cTBS) as a possible physiologic phenotype for autism spectrum disorders (ASD).
Methods: Twenty-one neurotypical controls (NT; age 39.8 ± 12.3, 19 males, all right-handed) and 12 adults with ASD (age 36.9 ± 15.2, 10 males, 10 right-handed) underwent a cTBS protocol

consisting of bursts of three pulses of 50Hz stimulation at 80% of individual active motor threshold, repeated at 200ms intervals for 40 seconds (for a total of 600 pulses). Corticospinal excitability was assessed before and after cTBS by sets of 30 single pulses of neuronavigated transcranial magnetic stimulation (TMS) applied to the left primary motor cortex at 120% of individual resting motor threshold. TMS procedures were performed with a MagPro X100 stimulator, an MC-B70 Butterfly Coil (outer diameter 97mm), and Brainsight TMS navigation system, using a brain MRI template.

Peak-to-peak amplitudes of motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle with a PowerLab 4/25T data acquisition device and measured with LabChart 8 software. Percentage change in MEP amplitudes relative to baseline (%Δ) 5 to 60 minutes after cTBS (T5–T60) were calculated for ASD and NT groups. The change in MEP amplitude induced by cTBS is a metric of the mechanisms of cortical plasticity.

Results: Complete-linkage cluster analyses revealed two subpopulations within each group with distinct responses to cTBS. Within the NT group, 13 exhibited an inhibitory response to cTBS, while 8 showed a paradoxical excitatory response. Within the ASD group, 9 and 3 participants exhibited either inhibitory or facilitatory responses, respectively.

Comparing the ASD and NT subgroups with inhibitory responses revealed a maximal difference in MEP modulation at T40 that was statistically significant ($p = .036$). The numerical difference between average ASD and NT facilitatory responses was also the largest at T40 and showed a trend toward larger facilitation in the ASD subgroup ($p = .097$).

Within 21 participants who showed inhibitory responses across both groups, a logistic regression analysis of Group (ASD vs. NT) found a significant model [likelihood-ratio χ^2 (2) = 6.58, $p = .037$] with %Δ at T10 ($p = .087$) and %Δ at T40 ($p = .058$) as predictors.

Conclusions: Our results provide further insight into interindividual differences in the effects of cTBS on the primary motor cortex, indicate the existence of subpopulations with distinct patterns of response to cTBS within both ASD and NT groups, and support the utility of cortical plasticity measures by cTBS as a physiologic ASD biomarker.

Disclosures: **A. Jannati:** A. Employment/Salary (full or part-time): Natural Sciences and Engineering Council of Canada (NSERC) Postdoctoral Fellowship. **G. Block:** None. **L.M. Oberman:** None. **A. Rotenberg:** None. **A. Pascual-Leone:** F. Consulting Fees (e.g., advisory boards); Neosync - Member of Scientific Advisory Board, Starlab - Member of Scientific Board, Neuroelectrics - Member of Scientific Board, Neuronix - Member of Medical and Scientific Advisory Board, Nexstim - Advisory Board Member, Magstim - Advisory Board Member, Axilum Robotics - Advisory Board Member.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.15/E14

Topic: A.07. Developmental Disorders

Support: KAKENHI (C; No. 15K09617)

Title: Interneuron dysfunction in syndromic autism

Authors: *T. TAKANO;
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Abstract: Purpose: Autism is an extremely heterogeneous disorder, but its frequent co-occurrence with epilepsy leads to the speculation that there may be common mechanisms associated with these disorders. Inhibitory interneurons are considered to be the main cellular elements that control hyperexcitability in the brain, and interneuron dysfunction can cause pathological hyperexcitability linked to seizure susceptibility or epilepsy. Methods: This study presents some of the recent advances that support the relationship between interneuron dysfunction and cognitive impairment in human syndromic autism with particular reference to the pathophysiological findings of murine experimental models of autism.Results: Alterations to GABAergic circuits include a wide variety of neurobiological dysfunction and do not simply involve the loss or gain of any given type of inhibitory mechanism. The characteristics of interneuron dysfunction in each murine model of autism differ among each syndrome, and these diversities may be due to differences in the genetic backgrounds or some other currently unknown variances.Conclusion: Future studies should bring us a greater understanding of the involvement of different classes of GABAergic interneurons and allow us to define the relationship between the precise pathophysiological mechanisms and the corresponding clinical phenotypes in autism.

Disclosures: T. Takano: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.16/E15

Topic: A.07. Developmental Disorders

Support: NIH R21 Grant MH100387

NIH R21 Grant HD083629

The Mosbacher Family Fund for Autism Research, and the Child Health Research Institute

Title: Identifying novel biomarkers of social deficits in children with autism spectrum disorder

Authors: *O. OZTAN, L. P. JACKSON, R. A. LIBOVE, R. D. SUMIYOSHI, J. M. PHILLIPS, A. E. URBAN, J. P. GARNER, A. Y. HARDAN, K. J. PARKER;
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Abstract: Autism spectrum disorder (ASD) is characterized by core social impairments, and is among the most devastating disorders of childhood, affecting 1 in 68 US children. Early and accurate diagnoses are difficult and there are no medications that effectively treat the social features of ASD. ASD is currently diagnosed using behavioral criteria because no biomarkers have been identified. Biomarkers improve our ability to make accurate diagnoses and provide biological targets for drug development and testing. Two promising biomarkers of ASD are the closely related neuropeptides oxytocin (OXT) and arginine vasopressin (AVP), which are critical for normal social functioning in animals and people. Experimentally-induced impairments in OXT and AVP neuropeptides and their receptors also produce social deficits in animal models with relevance to ASD. Our team is currently conducting OXT and AVP treatment trials in children with ASD. Treatment efficacy may be due to a patient's blood neuropeptide levels prior to treatment and the availability of OXT receptor (OXTR) and/or AVP_{v1a} receptor ($AVPR_{v1a}$) on which the drugs can effectively act to enhance social functioning. Despite the obvious need, no prior research has tested whether variation in expression of the OXTR and/or $AVPR_{v1a}$ genes has diagnostic and/or treatment implications in ASD. The aims of the present project therefore were: 1) to test whether expression of the OXTR and $AVPR_{v1a}$ genes differ between children with ASD and matched typically developing control children; and 2) to test whether OXTR and/or $AVPR_{v1a}$ gene expression predicts social functioning and disease symptoms or severity. Preliminary data showed that OXTR gene expression levels were lower in children with ASD compared to matched typically developing control children. Moreover, OXTR gene expression strongly predicted risk for ASD diagnosis and impaired social functioning in children with ASD. Findings

from this overarching research program may lead to the development of companion diagnostics and the first effective and personalized therapeutics to treat the social impairments of ASD.

Disclosures: O. Oztan: None. L.P. Jackson: None. R.A. Libove: None. R.D. Sumiyoshi: None. J.M. Phillips: None. A.E. Urban: None. J.P. Garner: None. A.Y. Hardan: None. K.J. Parker: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.17/E16

Topic: A.07. Developmental Disorders

Support: NSERC I3T Graduate Student Stipend

QE II Graduate Student Scholarship

Title: Limited somatosensory functional connectivity differences in youth with autism spectrum disorder

Authors: *B. CECHMANEK[1], H. JOHNSTON[2], C. LEBEL[1], S. BRAY[1];
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Abstract: A majority of children and adolescents with autism spectrum disorder (ASD) experience tactile sensory sensitivities. These sensory sensitivities can have a negative impact on quality of life, and appear to be more pronounced in younger children and adolescents relative to adults. Functional connectivity differences, measured through resting-state functional MRI (rs-fMRI), may increase our understanding of the brain basis of these sensory sensitivities, and guide interventions or pharmacologic treatments. We examined whether youth with ASD have altered connectivity of somatosensory brain regions and if connectivity varies with age differently in ASD relative to typically developing (TD) controls. rs-fMRI data from 188 participants aged 8-15 years (67 ASD, 121 TD) from 5 sites in the Autism Brain Imaging Data Exchange database were analyzed using functional connectivity measures. Subjects included had >5 minutes of rs-fMRI data with <0.2 mm framewise displacement. TD and ASD groups did not differ in age (ASD=12.7(2.0), TD=12.2(2.3), P=0.11) or IQ (ASD=105.8(17.7), TD=109.5(11.8), P=0.13). Data underwent standard fMRI preprocessing steps, with additional bandpass filtering ($0.01<f<0.1$Hz) and nuisance regression (white matter, cerebrospinal fluid and motion signals). Eight functional-connectivity parcellated regions of interest (ROIs) along the somatosensory

strip were analyzed in a seed-to-voxel approach across the whole brain, as well within *a priori* networks: salience, limbic, sensory and cerebellar. At the second level, general linear models were used to estimate effects of diagnosis, age, and their interaction, while controlling for IQ, handedness, sex, imaging site, and eye status (open/closed). Significant over-connectivity in ASD was observed between a left lateral somatosensory seed and the left superior parietal lobule ($p=0.008$). An age-diagnosis interaction effect survived small volume correction in the salience network, between a right lateral somatosensory ROI and the dorsal anterior cingulate ($p=0.008$), demonstrating stable connectivity with age in TD, and increasing connectivity with age in ASD participants. No significant associations were found with symptom severity (Social Responsiveness Scores, Autism Diagnostic Observation Schedule). Despite previous findings of distributed connectivity differences in ASD, our study does not support robust differences related to the somatosensory network in youth with ASD, compared to TD. However, this study may be limited by a bias for youth with ASD who are high-functioning and able to stay still in the scanner environment.

Disclosures: B. Cechmanek: None. H. Johnston: None. C. Lebel: None. S. Bray: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.18/E17

Topic: A.07. Developmental Disorders

Support: SickKids New Investigator Research Grant

Title: Typical emotional processing of special interest stimuli in adolescents with autism spectrum disorder (ASD)

Authors: *K. B. RIVARD[1], F. BURLES[1], M. SCHUETZE[1], I. CHO[1], S. VINETTE[2], F. CORTESE[1], A. PROTZNER[1], S. BRAY[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]Med., Univ. of Toronto Mississauga, Mississauga, ON, Canada

Abstract: It has been suggested that the core symptoms of ASD, impaired social communication and restricted, repetitive patterns of interest or behaviors, may be related to atypical engagement of the brain's reward system (e.g., social motivation hypothesis). We investigated whether visual reinforcers, images depicting restricted interests, are processed abnormally in ASD, as a potential marker for reward system dysfunction. We collected electroencephalography (EEG) while 20 individuals with ASD (4 female) and 20 typically developing (TD) control participants (4

female), aged 14-20, performed a visual target detection task. Non-verbal IQ was not significantly different between groups (ASD mean = 104.9, range = 63-115; TD mean = 110.3, range = 86-150). The Autism Diagnostic Observation Schedule (ADOS-2) was administered to confirm ASD diagnosis. Each participant was presented with a set of images customized to include things that they liked and disliked (High- and Low-value images), as well as Neutral images from the International Affective Picture System (IAPS), Gabor patch stimuli and Target images, a picture of a zebra, to which they responded with a button press. In total there were 200 High-value, 200 Low-value, 200 Neutral, 200 Gabor and 100 Target image trials, with each image centrally presented for 500ms. Target detection accuracy and reaction time did not differ between groups. We evaluated differences in the late positive potential (LPP; 400-700ms post-stimulus), an event-related potential related to emotional processing, between valence conditions and diagnostic groups for High-, Low- and Neutral-valued images. Average amplitude between 400 and 700ms were calculated for central (Cz) and frontal (Fz) midline electrode sites. Repeated measures ANOVAs showed a significant effect of condition (High, Low and Neutral) for both Cz ($p=0.007$) and Fz ($p=0.05$). This effect was driven by a larger LPP during the High- and Low-value, relative to Neutral conditions. We did not, however, find a significant main effect of, or interaction with, diagnostic group. Our finding that TD and ASD individuals show a similar modulation of the LPP in response to High- and Low-interest visual stimuli suggests that distributed emotional processing of these stimuli is relatively intact in ASD. This further suggests that atypical affective responses may be specific to task context and type of stimuli, which could be explored in future studies. Understanding dysfunction of the reward system in ASD is critical as behavioral treatment approaches typically rely on reinforcement learning.

Disclosures: K.B. Rivard: None. F. Burles: None. M. Schuetze: None. I. Cho: None. S. Vinette: None. F. Cortese: None. A. Protzner: None. S. Bray: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.19/E18

Topic: A.07. Developmental Disorders

Support: NIH Grant MH096773

NIH Grant MH086654

Title: Characterizing heterogeneity in autism spectrum disorders using a random forest algorithm

Authors: *E. J. FECZKO[1], N. BALBA[1], O. MIRANDA-DOMINGUEZ[1], M. CORDOVA[1], L. IRWIN[1], A. P. HILL[2], B. H. LANGHORST[1], J. GRIESER PAINTER[1], E. J. FOMBONNE[2,3], J. L. NIGG[1,3,4], D. A. FAIR[1,3,4];
[1]Behavioral Neurosci., [2]Pediatrics, [3]Psychiatry, [4]Advanced Imaging Res. Ctr., Oregon Hlth. Sci. Univ., Portland, OR

Abstract: Autism spectrum disorders (ASD) are characterized by deficits in social communication and restricted interests. Understanding the biological etiology of autism is critical for developing better diagnostic and treatment tools. Recently, studies using machine classification algorithms suggest that biological markers exist in this disorder and can be identified via magnetic resonance imaging (MRI). However, these models do not generalize to independent datasets, possibly because multiple etiologies may exist, i.e, distinct subgroups of individuals with ASD may have different underlying mechanisms. Classifiers, trained on behavioral data to identify discrete subpopulations in ASD, may assist in moving the field forward with this regard. In our study, we developed a random forest (RF) model to classify ASD (n = 68) from control (n = 65) subjects using accuracy and median reaction time (RT) measures from face identity, face affect, and vocal affect recognition tasks. RFs comprise many decision trees, where each tree divides groups of subjects into discrete subgroups using input variables. The RF was validated by measuring the classification accuracy of a random sample of 6 ASD participants and 6 controls removed from the training data. 1000 classifiers were trained to assess confidence intervals for accuracy. When compared to a null model, we found that the RF model was 71.6 percent accurate ($t(1998) = 38.7$, $p < 0.01$) with 73.3 ($t(1998) = 30.59$, $p < 0.01$) specificity and 69.9 ($t(1998) = 21.5$, $p < 0.01$) sensitivity. From the classifier, we can measure heterogeneity by measuring the proximity of each individual to another: the number of times two individuals end up in the same subgroup across all trees. Using a community detection algorithm on these proximity measures, we identified two subgroups of individuals with an ASD diagnosis. Relative to typical subjects, one group had very high performance in the visual face tasks but slow RTs, while the other group had worse performance in the visual face tasks but similar RTs. We examined whether the two subgroups showed differences within functional systems using resting state functional connectivity MRI. Chi-squared tests found a significantly greater number of between group differences ($p < 0.01$) within the cingulo-opercular, default, visual, and ventral attention systems. This finding hints at a potential approach to characterize heterogeneity in ASD, and under these conditions two subgroups of ASD children can be identified via performance on behavioral tasks. Importantly, these subgroups show differences in functional connectivity within multiple systems, highlighting potentially unique underlying etiologies.

Disclosures: E.J. Feczko: None. N. Balba: None. O. Miranda-Dominguez: None. M. Cordova: None. L. Irwin: None. A.P. Hill: None. B.H. Langhorst: None. J. Grieser Painter: None. E.J. Fombonne: None. J.L. Nigg: None. D.A. Fair: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.20/E19

Topic: A.07. Developmental Disorders

Support: K01MH087720

Title: Atypical laterality in visual sensory activation during simple sensorimotor tasks in Autism Spectrum Disorders

Authors: *Y. TAKARAE[1], F. VIEIRA[2], W. SONG[1], C. SARON[3];
[1]Univ. of Texas Southwestern, Dallas, TX; [2]Univ. of San Francisco, San Francisco, CA; [3]Univ. of California, Davis, Davis, CA

Abstract: Impairments in sensorimotor control are frequently observed in autism spectrum disorders (ASD). We investigated neural correlates of sensorimotor dysfunction in ASD using 124-channel ERPs. The participants were 12 to 18 year old children,18 with ASD and 17 that were typically developing (TD). Participants performed, in alternating blocks, an antisaccade (ANTI) task that required looking away from a suddenly appearing peripheral target, or a prosaccade (PRO) task that required looking toward the peripheral target. The most notable group difference was a lateral activation at occipitoparietal sites starting approximately at 100 ms after stimulus onset. This occipitoparietal positivity reflected the contralateral organization in the visual system, with greater activity evoked by stimuli contralateral to the occipitoparietal electrodes. For the TD group, this activity was symmetric for left and right target conditions. The activation was first observed on the contralateral side, followed by activation on the ipsilateral side with slight delay, following patterns observed for lateral P1 responses in studies of TD adults (Di Russo et al., 2002). In the ASD group, while the general contra to ipsi shift was maintained, the occipitoparietal activation was less symmetric in both tasks, with overall greater activation at the contralateral occipitoparietal location with left targets than with right targets. Further, the ASD group had bilateral occipitoparietal activation without the clear contra to ipsi shift in the left target condition in the PRO task. Thus, the left occipitoparietal activation was reduced when contralateral to the target and enhanced, and of earlier onset, when ipsilateral to the target location. A contralateral frontal negativity preceding the occipitoparietal activation was also reduced in the ASD group compared to the TD group in both ANTI and PRO tasks. The negativity was also asymmetric in the ASD group with greater negativity in the left target than the right target condition. Thus, both occipitoparietal positivity and frontal negativity show asymmetry favoring right hemispheric activation in the ASD group. The findings are consistent with earlier studies demonstrating rightward cerebral asymmetry in ASD (Eyler et al., 2012; Floris et al., 2016). While most of those studies address asymmetry in language and motor

systems that are strongly lateralized, the current study suggest the atypical lateralization may also be observed with visual processes. The current findings additionally suggest this disorganization of early sensory processing may contribute to sensorimotor dysfunction in ASD.

Disclosures: Y. Takarae: None. F. Vieira: None. W. Song: None. C. Saron: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.21/E20

Topic: A.07. Developmental Disorders

Support: MCubed

Title: Altered p50 neuromagnetic evoked responses and neural synchrony related to auditory attention in Autism Spectrum Disorder

Authors: A.-M. FLORES[1], K. MCFARLANE[1], C. SWICK[1], T. ANDERSEN[1], K. RUSINIAK[1], I. KOVELMAN[2], S. BOWYER[3], *R. LAJINESS-O'NEILL[1];
[1]Eastern Michigan Univ., Ypsilanti, MI; [2]Univ. of Michigan, Ann Arbor, MI; [3]Henry Ford Hosp., Detroit, MI

Abstract: Atypical auditory attention (AA) may be evident at the P50 latency in Autism Spectrum Disorder (ASD), affecting higher-order domains like social/language functioning. This study examined relationships between evoked response (ERP), synchrony and AA.
11 ASD (Age: M = 8.8; SD= 0.9) and 10 neurotypical (NT) children (Age: M = 9.4; SD = 1.4) underwent magnetoencephalography at rest and during an oddball paradigm of plausible (S1) and implausible syllables (S2). P50 amplitudes (amp) and latencies were averaged. Gating was calculated as an amplitude difference score (S1-S2). Synchronization was quantified by coherence between cortical sites during resting state. Kendall Tau correlations were used to examine relationships of interest.
No group differences in amp, latency or gating. *ERP*: ASD: longer S2 latencies were related to lower AA (p=.03). NT: better gating was related to increased inhibition (p=.02). *Coherence:* ASD: higher right intrahemispheric occipito-parieto-temporo coherence was related to lower S1 amp (τ=-.56- -.72). Increased interhemispheric temporo-parietal coherence was related to longer S1 latencies (τ=.57-.63). Higher interhemispheric and right intrahemispheric coherence was related to lower S2 amp (τ=-.56- -.83). Lower angular, cuneus and temporal coherence was related to longer S2 latencies (τ=-.54- -.82). Higher right intrahemispheric long range coherence was related to poorer gating (τ=.67-.78). NT: higher intrahemispheric temporo-cingulo coherence

was related to lower S1 amp (τ=.51- .64). Increased temporo-occipito-fronto-cingulo coherence was related to longer S1 latencies (τ=.49-.67). Increased interhemispheric and right intrahemispheric fronto-parieto-occipito coherence was related to longer S2 latencies (τ=-.56- -.72). Decreased right intrahemispheric angular coherence was related to poorer gating (τ=-.60- -.96).
ASD: response to novel language was characterized by delayed responses associated with lower AA, increased coherence between reading-related regions predicting lower amps, and increased right long range connectivity associated with decreased response suppression. Increased connectivity associated with reading disorders was predictive of delayed/lower response to familiar language. *NT*: increased suppression of response to novel language was related to increased inhibition and increased coherence between reading related regions. Increased connectivity associated with reading was related to delayed responses to novel language. Results suggest aberrant connectivity in reading disorders is implicated in neural differences between ASD and NT processing of novel language.

Disclosures: A. Flores: None. K. McFarlane: None. C. Swick: None. T. Andersen: None. K. Rusiniak: None. I. Kovelman: None. S. Bowyer: None. R. Lajiness-o'Neill: None.

Poster

212. Autism: Clinical Studies II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 212.22/E21

Topic: A.07. Developmental Disorders

Title: Atypical hippocampal functional organization with the neocortex and basal ganglia inchildren with autism

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Abstract: Autism spectrum disorder (ASD) is an early onset neurodevelopmental disorder, characterized by stereotyped or repetitive behavior and interests, social and communication deficits. ASD has also been linked to atypical memory processing, especially for the formation of restricted behavior and interests such as certain habits, and flexible remember and use of complex information. As DeLong wrote, “it is impossible to separate the study of memory from that of autism (DeLong,2003, p. 741). Understanding the brain basis of atypical memory processing in children with ASD will allow us to investigate influential neurobehavioral models of ASD and has the potential to provide new insight into the nature and etiology of the disorder.

We used resting state fMRI in conjunction with advanced analytic approaches to investigate functional integration and segregation of memory related brain networks in 45 children (aged 8-12 years old) with ASD and 45 matched neurotypical controls. A graph theory analysis of complex brain networks was implemented to parcellate the hippocampus based on resting state fMRI data. We identified three distinct clusters along the left and right hippocampal long axis, including the anterior, middle and posterior portions in TD children and children with ASD. Each portion shows distinct functional connectivity patterns with other regions on the whole brain level. Most importantly, when compared to TD children, children with ASD showed significantly weaker functional connectivity of the hippocampus with distributed brain regions in the primary visual cortex, the precuneus and thalamus, but significantly higher functional connectivity only with the right anterior caudate on the whole brain. Our study demonstrates that children with ASD show increased functional connectivity of the hippocampal memory system with brain systems important for visual sensory, default mode, and affective functions, but decreased connectivity with basal ganglia system important for the formation of procedural memory such as habits and rigid behavior. These findings provide important implications into the newly emerging view of autism as an early onset disorder of neural connectivity, thereby leading to atypical memory and cognitive development in late life for children with ASD.

Disclosures: S. Qin: None. R. Rehert: None. V. Menon: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.01/E22

Topic: A.07. Developmental Disorders

Support: P01AG14449

R01AG04375

P50AG16573

R01AG21912

R01HD065160

Title: Cortical tau pathology in non-demented and demented individuals with down syndrome

Authors: ***S. E. PEREZ**[1], B. HE[2], M. N. SABBAGH[3], I. T. LOTT[4], E. DORAN[4], E. J. MUFSON[2];

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Abstract: Down syndrome (DS), is a genetic disorder resulting from the triplication of chromosome 21, which carries the gene that encodes the amyloid precursor protein (APP) and is functionally characterized by intellectual disability. Brain tissue from individuals with DS displays an overexpression of APP as well as β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs), pathological hallmark lesions of Alzheimer's disease (AD). Brain amyloid plaques and NFTs appear between thirty and forty years of age and increases overtime in DS. Despite the fact that virtually all individuals with DS display AD lesions, most but not all individuals will develop AD dementia suggesting a disconnection between pathology and dementia related cognitive decline. Interestingly, of the two major AD lesions, NFTs are a better correlate of cognitive decline than amyloid plaques in AD. The main component of NFTs is the microtubule-associated protein tau that undergoes conformational and phosphorylational changes during the evolution of a tangle in the AD brain. Whether similar changes develop in non-demented compared to demented subjects with DS is unknown. Here, we examined the progression of NFT pathology in the frontal cortex in a total of 19 subjects with DS (n=7) and DSAD (n=12), ranging in age from 1-60 years. DS cases were confirmed either by blood karyotyping or in situ fluorescence hybridization for the detection of chromosome 21. Immunohistochemistry was used to detect the early tau phosphorylation (pS422 and AT8), conformational (Alz50 and MC-1) and the late tau marker (truncated TauC3) using free floating 40 micron thick frontal cortex sections. Semi-quantitation was used to estimate density of NFTs, neuropil threads (NTs) and amyloid plaques using antibodies against APP/Aβ (6E10), MOAB-2 (pan Aβ), Aβ42 and Aβ40 species. Findings revealed pS422 immunoreactive (-ir) NFTs and NTs were more abundant, followed by AT8, TauC3 and to a lesser extent MC-1 and Alz50-ir profiles in the frontal cortex of people with DSAD compared to DS. By contrast, Aβ pathology was comparable in the frontal cortex of DS and DSAD. The one year-old individual with DS did not display any tau or Aβ immunoreactivity. These data suggest that an increase in the early tau phosphorylation maker, pS422, plays a role in the onset of AD dementia in DS, rather than Aβ pathology.

Disclosures: S.E. Perez: None. B. He: None. M.N. Sabbagh: None. I.T. Lott: None. E. Doran: None. E.J. Mufson: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.02/E23

Topic: A.07. Developmental Disorders

Title: An anti-beta amyloid (Aβ) liposomal vaccine shows efficacy in a Down Syndrome (DS) mouse model

Authors: M. VUKICEVIC[1], R. MADANI[1], P. V. BELICHENKO[2], L. REY-BELLET[1], M. PIHLGREN[1], A. BECKER[2], A. PLASSARD[1], S. VUILLERMOT[1], A. SILVA[1], V. GIRIENS[1], R. L. NOSHENY[2], A. M. KLESCHEVNIKOV[2], J. VALLETTA[2], S. K. S. BENGTSSON[2], G. R. LINKE[2], M. T. MALONEY[3], D. T. HICKMAN[1], P. REIS[1], A. GRANET[1], D. MLAKI[1], M. LOPEZ-DEBER[1], L. H. DO[2], N. SINGHAL[2], E. MASLIAH[2], M. L. PEARN[2], A. PFEIFER[1], W. C. MOBLEY[2], *A. MUHS[1];
[1]AC Immune SA, Lausanne, Switzerland; [2]Univ. of California, San Diego, CA; [3]Stanford Med. Sch., Palo Alto, CA

Abstract: Down syndrome (DS) is caused by trisomy of chromosome 21, which contains the gene APP, encoding for the beta-amyloid (Aβ). In people with DS, the increased level of APP gene and the accumulation of Aβ are linked to the appearance of neuropathological markers of Alzheimer's disease-like (AD-like) characteristics, together with dementia. ACI-24, a liposomal vaccine containing human Aβ1-15 peptide, was developed for immunotherapy of AD patients. Immunization of APPxPS1 mice (AD mouse model) with ACI-24 led to a significant decrease of insoluble, plaque-related Aβ1-42 and Aβ1-40 and restoration of a cognitive memory. In addition, ACI-24 induced a T-cell independent antibody response, disabling a potentially harmful infiltration of antigen-specific T-cells in the brain. To address the efficacy of Aβ1-15 vaccine in vivo, a Ts65Dn DS mouse model was used, containing a trisomic portion of mouse chromosome 16 (homologous to human chromosome 21) with the murine gene for APP. Ts65Dn mice fail to develop neuritic Aβ plaques or neurofibrillary tangles, but demonstrate behavioral deficits in several memory tasks. The ACI-24 vaccine comprising the human Aβ1-15 amino acid sequence triggers a strong antibody response, which might be unable to recognize the murine Aβ sequence differing in three amino acids. In addition, we studied if the substitution from the human to the murine sequence would impact the antibody response due to the self-tolerance against the endogenous Aβ. To address these points, immunogenicity of vaccines containing human or murine Aβ1-15 (ACI-24 and DS-01, respectively) was tested in C57BL/6 mice. Both vaccines induced a strong antibody response against their respective antigen without cross-reactivity to the other sequence. Furthermore, the strong antibody response of DS-01 confirmed that it can break the tolerance against the endogenous mouse Aβ protein. 5 months-old Ts65Dn mice were immunized with the murine Aβ1-15 vaccine. Immunization resulted in robust anti-Aβ IgG titers, confirming the ability of the vaccine to break self-tolerance. Induced antibodies reacted with Aβ but not with APP. Immunization resulted in a trend to reduction of soluble Aβ levels in the brain. However, in vaccinated-Ts65Dn mice, the levels of Aβ were not different from those in vehicle- and vaccine-treated diploid (2N) mice. Importantly, vaccinated mice showed improved memory and a reduction in atrophy of cholinergic neurons compared to vehicle-treated mice, without any adverse effects. These data are the first evidence that an anti-Aβ immunotherapeutic approach may target plaque-free Aβ-related pathology, leading to the potential treatment of people with DS.

Disclosures: **M. Vukicevic:** A. Employment/Salary (full or part-time): AC Immune SA. **R. Madani:** A. Employment/Salary (full or part-time): AC Immune SA. **P.V. Belichenko:** None. **L.**

Rey-Bellet: A. Employment/Salary (full or part-time): AC Immune SA. **M. Pihlgren:** A. Employment/Salary (full or part-time): AC Immune SA. **A. Becker:** None. **A. Plassard:** A. Employment/Salary (full or part-time): AC Immune SA. **S. Vuillermot:** A. Employment/Salary (full or part-time): AC Immune SA. **A. Silva:** A. Employment/Salary (full or part-time): AC Immune SA. **V. Giriens:** A. Employment/Salary (full or part-time): AC Immune SA. **R.L. Nosheny:** None. **A.M. Kleschevnikov:** None. **J. Valletta:** None. **S.K.S. Bengtsson:** None. **G.R. Linke:** None. **M.T. Maloney:** None. **D.T. Hickman:** A. Employment/Salary (full or part-time): AC Immune SA. **P. Reis:** A. Employment/Salary (full or part-time): AC Immune SA. **A. Granet:** A. Employment/Salary (full or part-time): AC Immune SA. **D. Mlaki:** A. Employment/Salary (full or part-time): AC Immune SA. **M. Lopez-Deber:** A. Employment/Salary (full or part-time): AC Immune SA. **L.H. Do:** None. **N. Singhal:** None. **E. Masliah:** None. **M.L. Pearn:** None. **A. Pfeifer:** A. Employment/Salary (full or part-time): AC Immune SA. **W.C. Mobley:** None. **A. Muhs:** A. Employment/Salary (full or part-time): AC Immune SA.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.03/E24

Topic: A.07. Developmental Disorders

Support: LCI Grant PP-042014

DARPA-13-34-RTA-FP-007

Title: Cerebral organoids and quantitative proteomics reveal molecular mechanisms in down syndrome

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Abstract: To better understand the cellular and molecular processes associated with Down syndrome (DS) in the human central nervous system (CNS), we generated a model of early neuronal development using human induced pluripotent stem cells (IPSc) as a starting template. We obtained IPSc from an individual with Down syndrome and a line from an unrelated euploid individual. With some modifications to the method first described by Lancaster et al (Nature, 2013), we successfully generated human cerebral organoids from both cell lines and used them

for imaging experiments with whole-mount immunostaining and deep proteome profiling with label-free quantitation of over 8,500 proteins in each sample. Our imaging analysis shows neurons populating the outer edges of the tissue, with neuronal progenitors restricted to inner regions of the tissue; a cell type distribution of radial migration and differentiation similar to human cortex development. Our proteomics analysis shows many proteins changing in significant abundance due to Trisomy 21, with alterations in members of Wnt and Notch signaling pathways, catecholamine metabolism, axon guidance, and cell adhesion. A following experiment collected samples from each stage in organoid development: a.) IPSc growing in 2-dimensional standard maintenance culture, b.) embryoid bodies grown in suspension, 3.) neurospheres with fate-restricted neural progenitor populations and radial neuroectoderm, and 4.) organoids grown following embedding in extracellular matrix, cultured in suspension for 21 days and examined the effects of pharmacological inhibition of the protein kinase DYRK1A, located on the Down Syndrome Critical Region (DSCR). We observed changes in the proteome of drug treated organoids consistent with rescuing expression levels of several key signaling pathway members. These data are the first to interrogate cerebral organoids with proteomic approaches and targeted pharmacology in the study of complex genetic conditions with a spectrum of neurological phenotypes.

Disclosures: T.D. McClure-Begley: None. C.C. Ebmeier: None. M.J. Klymkowsky: None. K.E. Ball: None. W.M. Old: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.04/E25

Topic: A.07. Developmental Disorders

Support: PAPIIT RN309214 "Desarrollo del lenguaje en niños con SD: la comprensión temprana"

Foundation Jérôme Lejeune "Language comprehension in Down syndrome"

Title: Linguistic prediction in Down syndrome

Authors: J. RAMOS-SÁNCHEZ, J. B. BARRÓN-MARTÍNEZ, A. Q. ANGULO-CHAVIRA, D. R. CORTÉS-MONTER, *N. ARIAS-TREJO;
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Abstract: Previous literature has shown that adults and children are able to anticipate a referent when exposed to semantically informative verbs, articles and adjectives (Mani & Huettig, 2014; Mishra, Singh Pandey, & Huettig, 2012), given the relationship among words (e.g., eat-cake). However, currently there is no knowledge about the linguistic ability of prediction in people with Down syndrome (DS). Therefore, the present study aimed to identify if the relations of meaning stablished between verbs and nouns can allow people with DS to anticipate a referent.
Fifteen people with DS were evaluated (CA = 21, SD = 2.5 years, range = 18-25; MA = 6.7 years, SD = 1.8, range = 3.8-8.6 years). Participants performed a visual preference task by means of a Tobii eye-tracker X2-30. The task consisted of 10 trials with a duration of 6,000 ms each. Each trial involved the simultaneous display of two images on a screen (e. g., 'pencil'-'ball') and the auditory exposure to a sentence that contained - either a verb semantically related -RV- to one of the two images (e.g., 'I am going to write' related to 'pen') or an unrelated verb -NRV- ('I am going to see').
The total looking time was calculated for each of the images in a trial. Results showed that when exposed to a RV sentence (‘I am going to write’) participants looked proportionally more to the related target (‘pencil’) than to the non-related distracter (‘ball’) before the target was mentioned. In contrast, upon hearing the NRV sentence (‘I am going to see’) participants did not exhibit a preference for either image.
The findings of this study suggest that people with Down syndrome are able to obtain and use semantic information from verbs to predict a referent, which is an essential ability for the comprehension and establishment of predictions in oral speech.

Disclosures: J. Ramos-Sánchez: None. J.B. Barrón-Martínez: None. A.Q. Angulo-Chavira: None. D.R. Cortés-Monter: None. N. Arias-Trejo: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.05/E26

Topic: A.07. Developmental Disorders

Support: ALANA Foundation USA

Awakening Angels Foundation

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/MCTI)

Title: Isolation-induced ultrasonic vocalizations in the mouse model of Down syndrome Ts65Dn during pre-weaning postnatal development

Authors: *B. ZAMPIERI, M. W. JOHNSON, A. C. COSTA;
Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS), the phenotypic consequence of trisomy 21, is the most prevalent chromosomal cause of intellectual disability. An area of particular concern in children with DS is speech and language development, which is generally delayed when compared with typically developing children. These include deficits in semantic, phonological, syntactic, and pragmatic components of speech production. The causal mechanisms underlying these deficits are still not well understood. Recently, there has been significant progress in the field of the development of speech production through isolation-induced ultrasonic vocalizations (USV) in mouse models of various neurodevelopmental disorders. Therefore, it is not unreasonable to expect that mouse modeling of this uniquely human phenotype could shed some light into speech and language development in DS. Here, we recorded USVs from five Ts65Dn mice and eighteen of their euploid littermates every other day from postnatal days 3 to 21 for 5 min inside a sound attenuating chamber (SonoTrack SMART Chamber, Metris). Data were recorded at 250 kHz using a Sonotrack system (Metris). Acoustical analysis was performed using Avisoft Bioacoustics SasLab Pro software (Version 5.2.09) and IGOR Pro 6 (WaveMetrics). Spectrograms were generated with a Fast Fourier Transform (FFT)-length of 512 and a Flat top window with 87.5% overlap using Avisoft whistle detection algorithm and post-processed in IGOR Pro. Each syllable was classified as one of nine waveform categories according to previous work by our group (Zampieri et al., 2014). In the present study, Ts65Dn pups showed no significant delay in the onset of USV emissions compared to wild-type control mice, contradicting the only study in the literature using a mouse model for DS (Holtzman et al., 1996). Additionally, there was no evidence of genotype differences in total number of calls, number of calls per day, amplitude, types of call, or call duration. However, a histogram of call counts versus call frequency (pitch) emitted by the mice revealed subtle, but significant genotype-dependent differences. Ts65Dn mice showed a clear bimodal distribution, with approximately equal peaks at 62 and 94 kHz, whereas control mice showed a primary peak at 62 kHz and a very weak peak around 86 kHz (about 16% of the primary peak). These results held across the types of calls and postnatal days. Consistent with our previous study in adult mice (Zampieri et al., 2014), Ts65Dn pup mice did not show overt vocalization phenotypes that can be easily and directly correlated to quantitative features of the speech and language impairments affecting individuals with DS.

Disclosures: B. Zampieri: None. M.W. Johnson: None. A.C. Costa: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.06/E27

Topic: A.07. Developmental Disorders

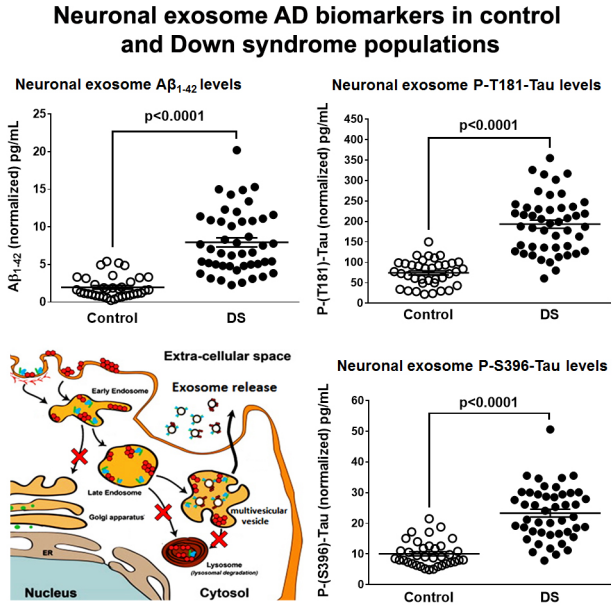
Support: DSADIIP-13-284845

Title: Neuronal exosome biomarkers of Alzheimer's dementia in Down syndrome

Authors: *E. D. HAMLETT[1], E. GOETZL[2], V. VASILEVKO[3], A. LEDREUX[1], H. BOGER[1], A. LAROSA[1], D. CLARK[1], S. CARROL[1], M. IRAGUI[4], E. MUFSON[5], M. SABBAGH[5], A. MOHAMMED[6], D. HARTLEY[7], J. FORTEA[4], E. DORAN[3], I. LOTT[3], A.-C. BENTLEY[1,8]; [1]MUSC, Charleston, SC; [2]Geriatric Res. Ctr. of the Jewish Home of San Francisco, CA, USA, San Francisco, CA; [3]Dept. of Pediatrics, Sch. of Medicine, Univ. of California, Irvine, Orange, CA, USA, Irvine, CA; [4]Hosp. de la Santa Creu i Sant Pau-Biomedical Res. Inst. Sant Pau, Bacelona, Spain; [5]Dept. Neurobio., Barrow Neurolog. Institute, Phoenix, AZ, USA, Phoenix, AZ; [6]Dept. of Psychology, Linnaeus University, Växjö, Sweden, Växjö, Sweden; [7]Alzheimer's Association, Chicago, IL, USA, Chicago, IL; [8]MUSC Ctr. on Aging, Charleston, SC

Abstract: Individuals with Down syndrome (DS) exhibit Alzheimer's disease (AD) neuropathology by 40 years of age and are at greater risk of developing AD dementia later in life. Due to increases in lifespan of individuals with DS, the prevalence of AD dementia has increased but few treatments are available in part because co-morbidities of intellectual and functional disability in DS make a diagnosis of dementia difficult. Blood biomarkers quantifying pathogenic proteins elevated at the onset of AD neuropathology may be particularly valuable. A possible marker may be exosomes which are defined as cell-derived vesicles that are found in biological fluids. Exosomes are actively secreted from neurons, contain proteins of their neuronal source, and enter the blood from which they can be isolated. In the general population, AD has a preclinical phase with high blood neuronal exosome levels of neurotoxic proteins prior to onset of symptoms. We hypothesized that neuronal exosomes obtained from blood of individuals with DS also would have elevated levels of amyloid-beta (Aβ) peptides and phosphorylated-Tau (P-Tau) that could document a preclinical stage. We therefore quantified levels of neuropathological biomarkers in blood neuronal exosomes from individuals with DS, including those with and without clinical symptoms of dementia, and from age-matched controls. Quantitation revealed that neuronal exosome levels of Aβ1-42 and P-Tau were significantly elevated in individuals with DS compared to age-matched controls. These biomarker levels were significantly increased at an early age, and continued to increase in older individuals. P-S396-Tau levels were significantly elevated in participants with early symptomatic dementia or fully symptomatic dementia compared to adults with DS without dementia. No significant gender differences were

observed. These findings suggest that the early increases in Aβ1-42 and P-Tau in individuals with DS provide a basis for early intervention as better treatments become available.



Disclosures: E.D. Hamlett: None. E. Goetzl: None. V. Vasilevko: None. A. Ledreux: None. H. Boger: None. A. LaRosa: None. D. Clark: None. S. Carrol: None. M. Iragui: None. E. Mufson: None. M. Sabbagh: None. A. Mohammed: None. D. Hartley: None. J. Fortea: None. E. Doran: None. I. Lott: None. A. Bentley: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.07/E28

Topic: A.07. Developmental Disorders

Support: IUPUI Research Support Grant

IUPUI: Department of Psychology Internal Support Funds

Title: Tissue specific effects of EGCG on Dyrk1a activity and cognitive and skeletal phenotypes in a Down syndrome mouse model

Authors: *M. STRINGER[1], J. LACOMBE[2], R. J. ROPER[2], C. R. GOODLETT[3];
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Abstract: Down syndrome (DS) is caused by three copies of human chromosome 21 (Hsa21) and results in phenotypes including intellectual disability and skeletal deficits. Ts65Dn mice, the most extensively studied DS model, have three copies of ~50% of the genes on Hsa21 and display many phenotypes associated with DS, including cognitive and skeletal deficits. *DYRK1A* is found in three copies in humans with Trisomy 21 and in Ts65Dn mice, and is involved in a number of critical pathways including CNS development and osteoclastogenesis. Epigallocatechin-3-gallate (EGCG), the main polyphenol in green tea, inhibits Dyrk1a activity. We have shown that a three-week EGCG treatment (~10mg/kg/day) during adolescence normalizes skeletal abnormalities in Ts65Dn mice, yet did not rescue deficits in the Morris water maze (MWM) spatial learning task or novel object recognition. A higher EGCG dose (50 mg/kg/day) failed to rescue skeletal abnormalities, or various motor and cognitive deficits. Therefore, we hypothesize that the effects of EGCG on various phenotypes are both dose and tissue-dependent. EGCG degrades in drinking water; thus, an oral gavage administration would control EGCG dosage. Furthermore, few studies have assessed EGCG's ability to inhibit Dyrk1a *in vivo,* or its inhibition in specific tissues. We tested whether a daily oral gavage administration of EGCG would inhibit Dyrk1a activity in specific tissues (cerebellum, hippocampus, cerebral cortex, femur). In addition, we anticipate that reductions in Dyrk1a activity in brain or bone tissue will correlate with improvements on behavioral and skeletal measures. Ts65Dn and euploid littermate mice were orally gavaged with 200mg/kg EGCG or PBS daily, beginning on postnatal day (PD) 42. Mice were tested on the Mulitvariate Concentric Square Field Maze (MCSF) task for two days. Mice then underwent three days of balance beam testing, followed by two days of rest. On PD57, mice were trained for eight days on the MWM. Mice were euthanized on PD65, and the cerebellum, hippocampus, cortex and femur were extracted. Proteins were isolated and subjected to a Dyrk1a activity assay. Preliminary data indicates that Ts65Dn exhibit behavioral deficits compared to euploid mice. Furthermore, we observed differential levels of Dyrk1a activity in various tissues. Ongoing analysis will focus on correlations between tissue-specific changes in Dyrk1a activity and behavioral and skeletal measures. The ability to correlate EGCG's inhibition on Dyrk1a activity in a specific tissue with a distinct phenotype may help identify more targeted pharmacotherapies for cognitive and skeletal deficits in individuals with DS.

Disclosures: M. Stringer: None. J. LaCombe: None. R.J. Roper: None. C.R. Goodlett: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.08/E29

Topic: A.07. Developmental Disorders

Support: CIHR grant M101111

Title: Infantile Spasms: Rescue of an animal model

Authors: ***K. JOSHI**[1], L. SHEN[2], A. MICHAELI[2], M. SALTER[2], J. H. EUBANKS[3], G. THIBAULT-MESSIER[2], S. HASHMI[2], M. A. CORTEZ[2], O. SNEAD, 3rd[2];
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Abstract: Infantile spasms (IS) is a catastrophic childhood seizure disorder characterized by extension and/or flexor spasms with a characteristic EEG abnormality and impairment of cognition. Children with Down Syndrome (DS) are quite vulnerable to IS. Similarly, the Ts65Dn (Ts) mouse model of DS is exquisitely sensitive to an IS phenotype induced by γ-aminobutyric acid$_B$ receptor ($GABA_BR$) agonists. The Ts mouse contains the core genomic triplication of the DS critical region, which includes three copies of the *kcnj6* gene that encodes the $GABA_BR$-coupled G protein-coupled inward rectifying potassium channel subunit 2 [GIRK2] channel. We test the hypothesis that GIRK2 is necessary for the $GABA_BR$ agonist-induced IS phenotype in Ts. We assessed the effect of genetic knockdown of the GIRK2 channel in Ts brain upon the IS phenotype in Ts, and upon $GABA_BR$ currents in hippocampal neurons prepared from GIRK2-trisomic and GIRK2-disomic Ts mice. The reduction of the copy number of *kcnj6* in Ts mice rescued the $GABA_BR$ agonist-induced IS phenotype and normalized $GABA_BR$-mediated GIRK2 currents. The radial arm maze task showed no differences between Ts and wild type mice, but Ts mice showed impaired recognition memory when tested with the novel object recognition task. This impairment also was rescued in the GIRK2-disomic Ts mice. GIRK2 is necessary for the IS phenotype in the Ts mouse model of DS. Further experiments are needed to determine if GIRK2 is sufficient for the IS phenotype. These data suggest that GIRK2 antagonists may have therapeutic utility for the treatment of IS in patients with Down syndrome.

Disclosures: K. Joshi: None. L. Shen: None. A. Michaeli: None. M. Salter: None. J.H. Eubanks: None. G. Thibault-Messier: None. S. Hashmi: None. M.A. Cortez: None. O. Snead: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.09/E30

Topic: A.07. Developmental Disorders

Support: ALANA Foundation USA

Ohio Department of Developmental Disabilities

Awakening Angels Foundation

Title: Noninvasive high-resolution *In vivo* imaging of retinal morphology and vasculature alterations in the mouse model of Down syndrome using optical coherence tomography and angiography

Authors: A. C. COSTA[1], *D. B. VICTORINO[2];
[1]Pediatrics, Case Western Reserve Univ., Cleveland, OH; [2]Pediatrics, Case Western Reserve Univ., Cleveland Heights, OH

Abstract: Ophthalmic disorders are among the most prevalent comorbidities of Down syndrome (DS). Therefore, in trying to use mice to model the effects of trisomy 21 in persons with DS, it is essential that we understand how the visual system is affected in mouse models of DS. As we have argued in the past, poor understanding of how vision is affected in mouse models of DS and other neurodevelopmental disorders can lead to misinterpretation of data obtained through behavioral test that are highly dependent on the integrity of the visual system. Here, we used the Micron IV system (Phoenix Research Laboratories) to perform slit lamp examinations, image-guided Optical Coherence Tomography (OCT), and fluorescein angiography in the Ts65Dn mice, which is the most commonly used mouse model of DS. Cohorts of 38 Ts65Dn and 37 control mice were categorized into three different age groups (P17, P35, and adult mice - P41 to P102). For the OCT assessment, the thickness of inner, outer, and total retinal layers were obtained by measurements of four lateral distances from the optic nerve disc on both sides (100, 200, 300, and 400 μm). Our results did not show any evidence of congenital or age-related cataracts in either Ts65Dn or control mice in all age groups. Similarly, the numbers of retinal arteries and veins did not differ significantly between both genotypes. Using OCT, we found that the retina of the Ts65Dn mice shows a normal layered organization. However, quantitate analyses, revealed that the total thickness of the retina is significantly increased in the Ts65Dn compared to control mice in all four lateral distances for which this parameter was measured. This genotype-dependent increase in total retinal thickness was primarily due to the increased thickness of the inner retinal layer, which was significantly greater in the Ts65Dn compared to control mice in all three age groups investigated. In conclusion, our results indicate that the total

retinal thickness is increased in the Ts65Dn mouse, and that a genotype-dependent increase in the thickness of the inner part of the retina is the primary contributor to this phenomenon. The actual functional significance of this finding is still under investigation in our laboratory. Nevertheless, it is important to notice that these differences parallel published reports of OCT evaluation of retinal thickness in young persons with DS, which provides yet another validation of the Ts65Dn mouse as a powerful animal model of DS.

Disclosures: A.C. Costa: None. D.B. Victorino: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.10/E31

Topic: A.07. Developmental Disorders

Support: ALANA USA Foundation

Title: High-density EEG assessment of mismatch negativity and 7T MRI whole-brain morphometric analysis of healthy adolescents and young adults with Down syndrome

Authors: *K. A. KOENIG[1], M. W. JOHNSON[2], M. R. STASKO[2], S.-H. OH[1], A. C. COSTA[2]; [1]Cleveland Clin., Cleveland, OH; [2]Div. of Pediatric Neurology, Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS), the phenotypic consequence of trisomy 21, is the most prevalent genetically defined cause of intellectual disability. To date, no pharmacotherapy aimed at counteracting either the neurodevelopmental or the neurodegenerative component of this genetic disorder has been approved. However, recent preclinical and clinical work on the N-methyl-D-aspartate (NMDA) receptor antagonist memantine give some reason for optimism. The results described here are preliminary data on a small subset of participants of a current Phase II clinical trial on the efficacy, tolerability and safety of memantine on enhancing the cognitive abilities of adolescents and young adults with DS (NCT02304302 at http://www.clinicaltrials.gov). These results refer to a subcomponent of this study in which we investigate the usefulness of mismatch negativity (MMN) and high-resolution, whole-brain morphometric analysis (with emphasis on volumetric assessments of hippocampal subfields), as potential biomarkers of the severity of the cognitive disability in persons with DS as well as potential surrogate markers for the efficacy of memantine in persons with DS. We describe results from two male and two female subjects with DS, and two male and one female typically developing control subjects. A whole-brain anatomical MP2RAGE (0.75mm isotropic voxel

size) was acquired on a Siemens 7T Magnetom with SC72 gradient (Siemens Medical Solutions, Erlangen) using a 32-channel head coil (Nova Medical). The hippocampus was manually segmented according to the HarP protocol. Whole brain white matter, grey matter, and intracranial volume (ICV) was estimated using Freesurfer. MMN studies were acquired on an Electrical Geodesics EEG System 400 (Eugene, OR) 128-channel EEG at 1 kHz synchronized to a SmartEP AEP system (Intelligent Hearing Systems, Miami, FL) for the auditory stimulation. The MMN auditory stimulation used 100msec triangular waveforms at 440 and 557 Hz, with an 80% standard and 20% oddball paradigm with a total of approximately 300 oddball and 1200 standard stimuli per study. MMN analysis was performed using Igor Pro 6 (Wavemetrics, Lake Oswego, OR). Overall, the participants with DS appear to have a depressed MMN response compared to the control subjects. In addition, raw volumetric measures showed a decrease in subcortical grey matter, mean hippocampal, and total brain volumes in individuals with DS ($p < 0.05$), though only mean hippocampal volume remained significant after correction for ICV ($p = 0.031$). Future work will include segmentation of the hippocampus and techniques for optimizing the signal-to-noise ratio in the MMN analysis.

Disclosures: K.A. Koenig: None. M.W. Johnson: None. M.R. Stasko: None. S. Oh: None. A.C. Costa: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.11/E32

Topic: A.07. Developmental Disorders

Support: NIH R01NS076503

Sponsored research grant from Illumina

Title: Comparative study of brain development and perinatal behavior in three mouse models of Down syndrome

Authors: *N. M. AZIZ[1], J. L. OLMOS-SERRANO[1], F. GUEDJ[2], W. A. TYLER[1], J. W. GOODLIFFE[1], D. W. BIANCHI[2], T. F. HAYDAR[1];
[1]Boston Univ. Sch. of Med., Boston, MA; [2]Tufts Med. Ctr., Boston, MA

Abstract: Down syndrome (DS), a genetic condition leading to intellectual disability, is characterized by triplication of human chromosome 21. Major neuropathological hallmarks of DS are abnormal brain growth and reduced neurogenesis that manifest during fetal development

and continue throughout life. At birth, newborns with DS exhibit major motor and reflexive deficits and fail to meet typical developmental milestones. A major outstanding question is how DS-related prenatal and postnatal phenotypes are recapitulated in different mouse models. To begin answering this question, we directly compared differences in embryonic development and brain morphogenesis, postnatal neuronal and oligodendroglial populations, and neonatal behavior in three cytogenetically distinct mouse models of DS—Ts1Cje, Ts65Dn and Dp16/1Yey (Dp16). At E15.5, gross morphological and histological measurements indicate that Ts65Dn mice are the most profoundly affected with respect to somatic growth, brain size and pallial thickness compared to Ts1Cje and Dp16 embryos. Histological abnormalities extend to P15 at which time Ts65Dn mice exhibit a misallocation of excitatory cortical neurons, inhibitory cortical and hippocampal interneurons, and a block in oligodendrocyte maturation. Additionally, Ts65Dn and Ts1Cje mice show delayed acquisition of both early and late developmental milestones, while Dp16 mice only show late delays. Taken together, our data show differences in behavioral and brain developmental phenotypes in these three most widely used mouse models. These findings illustrate unique potential applications for each model when studying different aspects of brain development and function in DS. Importantly, this study will help inform future model selection for studies aimed at elucidating how observed neurodevelopmental abnormalities contribute to cognitive impairment and whether prenatal and/or postnatal therapeutic intervention could help improve intellectual disability and, therefore, quality of life of individuals with DS.

Disclosures: N.M. Aziz: None. J.L. Olmos-Serrano: None. F. Guedj: None. W.A. Tyler: None. J.W. Goodliffe: None. D.W. Bianchi: None. T.F. Haydar: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.12/E33

Topic: A.07. Developmental Disorders

Support: Alana Foundation USA

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Awakening Angels

NIH NS074317

NIH NS083687

Title: Pharmacologically probing three modalities of CA1 hippocampal long-term potentiation in the Ts65Dn mouse model of Down syndrome

Authors: *J. J. SCOTT-MCKEAN[1], A. L. ROQUE[1], K. SUREWICZ[2], W. K. SUREWICZ[2], A. C. COSTA[1];
[1]Dept. of Pediatrics, [2]Dept. of Physiol. and Biophysics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS), the result of trisomy 21, is the most common genetically defined cause of intellectual disability. The Ts65Dn is the most studied mouse model of DS. Long-term potentiation (LTP) has been the main form of synaptic plasticity assessed in these mice, with long-term depression (LTD) being investigated to a lesser degree. A survey of different modalities of LTP, coupled with the use of pharmacological agents with different mechanisms of action, all in the same study, has been lacking in the literature. Here, we probed three modalities of LTP in CA1 hippocampal slices from Ts65Dn mice and investigated the effects of memantine, picrotoxin, and soluble (membrane anchor-free) recombinant human prion protein (PrP). First, we studied memantine (1 μM) on either single high-frequency stimulation (HFS; 100 Hz for 1 s) induced LTP or theta burst stimulation (TBS; 4 trains of 5 pulses at 100 Hz, 200-ms inter-train interval) induced LTP in Ts65Dn and euploid control derived slices. In agreement with our previous work, we found no significant difference in HFS LTP between Ts65Dn and euploid control-derived slices, and detected a reduction in TBS LTP in Ts65Dn-derived slices. When used at the therapeutically relevant concentration of 1 μM, memantine did not alter the induction or maintenance of HFS LTP, and seems to rescue TBS LTP to control euploid levels in slices from Ts65Dn mice. Next, we studied LTP induced by four HFS (4xHFS), with 5-min inter-train intervals. We found decreased LTP in Ts65Dn compared with control euploid derived slices both at 60 and 180 min post-induction (the latter being known as late-phase or L-LTP). Then, we probed the effects of memantine (1, 3, and 10 μM) and picrotoxin (10 and 100 μM) on L-LTP. Memantine (1 μM) pretreatment did not inhibit L-LTP in either Ts65Dn or euploid control slices, whereas 10 μM of picrotoxin seems to rescue L-LTP to control levels while causing severe oscillations in postsynaptic potentials. At 10 μM, memantine inhibited 4xHFS in both euploid control and Ts65Dn-derived slices. Our research had recently shown that PrP blocks Aβ oligomer-induced inhibition of 4xHFS and that PrP rescues this inhibition in euploid slices. In Ts65Dn slices, which already shows a deficit in 4xHFS when compared to euploid controls, Aβ oligomers further depressed this modality of LTP. PrP rescued Aβ oligomer-induced inhibition 4xHFS in Ts65Dn slices, however, only to untreated levels, and PrP alone had no effect on Ts65Dn-derived slices. This research should bring us closer to understanding the mechanistic basis for previous electrophysiological and behavioral studies in this important mouse model of DS.

Disclosures: J.J. Scott-McKean: None. A.L. Roque: None. K. Surewicz: None. W.K. Surewicz: None. A.C. Costa: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.13/E34

Topic: A.07. Developmental Disorders

Support: Fondecyt (Chile) #1130241

The Fondation pour la Recherche sur le Cerveau (France)

Fondation Jérôme Lejeune (France)

Title: Knockdown of overexpressed Down Syndrome Cell Adhesion Molecule results in the reversal of increased P21-Activated Kinase (PAK) activity in a neuronal model of Down Syndrome. Consequences on F/G actin ratio, cell process formation and extension.

Authors: R. PÉREZ-NUÑEZ[1], N. BARRAZA[1], J.-V. BARNIER[2], A. CÁRDENAS[3], *P. A. CAVIEDES[1];
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Abstract: Down Syndrome (DS), or trisomy 21 in humans, results from the presence of an extra copy of autosome 21. The most striking finding is mental retardation. The CTb cell line, derived from the cerebral cortex of a trisomy 16 fetal mouse (Ts16), an animal model of DS, constitutes a permanent *in vitro* model to study DS-related cell pathophysiological phenomena. Using this cell model, and comparing with a cell line derived from the cerebral cortex of a normal animal, named CNh, multiple impairments in neuronal function have been studied, all linked to the overexpression of specific DS-related genes. One of such genes, which reportedly is involved in proliferation, maturation, synaptogenesis and neural development is *dscam*, which codes for the protein DSCAM (Down Syndrome Cell Adhesion Molecule), DSCAM recognizes netrin at the membrane level, which leads to a correct morphological development, through cellular signaling pathways involving the serine / threonine p21-activated kinases (PAK), acting on downstream effectors such as LIMK and cofilin, leading to reorganization of actin filaments. We have shown that CTb cells overexpress DSCAM by 40%, compared to CNh cells. Further, Sholl analysis has revealed that trisomic cells exhibit reduced process number and length compared to controls, and also an increased F/G actin ratio. We then evaluated the response of the PAK signaling pathway by stimulating DSCAM with its agonist, netrin. After stimulation, PAK, LIMK and cofilin exhibited increased phosphorylation (2 fold compared to basal, t_0) in the trisomic line, and for longer periods of time (10 minutes for PAK and LIMK, and up to 20 minutes for cofilin),

compared to CNh cells. In CTb cells, these effects were completely reversed in all components of the PAK pathway, after siRNA-induced *knockdown* of DSCAM to levels comparable to those of CNh cells. Our work confirms that PAK kinases are effectively deregulated in the trisomic condition of DS, due at least in part to DSCAM overexpression. Considering the existence of PAK inhibitors, this kinase could represent an interesting therapeutical target in DS.

Disclosures: R. Pérez-Nuñez: None. **N. Barraza:** None. **J. Barnier:** None. **A. Cárdenas:** None. **P.A. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PC declares patent protection on CNh and CTb cell lines.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.14/E35

Topic: A.07. Developmental Disorders

Support: Wellcome Trust Strategic Award 098330/Z/12/Z

Baily Thomas Charitable Fund TRUST/RNA/AC/KW/3111/5776

Title: Age-related changes in cognitive abilities and related brain activity in Down syndrome

Authors: *C. M. STARTIN, S. HAMBURG, R. HITHERSAY, T. AL-JANABI, K. MOK, J. HARDY, M. DE HAAN, A. STRYDOM;
UCL, London, United Kingdom

Abstract: People with Down syndrome (DS) show large variability in their cognitive profiles. Some people have a mild intellectual disability (ID), while others a more severe ID. People with DS are also at an ultra-high risk of developing dementia, again with large individual differences; some people receive a dementia diagnosis in their 40s while others do not show cognitive decline in their 60s. The London Down Syndrome Consortium (LonDownS) is investigating factors influencing these differences across the lifespan. We have assessed over 300 adults (aged 16+ years) with DS. All adults were administered a cognitive battery, assessing both general abilities and specific aspects of cognition (mainly memory and executive function), and data was collected from informants regarding everyday adaptive abilities and relevant medical information. All participants also gave a blood or saliva sample for genetic analysis. A subgroup of participants underwent EEG assessments to investigate memory-related processes using event related potentials (ERPs) during auditory oddball and visual old-new paradigms. Cross-sectional

analyses revealed people with DS show decline in their early 40s for tasks requiring sustained attention. Other measures of cognition assessing general abilities, memory and executive function showed later changes, often in the early 50s. ERPs assessing memory processes showed earlier changes, with changes in amplitudes and latencies of relevant components starting in the late 30s. APOE4 genotype was associated with earlier onset of cognitive decline. Individuals who have recurrent infections showed poorer everyday abilities in young adulthood and cognitive decline at an earlier age. Our results describe changes in cognitive abilities across the lifespan in adults with DS. Changes in sustained attention occurred earlier than changes in general abilities, memory and executive function. Changes in memory-related ERPs were also seen earlier than changes in cognitive abilities. The presence of an APOE4 allele was associated with earlier cognitive decline, while presence of recurrent infections was associated with both poorer abilities in young adulthood and earlier decline in older adults.

Disclosures: C.M. Startin: None. S. Hamburg: None. R. Hithersay: None. T. Al-Janabi: None. K. Mok: None. J. Hardy: None. M. de Haan: None. A. Strydom: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.15/E36

Topic: A.07. Developmental Disorders

Support: NIH, NIDCD F32 DC014885-01A1

NIDCD T32 DC009401

NIDCD R01 DC005935

NIDCD R01008149

Jerome Lejeune Foundation, Agreement 1326

Title: Oromotor characterization of the Ts65Dn mouse model of Down syndrome

Authors: *T. J. GLASS[1], N. P. CONNOR[2];
[1]Surgery, Univ. of Wisconsin, Madison, WI; [2]Univ. of Wisconsin Madison, Madison, WI

Abstract: Down syndrome (DS) is often associated with oromotor impairment, which can impact vocal communication, feeding, and swallowing. Difficulties in feeding and swallowing in DS are complex and likely involve alterations in underlying muscle systems. Our prior work

found reduced levels of myosin heavy chain (MyHC) 2b in the digastric muscles of Ts65Dn mice, a commonly used model of DS. However, the biochemical composition of tongue muscles in this murine model of DS is not known. Because the tongue has an important role in bolus containment and transport during the swallow, this information is necessary for identification of putative mechanisms of swallowing impairment in DS. In this work, the Ts65Dn mouse model was used to address the hypothesis that myosin heavy chain isoform (MyHC) alterations in extrinsic tongue muscles contributes to deficits in feeding and swallowing. Videofluoroscopic swallowing and mastication assays were performed in 27 adult Ts65Dn mice and euploid controls ranging in age from 9 to 36 weeks of age (n = 7 male mice per genotype, and 6-7 female mice per genotype). Adult Ts65Dn collectively showed slower swallow rates and increased inter-swallow-intervals. MyHC isoform analysis was conducted of the genioglossus muscle (a tongue protrusor) and the styloglossus muscle (a tongue retrusor) across this age range. We found similar MyHC 2b levels in these muscles between genotypes. Collectively, these findings suggest that the adult Ts65Dn mouse model has utility for the study of functional feeding and swallowing differences in this syndrome. However, findings of typical MyHC isoform profiles of the GG and SG suggest that swallowing differences in adult Ts65Dn may not primarily involve biochemical alterations of extrinsic tongue muscles. Work is ongoing to increase sample sizes for discrete age points and to characterize the relationship between aging and swallow function in this model. Avenues for future study of altered swallowing in adult Ts65Dn include examination of the intrinsic tongue muscles and esophagus, both of which are implicated in altered feeding and swallowing in humans with DS.

Disclosures: T.J. Glass: None. N.P. Connor: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.16/E37

Topic: A.07. Developmental Disorders

Support: LuMind Foundation

LeJeune Foundation

Title: Inhibitory GABA mode in the CA1 region of adult Ts65Dn mice, a genetic model of Down syndrome

Authors: J. YU[1], F. MADAMBA[1], A. TYRTYSHNAIA[2], *A. M. KLESCHEVNIKOV[1];
[1]Neurosciences, Univ. of California San Diego, La Jolla, CA; [2]Sch. of Biomedicine, Far Eastern Federal Univ., Vladivostok, Russian Federation

Abstract: Down syndrome (DS) is a genetic disorder characterized by developmental delays and profound cognitive impairment in children and adults. Studies in mouse genetic models suggested that deficient cognition in DS is due, in part, to an increased efficiency of the GABAergic system that restricts synaptic plasticity. Recently this view was challenged by a study, in which an excitatory mode of GABA action was observed in the hippocampus of adult Ts65Dn mice, a genetic model of DS (Deidda et al., Nature Medicine, 2015). Because this study used mostly invasive electrophysiological techniques, the results could be affected by damage of neural tissue during the experiments. Here we re-examined the excitatory/inhibitory mode of GABA action in the CA1 region of hippocampal slices of 3-4 month old Ts65Dn mice using a 'non-invasive' approach - measurements of multi-unit activity (MUA) during bath application of the GABAa receptor agonist isoguvacine (10 μM, 90 s). We observed that applications of isoguvacine resulted in significant reduction of MUA frequencies in slices from both Ts65Dn and WT mice (Ts65Dn: 35.4 ± 10.2%, n = 8; WT: 42.4 ± 11.4%, n = 8; p = 0.63). This result suggests that the primary mode of GABA action in the Ts65Dn hippocampus is inhibitory.

Disclosures: J. Yu: None. F. Madamba: None. A. Tyrtyshnaia: None. A.M. Kleschevnikov: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.17/E38

Topic: A.07. Developmental Disorders

Support: MRC

Rosetrees Trust

Wellcome Trust

Title: Increased GABA inhibition in the Dentate Gyrus in a mouse model of Down syndrome

Authors: *S. HANNAN[1], S. WATSON-SCALES[3], E. LANA-ELOLA[3], F. WISEMAN[2], E. M. C. FISHER[2], V. TYBULEWICZ[3], T. SMART[1];
[1]Neuroscience, Physiol. and Pharmacol., [2]Dept. of Neurodegenerative Dis., Univ. Col. London, London, United Kingdom; [3]Francis Crick Inst., London, United Kingdom

Abstract: Down syndrome (DS) is characterised by severe cognitive deficits that are considered to arise from dysfunctional GABA-mediated inhibition in the brain[1]. In the hippocampus, increased inhibition impairs the development of long-term potentiation[2] (LTP), a potential cellular correlate of learning and memory at the circuit level. Here we used the Dp1 Tyb mouse model that contains an extra copy of the region of mouse chromosome-16 that is syntenic with human chromosome-21 to study the nature of GABAergic inhibition in the dentate gyrus of the hippocampus, using whole-cell electrophysiology of adult (P100-135) animals.
Using acute brain slices, we observed that the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in dentate granule cells is increased in trisomic animals compared to their disomic controls, without changing sIPSC amplitude. In addition, trisomic cells also exhibited increased levels of tonic inhibition. Overall, such findings are consistent with increased GABA-mediated inhibition in trisomic neurons.
In regard to granule cell excitability, there was no change in resting membrane potential between trisomic and disomic neurons. However, the threshold for spike firing was increased for trisomic cells in accord with the increased synaptic and tonic inhibition suppressing spike firing. These effects may contribute to the cognitive impairment observed in DS and the reduced level of LTP in DS mouse models.
By dye filling single granule cells, we also show that trisomic neurons undergo dendritic remodelling and from immunolabelling, we reveal that the number of parvalbumin positive interneurons is significantly increased in the dentate suggesting the mechanisms by which the increase of phasic frequency and tonic current is orchestrated.
This work was supported by the MRC, The Wellcome Trust and the Rosetrees Trust
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Disclosures: S. Hannan: None. S. Watson-Scales: None. E. Lana-Elola: None. F. Wiseman: None. E.M.C. Fisher: None. V. Tybulewicz: None. T. Smart: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.18/F1

Topic: A.07. Developmental Disorders

Support: Wellcome Trust Strategic Award (grant number: 098330/Z/12/Z)

Baily Thomas Charitable Fund (TRUST/RNA/AC/KW/3111/5776)

Title: EEG in adults with Down syndrome: how resting-state characteristics relate to cognitive ability

Authors: *S. HAMBURG[1], C. M. STARTIN[1], M. DE HAAN[2], A. STRYDOM[1];
[1]Div. of Psychiatry, Univ. Col. London, London, United Kingdom; [2]Ctr. for Developmental Cognitive Neurosci., UCL Inst. of Child Hlth., London, United Kingdom

Abstract: Electroencephalography (EEG) characteristics, particularly alpha activity, have been associated with cognitive ability in the typically developing (TD) population. Atypicalities in the EEG of individuals with Down syndrome (DS) are common and include power, frequency, topographical and connectivity measures; 'slowing' of the EEG is commonly reported in DS. However, the relationship of these characteristics with cognitive ability in DS remain unclear. Exploring these relationships may be important for understanding brain development and maturation in DS, as well as for identifying potential EEG characteristics indicative of cognitive decline (biomarkers). We used 128-channel EEG in two resting state paradigms (eyes-open and eyes-closed; 5.5 min each) to explore individual differences in EEG characteristics and their relationship with cognitive ability in younger adults with DS (n=39; aged 16-35). Raw K-BIT 2 scores provided an estimate of general cognitive ability and memory was assessed using a seven object immediate recall test. We report that individual alpha peak frequency (iAPF) was significantly negatively correlated with both memory ability and general cognitive ability during the eyes-open paradigm. In conclusion it appears that slower alpha-band activity while eyes are open confers a cognitive benefit in younger adults with DS.

Disclosures: S. Hamburg: None. C.M. Startin: None. M. de Haan: None. A. Strydom: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.19/F2

Topic: A.07. Developmental Disorders

Support: F30MH108321 (VM)

NIH NRSA F31NS084451 (HM)

Title: Implications for chromosome 21-encoded miRNAs and MeCP2 in synaptic dysfunction in Down syndrome

Authors: *V. R. MIRABELLA, H. MCGOWAN, A. VILLEGAS, Z. PANG;
Neurosci. and Cell Biol., Child Hlth. Inst. of NJ, Rutgers-Rwjms, New Brunswick, NJ

Abstract: Trisomy 21 (T21) is characterized by a number of clinical hallmarks, including cognitive impairment and mild to moderate intellectual disability. Animal models have demonstrated synaptic aberrations, including altered synaptic density, impaired endocytosis and excessive inhibitory signaling. Additionally, human induced pluripotent stem (iPS) cell models have demonstrated reduced synaptic connectivity. The formation and maintenance of synaptic connections is paramount to central nervous system function and is highly regulated. Emerging evidence implicates non-coding RNAs, including microRNAs (miRNAs), in regulating synaptic function. For example, several miRNAs have been implicated in spinogenesis, dendritic arborization, and synaptogenesis. Thus, over- or under-expression of miRNAs may contribute to synaptic dysfunction in neurodevelopmental disorders. T21 results in increased gene dosage of several miRNAs encoded by human chromosome 21 (HSA21), and our preliminary evidence suggests that HSA21 miRNAs target methyl CpG binding protein 2 (MeCP2), the protein mutated in Rett syndrome. These data suggest that HSA21-encoded miRNAs may regulate neuronal maturation and synapse formation via MECP2. Utilizing innovative induced neuronal (iN) cell technologies, we are studying the effects of HSA21 miRNAs on synaptogenesis in human neurons derived from several isogenic T21 patient iPS cell lines. We discovered MeCP2 as a target of these miRNAs by a dual luciferase assay and have confirmed the overexpression of HSA21 miRNAs in T21 iNs. Our preliminary data suggest that reduced MeCP2 expression in these neurons is also reduced. Synaptic function in T21 iNs will be assessed by morphological and functional analyses, including electrophysiology and calcium imaging. Preliminary electrophysiological data suggest that T21 iNs are less synaptically active. By combining interdisciplinary analytical methodologies with the iN cell and iPS cell technologies to examine the functions of HSA21 miRNAs in the nervous system, we will test causal-effect relationship of HSA21 miRNAs and synaptic alterations in T21 human neurons. This study will broaden our knowledge of the biological functions of these important and versatile regulatory molecules and provide important insight into the mechanistic and molecular basis of synaptic dysfunction in T21.

Disclosures: V.R. Mirabella: None. H. McGowan: None. A. Villegas: None. Z. Pang: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.20/F3

Topic: A.07. Developmental Disorders

Title: Microglial defects are present in the dp16 murine model of down syndrome.

Authors: *B. PINTO[1,2], L. PERLINI[1], L. CANCEDDA[1];
[1]NBT, IIT, Genova, Italy; [2]Neurosci., Scuola Normale Superiore, Pisa, Italy

Abstract: Down syndrome (DS) is a neurodevelopmental disorder caused by the presence of a supernumerary chromosome 21 (Chr21), and it represents the most frequent cause of genetic intellectual disability. Interestingly, some of the genes located on Chr21 are essential for the correct function of the immune system, and DS individuals often suffer from immunological disorders.
Microglia are the main immune cells of the brain and play important roles in its development. In particular, they are involved in neuronal progenitor phagocytosis and synaptic pruning, which are mechanisms essential for cognitive functions. However, it is not known whether microglial defects are involved in the development of cognitive deficits associated with DS.
Here, we investigated the presence of microglial alterations during brain development in a DS murine model (DP16). We found no difference in the density of microglial cells in the embryonic cortex of DP16 animals when compared to WT littermates. However, at postnatal day 1, we found an increased density of microglia that accumulated in the periventricular zone of the forebrain in DP16 mice. Interestingly, this increase was followed by a decreased microglia density that persisted into adulthood. Moreover, the distribution defects were accompanied by morphological alterations.
The accumulation of microglia in neurogenic locations of the developing DS brains together with microglial morphological alterations suggest a possible involvement of these cells in cerebral defects typical of trisomic brains. Thus, microglia may be a potential future target for early interventional therapies for the treatment of cognitive impairment in DS individuals.

Disclosures: B. Pinto: None. L. Perlini: None. L. Cancedda: None.

Poster

213. Molecular, Cellular, and Physiological Mechanisms of Down Syndrome

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 213.21/F4

Topic: A.07. Developmental Disorders

Support: ALANA USA Foundation

Ohio Department of Developmental Disabilities

Awakening Angels Foundation

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/MCTI)

Title: Memantine rescues delayed development of visual acuity in the Down syndrome mouse model Ts65Dn

Authors: D. B. VICTORINO, M. R. STASKO, J. J. SCOTT-MCKEAN, B. L. ZAMPIERI, *A. C. COSTA;
Div. of Pediatric Neurology, Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS) is the most common genetically defined cause of intellectual disability. This genetic disorder also affects the visual system in a variety of ways, which include high incidence of refractive errors, accommodative inaccuracy, amblyopia, strabismus, nystagmus, abnormal oculomotor and vestibular functions, decreased visual acuity, and decreased color and contrast sensitivities. In a previous study (Scott-McKean et al., IOVS 51: 3300- 3308, 2010), we demonstrated electrophysiologically that adult Ts65Dn mice (a murine model of DS) exhibit deficits in luminance threshold, spatial resolution, and contrast threshold, compared with euploid control mice. Here, we investigated visual thresholds of optokinetic tracking (OKT), a visual behavior that facilitates the relative stabilization of retinal images. Using the methods of Prusky el al. (IOVS 45:4611-4616, 2004), we quantified OKT thresholds of untrained, freely moving Ts65Dn and control euploid mice, daily, from eye opening (postnatal day 14; or p14) to 35 days of age, and then in longer intervals (5-10 days), until the animals were 60-day old. We found that Ts65Dn mice show a significant delay in the maturation of the visual system, which translated to detectable significant differences from p14 to p26. For example, whereas the mean spatial frequency sensitivity to a 100% contrast grating projected on a virtual cylinder for 14-day old euploid control mice was 0.20 c/deg, the mean value of this measure was 0.08 c/deg for Ts65Dn mice. Ts65Dn mice only achieved mean spatial frequency sensitivities of 0.20 c/deg at 16 days of age. At p35, the measured values of mean spatial frequency sensitivity reached mature levels at 0.46 and 0.44 c/deg for control euploid and Ts65Dn mice, respectively. The development of contrast sensitivity was also delayed in Ts65Dn mice. At 17 and 35 days of age, contrast sensitivity was 6% and 17% in Ts65Dn mice versus 9% and 26% in control mice, respectively. We tested the effect of a single dose of the NMDA-receptor antagonist memantine (5mg/kg i.p.) on Ts65Dn and control euploid mice at p17 and p35. Using this treatment, we found that memantine produces an enhancement of the mean spatial frequency sensitivity in Ts65Dn mice to control euploid levels, and does not have any significant effect in control euploid mice. We are currently attempting to replicate these behavioral-based findings with objective, *in vivo* electrophysiological experiments. Given that the observed delay in the maturation of the visual system in a mouse model of DS mimics the qualitative features of the same phenomenon seen in young persons with DS, this finding may have important therapeutic applications.

Disclosures: D.B. Victorino: None. M.R. Stasko: None. J.J. Scott-McKean: None. B.L. Zampieri: None. A.C. Costa: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.01/F5

Topic: A.07. Developmental Disorders

Support: 1R03AA021545-02

Title: Postnatal neuro-behavioral effects of prenatal ethanol exposure in a mouse model of FASD.

Authors: O. O. KOZANIAN, *K. J. HUFFMAN;
Psychology/Neuroscience, Univ. of California, Riverside, Riverside, CA

Abstract: Human ethanol consumption during pregnancy results in developmental abnormalities in offspring brain and behavior that often persist into adulthood. Fetal alcohol spectrum disorders (FASD) is an umbrella term that describes a wide range of adverse developmental conditions caused by prenatal ethanol exposure (PrEE). Children diagnosed with FASD exhibit a range of deleterious phenotypes including, but not limited to, growth retardation, facial dysmorphologies, neurological anomalies and cognitive-behavioral deficits. Given the cognitive and behavioral abnormalities present in children with FASD, the palette of PrEE-related developmental deficits in humans have led to hypotheses centering around neocortical dysfunction, a structure within the nervous system that is responsible for complex cognitive function, thought and behavior. Our laboratory was first to demonstrate the disruption of the network of intraneocortical connectivity within sensori-motor regions of neocortex in newborn PrEE mice. Specifically, disrupted targeting of ipsilateral intraneocortical connections and altered gene expression patterns were observed in newborn PrEE mice (El Shawa et al., 2013). The current study extends our laboratory's initial findings by examining whether PrEE-related phenotypes observed in newborns are maintained throughout 'childhood'. Here, we investigate whether aberrant INCs and altered gene expression patterns found in the PrEE newborn are transient or persist into pre-pubescence (P20). We also implement a battery of behavioral assays in order to correlate cortical development after PrEE with behavioral outcomes. Results indicate that PrEE-induced changes in newborn gene expression are maintained through pre-pubescence, while PrEE-induced ectopic cortical connectivity observed at P0 is rescued by P20. Although ectopic cortical connectivity is rescued by P20, pre-pubescent PrEE mice continue to show significant behavioral deficits including poor motor coordination and sensori-motor integration, as well as depression. Outcomes from this study will help identify long-term neurobiological and behavioral effects resulting from prenatal ethanol-exposure related brain dysfunction in humans with FASD.

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

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.02/F6

Topic: A.07. Developmental Disorders

Support: NSERC 6362-2012

Title: Effect of aging and fetal alcohol syndrome on retinal function

Authors: *V. HARRAR[1], L. ELKRIEF[1], J. BOUSKILA[1], R. KUPERS[1,2], A. FINK-JENSEN[3], J.-F. BOUCHARD[1], R. PALMOUR[4,5], M. PTITO[1,2,3];
[1]Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada; [2]BRAINlab, Dept. of Neurosci. and Pharmacol., [3]Lab. of Neuropsychiatry-Psychiatric Ctr. Copenhagen, Univ. of Copenhagen, Cophenhagen, Denmark; [4]Dept. of Human Genet., McGill Univ., Montreal, QC, Canada; [5]St-Kitts Behavioral Sci. Foundations, Basseterre, Saint Kitts and Nevis

Abstract: Fetal Alcohol Spectrum Disorder (FASD) is a developmental disease characterized by various behavioural problems and physical defects. FASD has been shown to affect all parts of the eye, from extra-ocular structures to the optic nerve [1]. The results of ERG studies in children suffering from FASD are similar to those carried out in aging populations [2-4]. Here, we directly compared these effects by testing Fetal Alcohol Exposure (FAE) in monkeys (*Chlorocebus sabaeus*) spanning a large age range, from 3 to 12 years old, as well as their matched controls. We measured ERGs in 78 monkeys, across 12 flash intensities in photopic conditions, and 14 flash intensities in scotopic conditions - after a period of dark adaptation. FAE and age both affected electroretinographic waves. More specifically, in photopic conditions amplitudes of a-waves and b-waves increased with age, and were higher in FAE than age matched controls. In scotopic conditions, the amplitude of the a-wave was affected by age, but the effect depended on the intensity of the flash. In contrast, the amplitude of the b-wave decreased with age in controls with little or no difference in the FAE sample, which overall had smaller b-wave amplitudes compared to controls. The differences in retinal responsiveness from these populations suggest a different maturation in the FAE population and should be followed up with anatomical explorations.

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Disclosures: V. Harrar: None. L. Elkrief: None. J. Bouskila: None. R. Kupers: None. A. Fink-Jensen: None. J. Bouchard: None. R. Palmour: None. M. Ptito: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.03/F7

Topic: A.07. Developmental Disorders

Support: NIH Grant MH103775

Title: Alterations in cognitive function in male and female rats in MAM rodent model of schizophrenia

Authors: *M. GHASEMZADEH, C. BOLDIG, K. BOLDIG, V. SCHLOEGEL, R. DIDOMINICIS, C. ALBRECHT, B. PAHLAVAN;
Dept Biomed. Sci., Marquette Univ. Dept. of Biomed. Engin., Milwaukee, WI

Abstract: Schizophrenia (SZ) is a neurodevelopmental mental disorder with devastating personal and family consequences. The MAM animal model (E17 gestational methylazoxymethanol acetate administration) is a neurodevelopmental model that displays many of the anatomical, neurochemical, and functional deficits associated with schizophrenia. We have examined the performance of MAM-treated animals (Sprague-Dawley rats) in several behavioral cognitive tasks to evaluate the extent and nature of the deficits in this model. Rats were bred on site and MAM (22 mg/kg, ip) or saline (1 ml/kg, ip) was administered on gestation day 17. Male and female offspring were housed 3-4 per cage and were provided with enrichment at all times. All animals were tested between 3-8 months of age. All efforts were made to eliminate all sources of stress in these animals during development and behavioral testing. Both male and female MAM-treated rats responded with higher locomotor activity to a novel environment. In an open field test, both male and female MAM-treated rats spent more time in the center of the arena compared to the saline-treated animals. In addition, both male and female MAM-treated animals spent more time on the open arm of an elevated plus maze compare to saline-treated rats. Surprisingly, while male MAM-treated rats engaged in higher levels of social interaction, female MAM-treated rats were not different from saline-treated animals. Both gender performed the same as the saline-treated rats in the novel object recognition task. The overall pattern of behaviors displayed by MAM animal model is suggestive of a decrease in behavioral inhibition.

Some aspects of the schizophrenia symptoms also suffer from the loss of behavioral inhibition. The data suggest that the MAM model may be useful in examining the neuronal basis of behavioral inhibition. Studies to further characterize these behavioral abnormalities and their developmental timeline are ongoing and results will be presented.

Disclosures: M. Ghasemzadeh: None. C. Boldig: None. K. Boldig: None. V. Schloegel: None. R. DiDominicis: None. C. Albrecht: None. B. Pahlavan: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.04/F8

Topic: A.07. Developmental Disorders

Support: NIH 1R01HD083001-01A1

NIH grant P51 OD011106

Title: Sevoflurane anesthesia causes apoptosis in the developing nonhuman primate brain

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Abstract: Anesthetics, sedatives and antiepileptics utilized in neonatal and pediatric medicine, can be harmful to the developing brain. They have been shown to cause widespread active cell death (apoptosis), impair synaptic maturation and plasticity and inhibit neurogenesis in the brains of rodents and nonhuman primates (NHP). Studies in rodents and in NHP have provided compelling evidence that early life exposure to these drugs also triggers behavioral toxicity (i.e., they cause long-term behavioral and cognitive deficits that persist when the animals mature). Furthermore, retrospective clinical studies raise serious concerns that exposure of human infants to these classes of drugs may lead to neurocognitive and behavioral disorders. Sevoflurane (SEVO) is becoming one of the most frequently used general anesthetics in pediatric medicine. In this study, we exposed neonatal rhesus macaque monkeys (n=2) to sevoflurane anesthesia over 5 hours according to current clinical standards in pediatric anesthesia. Brains were collected 3 hours later and examined immunohistochemically (activated caspase 3 immunohistochemistry) to analyze apoptotic neuronal and glial death. Sevoflurane anesthesia was well tolerated with

oxygen saturation, end tidal CO_2 and electrolyte levels remaining at optimal levels throughout the procedures. Brains of rhesus monkeys exposed to SEVO on postnatal days 2-6 displayed significant apoptosis in both the white and gray matter throughout the central nervous system. Areas predominantly affected included the visual, frontoparietal, cingulate and retrosplenial cortex, and the thalamus, globus pallidum and putamen. Within the white matter apoptosis was seen to affect the corpus callosum and diffusely the subcortical white matter. Approximately 50% of the dying cells were glia and 50% were neurons. Oligodendrocytes (OLs) engaged in myelinogenesis were selectively vulnerable. As expected, the apoptosis response to sevoflurane is very similar to the response to isoflurane, reported by Brambrink et al. (Ann Neurol 2012;72:525-535) in infant rhesus monkeys. Our findings indicate that exposure of the infant rhesus macaque to sevoflurane for 5 hours is sufficient to cause widespread apoptosis of neurons and OLs throughout the developing brain.

Disclosures: C. Ikonomidou: None. K. Crosno: None. V. Elam: None. S. Woelffer: None. H. Simmons: None. A. Meija: None. J.M. Hayes: None. S. French: None. K. Brunner: None. S. Capuano III: None. K. Noguchi: None. C.A. Turski: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.05/F9

Topic: A.07. Developmental Disorders

Support: KAKENHI

CREST

Title: Paternal aging-induced differential DNA methylation in sperm: possible transgenerational effects on gene expression and behavior in offspring

Authors: *R. KIMURA[1], K. YOSHIZAKI[1], T. KOIKE[2], R. YAMASHITA[3], K. KOIKE[1], T. KIKKAWA[1], H. INADA[1], Y. MATSUI[4], T. KONO[2], N. OSUMI[1];
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Abstract: Recent studies suggested that advanced paternal age could be a risk for some psychiatric diseases like autism and schizophrenia, and how paternal aging affects their offspring's health is becoming a fundamental issue. In this study, we established a model to

examine the biological basis for how paternal aging affects specific traits in their offspring. First we confirmed behavioral abnormalities in offspring derived from old male mice. The offspring showed defects in isolation induced vocal communication and sensorimotor gating function, but their social communication and repetitive behavior were comparable to those derived from young male mice. Because several literatures have indicated age-associated DNA methylation changes in sperm as a possible risk for the health problem in offspring, comprehensive targeted DNA methylome analysis was carried out using young and old mice sperm. We found in old sperm 16 hypermethylated and 96 hypomethylated genome loci, in which several autism/schizophrenia related genes are included. Further analysis revealed that profiles of these differentially methylated regions (DMRs) were similarly observed among individual sperm samples. We also revealed conserved motives potentially bind to a transcriptional repressor within hypomethylated loci. These findings suggest that age-associated DNA methylation changes may cause deterioration of transcriptional regulation of target genes/loci during brain development. We are now investigating, in the offspring's brain, expression profiles of genes related with DMRs in old sperm.

Disclosures: R. Kimura: None. K. Yoshizaki: None. T. Koike: None. R. Yamashita: None. K. Koike: None. T. Kikkawa: None. H. Inada: None. Y. Matsui: None. T. Kono: None. N. Osumi: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.06/F10

Topic: A.07. Developmental Disorders

Support: PICT 2013-1362

IBRO USCRC Travel Grant

CONICET Research Fellowship

Title: Sex-specific neuroinflammatory and sociability alterations in a mouse model of autism

Authors: *N. KAZLAUSKAS[1], M. CAMPOLONGO[2], C. ZAPPALA[2], A. DEPINO[2]; [1]Inst. For Physiology, Mol. Biol. and Ne, Argentina; [2]Inst. For Physiology, Mol. Biol. and Ne, Buenos Aires, Argentina

Abstract: Autism is a neurodevelopmental disorder characterized by decreased sociability, impaired communication and the presence of stereotyped or restrictive behaviors. These

symptoms typically appear during the first years of childhood and affect 1 in 100-200 children, with a 4:1 male:female ratio. Although many factors have been implicated in this disease, the exact underlying causes are still unclear. However, previous studies have shown a link between autism and neuroinflammation. VPA administration at GD12.5 produced decreased sociability in adult male mice, but not in females. Interestingly, we have found that adult female mice show increased micro and astroglial cell density in the cerebellum and an exacerbated peripheral inflammatory response upon a LPS challenge. However, these alterations are not present in males. We hypothesized that there is a developmental critical window in which maturation and consolidation of the neural systems responsible for autism symptoms typically occur. In order to define this temporal window, we characterized the peripheral and neuroinflammatory state of female mice from P7 to P42. We found glial alterations in the hippocampus and cerebellum of VPA mice at early ages (P21, P28 and P35). We then administered LPS during this period (P21 to P35) to further explore the direct effect of neuroinflammation on sociability in male and female mice. We found that eliciting postnatal inflammation produces distinct behavioral outcomes depending on sex.

Disclosures: N. Kazlauskas: None. M. Campolongo: None. C. Zappala: None. A. Depino: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.07/F11

Topic: A.07. Developmental Disorders

Support: HD 080910

Title: The metabolic effects of creatine loss in adult creatine transporter conditional knockout mice

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Abstract: Creatine (Cr) transporter (CrT) deficiency (CTD) is a leading cause of X-linked intellectual disability. CTD is characterized by a lack of brain Cr, severe intellectual disability and aphasia. We generated mice with exons 2-4 of *SLC6A8* flanked by *loxP* sites ($CrT^{flox/flox}$). Ubiquitous CrT knockout mice ($CrT^{-/y}$) and conditional knockout mice (cKO) generated from this line exhibit cognitive and metabolic deficits, mimicking the human disorder. While it is clear that Cr is essential for proper cognitive and metabolic function as evidence by cognitive deficits

present in $CrT^{-/y}$ mice, it is still unknown if these deficits result from an absence of Cr during development or the persistent absence of Cr. A better understanding of the role of Cr in cellular metabolism is essential for the development of treatments and treatment strategies for CTD. The purpose of this study is to determine if the lack of Cr leads to changes in whole body metabolism in adult CrT knockout mice. To better understand if the metabolic deficits in $CrT^{-/y}$ mice are due to developmental changes resulting from an absence of cellular Cr, the CrT was deleted in adult mice. $CrT^{flox/flox}$ mice were crossed with mice expressing a ubiquitous tamoxifen-inducible Cre recombinase to create a temporally mediated CrT knockout mouse (uKO). Recombination was initiated by tamoxifen administration from postnatal day 60-65. Brain CrT transcripts were undetectable 5 days following Cre recombinase induction. Following the induction of Cre recombinase activity, cKO mice exhibit a significant reduction in body weight compared to wildtype (WT) mice. Following validation of the knockout, cognitive and metabolic function were assessed based on deficits observed in the $CrT^{-/y}$ mice. In the visible platform testing, cKO mice show no significant difference in latency to the platform compared with controls. There were no differences between groups in latency and path length during the Morris Water Maze (MWM). No reference memory deficits were present. Control and cKO mice performed similarly in tasks of object recognition and fear memory. Oxygen consumption and carbon dioxide waste were measured for a 24 hour period using the Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS). The adult uKO mice showed no observable differences in oxygen consumption and carbon dioxide waste compared to controls. The absence of cognitive and metabolic deficits in the adult uKO mice suggest that the deficits seen in the $CrT^{-/y}$ mice are likely due to a lack of Cr during critical points in development.

Disclosures: K.C. Udobi: None. M.R. Skelton: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.08/F12

Topic: A.07. Developmental Disorders

Support: Polish National Science Centre Grant DEC-2014/15/N/NZ4/04844

Title: The influence of the electrical stimulation of the raphe magnus on neuronal structure of the serotonergic system in rats

Authors: *K. K. PTASZEK, K. PLUCINSKA, G. JERZEMOWSKA, E. JURKOWLANIEC; Dept. of Animal and Human Physiol., Univ. of Gdansk, Fac. of Biol., Gdansk, Poland

Abstract: Serotonin (5-HT) is the endogenous amine present in the tissue of vertebrates, invertebrates and even the plants. In humans, 5-HT is located in the blood platelets, intestine cells and brain, mainly in the raphe nuclei and pineal gland. The serotonergic system develops earliest of the neurotransmitter systems and become the most expanded, linking together many regions in the brain. The influence of 5-HT on the organism is strictly connected with the interaction between this neurotransmitter and the specific serotonergic receptors.
Our previous study indicated that electrical stimulation of the raphe magnus (RMg) increased rats locomotor, anxious and reduced sociability. Now, we would like to verify the influence of the RMg stimulation on serotonergic neurons in different brain structures, especially those that are responsible for previously observed rats behaviors.
Male Wistar rats (3-months old) were implanted with electrodes into the RMg. After 10-day convalescence, 13-day electrical stimulation was performed with the stimulation current determined individually for each animal (60-140 μA). Rats were divided into stimulated (n=6) and non-stimulated (sham; n=7) groups. Every day, each animal had 25 stimulation trials (30-s stimulation followed by 20-s break). One hour after the last stimulation, rats were sacrificed, their brains were perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). Next, brains were removed and stored in the PFA for 24 h, then for 48 h in 30% sucrose. After that the frozen brains were cut into 30 μm thick sections and collected slices were stained during immunofluorescence procedure, especially were incubated with primary antibody to 5-HT (*ab66047*, Abcam, UK) and probed with secondary antibody (*ab150129*, Abcam, UK). Images were taken by fluorescent microscope (*PrimoStar*, Carl Zeiss, Germany) and processed using the imaging software (*Axio Vision*).
Results were analyzed as total numbers of 5-HT positive cells per structure at magnification 100 × 10. The RMg stimulation increased number of 5-HT cells in hypothalamic nuclei: arcuate nucleus (77 vs. 56 in sham) and periventricular nucleus (65 vs. 0), motor cortex (63 vs. 36), reticulotegmental nucleus of the pons (16 vs. 12) and RMg (44 vs. 29). In turn, the number of 5-HT cells were decreased in supraoptic nucleus (31 vs. 41), reticular thalamic nucleus (9 vs. 22) and hippocampus (34 vs. 47).
Summarizing, the RMg stimulation affected the serotonergic projections either to the limbic structures or motor cortex. It can be assumed that hyperactivity of the serotonergic system may correlate with behaviors observed in different disorders, such as Autism Spectrum Disorder.

Disclosures: **K.K. Ptaszek:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; This project was financed by the National Science Centre Poland on the basis of decision DEC-2014/15/N/NZ4/04844. **K. Plucinska:** None. **G. Jerzemowska:** None. **E. Jurkowlaniec:** None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.09/F13

Topic: A.07. Developmental Disorders

Support: Support from The Autism Research Foundation

Support from The Wallace Research Foundation

Title: GABA receptor involvement in sensorimotor gating in the rat model: an autoradiography study

Authors: E. T. CHOW[1], M. FAZAL[1], C. R. CLANCY[1], J. SKEFOS[1], E. D. LEVIN[2], *M. L. BAUMAN[1];
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Abstract: Sensorimotor gating relates to the process of filtering irrelevant external and internal stimuli, in order to modulate a motor response to salient stimuli. Sensorimotor gating can be gauged behaviorally by indexing prepulse inhibition (PPI), a measure of startle response suppression to a strong sensory stimulus (e.g., 110 dB acoustic pulse) when preceded by a weaker sensory stimulus (e.g., 68-77 dB acoustic pre-pulse). Abnormal sensorimotor gating has been implicated in many neurological disorders, including autism spectrum disorders, sensory processing disorders and schizophrenia. The neural circuitry underlying PPI is complex and has been shown to include dopaminergic, GABAergic, cholinergic and histaminergic systems. Our prior work in a rat model explored the role of cholinergic/histaminergic systems in PPI. We utilized the NMDA glutamate receptor antagonist, dizocilpine, to induce PPI impairment, and administration of the histamine H1 receptor antagonist, pyrilamine, to reverse this PPI impairment, as had been previously demonstrated. In this autoradiographic study, we extend these findings by investigating potential GABAergic involvement in the PPI disruption induced by treatment with dizocilpine, as well as PPI improvement induced by treatment with pyrilamine. We explore key brain regions that participate in the sensorimotor gating network, e.g., the basolateral amygdala (BLA), anterior cingulate cortex, insular cortex, hippocampus and striatum. Four treatment groups were studied, with 9 Sprague-Dawley rats per group. Control animals were administered saline, and treatment animals were administered either dizocilpine, pyrilamine, or the combination of dizocilpine and pyrilamine for 4 weeks. Specific binding of the GABA-A and GABA-B receptors was determined using [3H]Muscimol and [3H]CGP54626, respectively. Preliminary results found no differences in GABA-A/B receptor binding density between the PPI impaired treatment groups with and without pyrilamine in the BLA ($p=0.62$), although GABA-B receptor density was significantly decreased in dizocilpine-exposed groups in

the BLA (p=0.01). GABA-B receptors may thus play a mechanistic role in dizocilpine's effect on PPI in this model. Further studies are ongoing to explore additional brain regions and confirm the role of GABA in PPI impairment/recovery, which may aid in the development of treatments for disorders such as autism, where GABAergic abnormalities are widely reported.

Disclosures: E.T. Chow: None. M. Fazal: None. C.R. Clancy: None. J. Skefos: None. E.D. Levin: None. M.L. Bauman: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.10/F14

Topic: A.07. Developmental Disorders

Title: Inheritance of neocortical molecular layer heterotopia

Authors: *A. R. TOIA[1], J. CUOCO[1], A. JOSHI[1], J. AHSAN[1], G. TORRES[1], V. BOLIVAR[2], R. RAMOS[1];
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Abstract: Neocortical molecular layer heterotopia (MLH) in mouse models, such as C57BL/6 inbred mice, contain diverse cellular and axonal constituents. First, diverse excitatory neurons are present in heterotopia, including neurons normally destined for deep (layers VI and V) and superficial layers (layers II and III) as well as layer IV. These data indicate that pyramidal neurons born throughout corticogenesis are found in MLH. Second, diverse GABAergic neurons are present in heterotopia including those born in distinct regions of the ganglion eminence. Third, all three types of glial cells are found in MLH including astrocytes, oligodendrocytes, and microglia; however, there is no evidence of reactive astrocytes or activated microglia. Finally, diverse projections to and from MLH are present including those from brainstem nuclei as well as intracortical and callosal connections.
The genetic mechanisms of MLH formation are poorly understood and the extent to which MLH are present in other inbred strains of mice is unclear. In the present report, a neuroanatomical survey was performed of the neocortex of 7 widely-used inbred mouse strains for the presence of MLH, including strains commonly used in the production of genetically-engineered mice. In order to understand the basic principles of inheritance of MLH in C57BL/6 mice, the presence of heterotopia was examined in 2 first filial generation (F1) hybrids, numerous different recombinant inbred strains, one consomic strain, and several genetically-engineered mouse lines. Our results indicate that MLH formation is a weakly penetrant trait which requires homozygosity

of one or more C57BL/6 alleles outside of chromosome 1. These data are relevant for understanding normal neocortical development and the mechanisms of cortical lamination. In light of the fact that C57BL/6 mice are the most popular strain used as animal models of neurological disorders, our results also have broad implications for the use of these mice in diverse studies as well as in the creation of knockout and transgenic mice.

Disclosures: A.R. Toia: None. J. Cuoco: None. A. Joshi: None. J. Ahsan: None. G. Torres: None. V. Bolivar: None. R. Ramos: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.11/F15

Topic: A.07. Developmental Disorders

Title: Magnetic resonance and diffusion tensor imaging of basal ganglia circuitry in an animal model of repetitive behavior

Authors: B. J. WILKES[1], L. M. COLÓN-PÉREZ[2], A. M. MUEHLMANN[2], M. FEBO[2], *M. H. LEWIS[2];
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Abstract: Restricted, repetitive behavior is a prominent feature of a number of neurodevelopmental disorders and diagnostic for autism spectrum disorder (ASD). Our lab utilizes an inbred mouse strain (C58) that demonstrates robust repetitive motor behavior phenotype (vertical jumping, backward somersaulting). Previous histochemical work in our lab suggests that alterations in basal ganglia circuitry mediate the expression of this repetitive behavior. We previously found reduced cytochrome oxidase (CO) expression, a marker of cellular activity, and decreased dendritic spine density in the sub-thalamic nucleus (STN) in animals with high levels of repetitive behavior (Tanimura et al., 2011). Furthermore, animals that exhibit attenuated repetitive behavior following rearing in an enriched environment show increased CO and dendritic spine density in the globus pallidus (GP) and STN, compared to standard housed controls (Bechard et al., 2016). In order to further investigate the role of basal ganglia circuitry alterations in repetitive behavior, we performed *ex vivo* magnetic resonance imaging (MRI) on C58 mice and C57BL/6 controls. We used 3D FLASH gradient echo and 2D diffusion weighted spin echo scans to assess regional morphology and structural connectivity, respectively. Volumetric comparisons and probabilistic fiber tracking were performed for structures of the basal ganglia and the associated white matter tracts. Consistent with our hypothesis of indirect pathway dysfunction, we found significantly smaller volumes of the GP

and STN in C58 mice, as well as trends for lower fractional anisotropy and mean diffusivity in fiber tracts connecting the GP and STN. These findings are consistent with human neuroimaging findings indicating altered basal ganglia morphology that is correlated with repetitive behavior symptoms in ASD (Herbert et al., 2003; Hollander et al., 2005; Langen et al., 2009). These findings provide hypotheses that can be tested using diffusion tensor imaging investigations of intra basal ganglia connectivity in ASD and related neurodevelopmental disorders.

Disclosures: B.J. Wilkes: None. L.M. Colón-Pérez: None. A.M. Muehlmann: None. M. Febo: None. M.H. Lewis: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.12/F16

Topic: A.07. Developmental Disorders

Support: Swebilius

NINDS grant R21 R21NS093510

NINDS grant R15NS072879

Citizens United for Research in Epilepsy

R01 MH-067528

P30 NS-052519

NINDS grant K08NS069667

Title: Convulsive seizures from experimental focal cortical dysplasia occur independently of cell misplacement

Authors: *L. S. HSIEH[1], J. WEN[1], K. CLAYCOMB[2], Y. HUANG[2], F. HARRSCH[3], J. NAEGELE[3], F. HYDER[2], G. BUCHANAN[4], A. BORDEY[1];
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Abstract: Focal cortical dysplasia (FCD), a local malformation of cortical development, is the most common cause of pharmacoresistant epilepsy associated with life-long neurocognitive impairments. It remains unclear whether neuronal misplacement is required for seizure activity.

Here we show that dyslamination and white matter heterotopia are not necessary for seizure generation in a murine model of type II FCDs. These experimental FCDs generated by increasing mTOR activity in layer 2/3 neurons of the medial prefrontal cortex are associated with tonic-clonic seizures and a normal survival rate. Preventing all FCD-related defects, including neuronal misplacement and dysmorphogenesis,with rapamycin treatments from birth eliminate seizures, but rapamycin withdrawal is followed by the recurrence of seizures. In addition, bypassing neuronal misplacement and heterotopia using inducible vectors ddo not prevent seizure occurrence. Collectively, data obtained using our new experimental FCD-associated epilepsy suggest that life-long treatment to reduce neuronal dysmorphogenesis is required to suppress seizures in individuals with FCD.

Disclosures: L.S. Hsieh: None. J. Wen: None. K. Claycomb: None. Y. Huang: None. F. Harrsch: None. J. Naegele: None. F. Hyder: None. G. Buchanan: None. A. Bordey: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.13/F17

Topic: A.07. Developmental Disorders

Title: Late adolescent shift from local to distributed monosynaptic inputs onto fronto-posterior cortical projection neurons.

Authors: *E. NABEL[1], M. DEMARS[2], S. LOPEZ[2], G. TACCHERI[2], H. KOIKE[2], H. MORISHITA[2];
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Abstract: Visual attention, the cognitive ability that enhances the perception of selected stimuli, develops postnatally and is disrupted in neurodevelopmental disorders such as autism. A direct long-range projection from the anterior cingulate cortex (ACC) to primary visual cortex is a key circuit mediating “top-down” modulation of visual processing, which is a hallmark component of attention. However, little is known about this projection’s input sources, and if inputs undergo developmental modifications. In this study, an intersectional viral genetic technique was employed to map the monosynaptic inputs onto top-down cells in adolescence and adulthood. Whole brain maps of top-down inputs were generated, and regions of interest were further characterized by cell type. Cortical areas contributed the majority of monosynaptic inputs onto top-down cells. Local inputs from the ACC and secondary motor area comprise the large source; other major sources of cortical contributions include the retrosplenal, prelimbic, and infralimbic

cortex. Non-cortical regions provide distributed inputs from the basal forebrain, dorsal thalamus, and hypothalamus. Inputs from the basal forebrain, the major neuromodulatory source for this projection, comprise of approximately 80% cholinergic, 14% putative glutamatergic, and 6% inhibitory neurons. Comparison of the adult and adolescent whole brain maps revealed a larger number of inputs from local cortical regions during adolescence lost in adulthood. No age-related differences were observed in other cortical and non-cortical areas. This result suggests that local pruning during late adolescence causes a relative shift in input weight from local to distal brain regions between adolescence and adulthood. This cortical maturation may be required to establish effective top-down control of attention.

Disclosures: E. Nabel: None. M. Demars: None. S. Lopez: None. G. Taccheri: None. H. Koike: None. H. Morishita: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.14/F18

Topic: A.07. Developmental Disorders

Support: P50MH096890

R01MH104341

R21MH106919

R01 EY026053

R01 EY024918

5T32MH087004-07

Title: Chemogenetic targeting of prefrontal parvalbumin interneurons affects social behavior in mice

Authors: *L. BICKS[1], H. KOIKE[1], C. MILLER[3], M. PENG[1], S. AKBARIAN[2], H. MORISHITA[2];
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Abstract: Social processing is a domain that is commonly dysregulated in psychiatric disorders, and is poorly treated by available psychiatric medications. In humans and rodents, portions of the

evolutionarily conserved medial prefrontal cortex (mPFC) are part of a network that regulates social behavior. Many disorders with shared social processing deficits show impairments in inhibitory neurotransmission within the brain, particularly in the mPFC, suggesting a role for PFC inhibitory action in regulating social behavior. We investigated the role of prefrontal parvalbumin (PV) interneurons, a major class of cortical inhibitory neurons, in social behavior of adult mice by leveraging chemogenetic technologies. We selectively expressed hM4Di, an inhibitory DREADD (Designer Receptor Exclusively Activated by Designer Drugs), in PV interneurons in the mPFC. Acute selective suppression of mPFC PV interneurons decreased sociability in a 3-chamber test and disrupted social recognition in a habituation-dishabituation paradigm. Suppression of PV interneurons did not affect spatial working memory, olfactory discrimination, or anxiety-related behaviors, suggesting a specific effect of PV interneuron suppression on social behavior. These results demonstrate that PV interneuron activity in the mPFC is necessary for appropriate social behavior in mice.

Disclosures: L. Bicks: None. H. Koike: None. C. Miller: None. M. Peng: None. S. Akbarian: None. H. Morishita: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.15/F19

Topic: A.07. Developmental Disorders

Support: NINDS Grant P30NS069266

NEI Grant R00EY019547

Title: Minor spliceosome inactivation in the mouse pallium causes progenitor cell death and microcephaly

Authors: *A. OLTHOF[1], M. BAUMGARTNER[1], C. LEMOINE[1], S. AL SEESI[2], K. HYATT[1], N. STURROCK[1], N. NGUYEN[1], K. DRAKE[1], R. GOZ[1], J. LOTURCO[1], R. KANADIA[1]; [1]Physiol. and Neurobio., [2]Computer Sci. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Microcephaly in microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1) is linked to U4atac mutations. U4atac is a crucial component of the minor spliceosome, which is responsible for splicing of <0.5% of introns. This suggests that inactivation of the minor spliceosome can lead to loss of expression of minor intron-containing genes (MIGs). To test whether this is the underlying cause for the MOPD1 phenotype, we ablated the U11 snRNA,

another crucial component of the minor spliceosome, in the mouse dorsal telencephalon. Here we report that minor spliceosome inactivation in the developing neocortex resulted in microcephaly. We found that loss of U11 caused death of self-amplifying neural progenitor cells (NPCs) at E12, due to defects in mitosis. Intermediate progenitor cells (IPCs) were not produced in the U11 knockout mouse. In contrast, neuron-producing NPCs were unaffected, since U11-null neurons were produced and survived postnatally. RNAseq data revealed drastic downregulation of non-coding RNAs, specifically small nucleolar RNAs (snoRNAs) in the U11 knockout mouse. No transcriptional changes in MIGs were observed, except for *Spc24*, which is a MIG involved in kinetochore assembly. Since we disrupted the minor spliceosome activity, we tested for splicing efficiency. We found that loss of U11 caused defective minor intron splicing of MIGs, leading to minor intron retention. This could introduce premature stop codons and degradation of the transcript by the non-sense mediated decay (NMD) pathway. However, we observed that the MIG *Upf1,* an essential component for the NMD pathway, also has increased minor intron retention in the U11 knockout mouse. This might cause inactivation of the NMD pathway, explaining why the majority of the MIGs did not change in their net expression. In all, we show the underlying molecular and cellular defects caused by inactivation of the minor spliceosome in the developing mouse neocortex. These findings reveal a potential mechanism for the pathogenesis of MOPD1.

Disclosures: A. Olthof: None. M. Baumgartner: None. C. Lemoine: None. S. Al Seesi: None. K. Hyatt: None. N. Sturrock: None. N. Nguyen: None. K. Drake: None. R. Goz: None. J. LoTurco: None. R. Kanadia: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.16/F20

Topic: A.07. Developmental Disorders

Support: EBIRE

Title: Missense polymorphisms in the brain-derived neurotrophic factor binding region of nTRK2 in randomly selected pools of human trace DNA

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Abstract: The NTRK2 gene encodes a high affinity transmembrane receptor for brain-derived neurotrophic factor (BDNF). Binding of BDNF to NTRK2 triggers numerous biological responses in multiple systems including nervous, immune and skeletal systems. The extracellular BDNF binding site of NTRK2 is known from mutation analysis, crystallography, and functional studies. The binding site is encoded by three conserved exons of the NTRK2 gene - coding exons 6, 7, and 8 spanning amino acids 196 - 386. Numerous single nucleotide polymorphisms have been identified in the NTRK2 gene, among them 199 missense SNPs in the coding region. So far only 1 SNP has been clearly associated with a phenotype, an A to G transition that changes the functionally critical tyr722 in the intracellular kinase domain into cys722. The remaining SNPs have not yet been associated with any phenotypes nor have their distributions in non-selected populations been determined. We focused on exons 6-10 because changes in these regions may affect binding of BDNF, intracellular signaling, and consequently downstream effector pathways during development, adulthood, and aging.
To identify known and new SNPs in anonymous phenotypically unselected populations we collected samples from commonly touched objects in our healthcare setting and extracted trace DNA using a kit from Norgen BioTek. The extracted DNA was then amplified using primer pairs that spanned each of the target exons. The experimental system was verified by nucleotide sequencing of DNA amplified from saliva of 12 anonymous volunteer subjects. Using this procedure, we successfully amplified NTRK2 exons from trace DNA obtained by saline rinses of door and cabinet handles, computer mice, and telephone touch pads. We are in the process of sequencing these samples and searching their sequences for SNPs. Future efforts will expand the sample collection to public objects, developing linear statistical analyses of nucleotide sequences from pooled DNA to identify potential SNPs, and functional in vitro and in silico studies of the effects of missense SNPs in exons 6-10 on BDNF binding and signaling.

Disclosures: G. Heinrich: None. I. Yao: None. A.J. King: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.17/F21

Topic: A.07. Developmental Disorders

Support: CIHR MOP119421

NSERC RGPIN341498

China 973 Program 2012CB517903

NSFC 31571040

CIHR Joint Health Research Initiative Program 81161120543

Title: PAK1(p21-activated kinase 1) regulates GABAergic synaptic transmission via endocannabinoid signaling in mouse hippocampus

Authors: *S. XIA[1], Z. ZHOU[1], C. LEUNG[2,3], Y. ZHU[1], X. PAN[1], J. QI[1], M. MORENA[4], M. HILL[4], W. XIE[1], Z. JIA[2,3];
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Abstract: Normal brain function, including learning and memory, requires balanced excitation and inhibition, but the mechanisms underlying such balance remain unclear. p21-activated kinases (PAKs) are a family of protein kinases known to play critical roles in neuronal development, synaptic regulation and behaviors. In addition, recent studies show that PAK inhibitors can rescue the neurobiological and behavioral deficits in the mouse models of a number of neurodevelopmental disorders including autism and schizophrenia. However, how PAKs impact synaptic function and mental dysfunction remains elusive. In this study, we have found that sustained kinase activity of PAK1, a major form of PAKs expressed in the brain, is indispensable for a proper excitation/inhibition ratio in the hippocampus and this function of PAK1 is mediated by affecting GABAergic synaptic transmission and the retrograde endocannabinoid signaling. These results suggest a novel process by which PAK1 regulates brain function. This study also provide potential therapeutic targets to treat related brain disorders.

Disclosures: S. Xia: None. Z. Zhou: None. C. Leung: None. Y. Zhu: None. X. Pan: None. J. Qi: None. M. Morena: None. M. Hill: None. W. Xie: None. Z. Jia: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.18/F22

Topic: A.07. Developmental Disorders

Title: Comparison of seric and cerebroventricular GLP-1 levels induced by ileal interposition in wistar male rats

Authors: *M. S. SALGADO[1], H. L. HERNÁNDEZ MONTIEL[2], J. C. SOLÍS SÁINZ[3], P. GARCÍA SOLÍS[4], N. G. HERNÁNDEZ CHAN[2], M. RAMOS GÓMEZ[2], M. C. ABURTO FERNÁNDEZ[2];
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Abstract: Glucagon-like peptide 1 (GLP-1) is a hormone synthesized in gut by L cells and in brain by Preproglucagon neurons. GLP-1 binds to a specific receptor with a ubiquitous localization and with multiples effects. It is known that GLP-1 secretion is induced by the presence of food bolus in gut and also an indirect activation on its secretion can be induced by neuroendocrine mechanisms. A significant increase in GLP-1 circulating levels has been observed in patients after ileal interposition surgery. A neuroprotective effect induced by GLP-1 has been reported in neurodegenerative alterations like Alzheimer and Parkinson disease. Our hypothesis is that after ileal interposition surgery GLP-1 levels will increase both in serum and cerebroventricularly. Ileal interposition surgery consists in the transposition of the ileal proximal segment at the level of the ligament of Treitz, in order to enhance the contact of food bolus with intestinal L cells. Male Wistar rats (350-400g) GLP-1 levels in serum and cerebroventricular fluid will be analyzed after three weeks of the ileal interposition surgery by enzyme-linked immunoassays. The aim of this work is to evaluate the degree of peripheral and cerebroventricular elevation of GLP-1 levels after Ileal interposition surgery.

Disclosures: M.S. Salgado: None. H.L. Hernández Montiel: None. J.C. Solís Sáinz: None. P. García Solís: None. N.G. Hernández Chan: None. M. Ramos Gómez: None. M.C. Aburto Fernández: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.19/F23

Topic: A.07. Developmental Disorders

Support: NIH 1R21NS091724-01

Title: DEAF1 regulates expression of UBE2M

Authors: P. J. JENSIK[1], S. RAJAMANICKAM[1], L. A. ARBOGAST[1], *G. M. ROSE[2], M. W. COLLARD[1], J. I. HUGGENVIK[1], S. R. MCGEE[1];

[1]Southern Illinois Univ. Sch. of Med., Carbondale, IL; [2]Neurosci. Res. Ctr., Southern Illinois Univ., Carbondale, IL

Abstract: Deformed epidermal autoregulatory factor 1 (DEAF1) is a transcription factor that binds TTCG motifs and acts as either a transcriptional repressor or activator on target gene expression. Several mutations in the DNA binding domain of the *DEAF1* gene have been identified in individuals with moderate to severe intellectual disabilities. Mice with conditional knockout of *Deaf1* in the brain (NKO mice) have altered cognitive function indicating DEAF1 has conserved roles in learning and memory. The objective of this study was to identify DEAF1 target genes. RNA-sequencing, using RNA isolated from hippocampal tissue of NKO and WT mice, identified 348 genes that were differentially expressed in the hippocampus of NKO mice. Ubiquitin-Conjugating Enzyme E2M (UBE2M, also referred to as UBC12) acts as a NEDD8 conjugation enzyme and the expression of *Ube2m* was reduced 2-fold in the hippocampus of NKO mice compared to WT mice. Similar reductions in *Ube2m* expression were also observed in RNA isolated from frontal cortex and cerebellum tissue of NKO mice. In order to ascertain if *UBE2M* is a direct target of DEAF1 transcriptional regulation, the CRISPR-Cas9 system was used to generate a DEAF1 knockout (KO) human cell line. Compared to control lines, there was a similar 2-fold reduction in both *UBE2M* RNA expression and UBE2M protein levels in the DEAF1-KO cell lines. A conserved putative DEAF1 TTCG-containing DNA consensus sequence was identified in the mouse and human *UBE2M* promoter. Chromatin immunoprecipitations (ChIP) indicate DEAF1 is bound to the *UBE2M* promoter in mouse neuronal and human neuronal and kidney cell lines. DEAF1 interactions with the *UBE2M* promoter were not detected in the DEAF1-KO cell line demonstrating specificity of the DEAF1 antibody in ChIP analysis. A dsDNA probe encompassing the *Ube2m* promoter putative DEAF1 consensus sequence was generated and used in electrophoretic mobility shift assays to determine if DEAF1 can bind directly to this sequence. The influence of an identified human DEAF1 mutation p.Q264P and corresponding mouse p.Q265P mutation on DEAF1 interactions with this putative consensus sequence was also assessed. Both purified mouse and human DEAF1 proteins bound to the *Ube2m* promoter DEAF1 consensus sequence; however the human p.Q264P and mouse p.Q265P DEAF1 mutant proteins did not bind to the consensus sequence. Taken together, these data indicate that DEAF1 regulates *UBE2M* expression and changes in DEAF1 activity may have resultant effects on UBE2M levels and the neddylation pathway.

Disclosures: P.J. Jensik: None. S. Rajamanickam: None. L.A. Arbogast: None. G.M. Rose: None. M.W. Collard: None. J.I. Huggenvik: None. S.R. McGee: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.20/F24

Topic: A.07. Developmental Disorders

Support: NIH Grant MH18501

Title: Inositol hexakisphosphate kinase-1 physiologically associates with alpha actinin and regulates focal adhesion kinase activity

Authors: *C. FU, J. XU, A. M. SNOWMAN, S. H. SNYDER;
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Abstract: Inositol hexakisphosphate kinase-1 (IP6K1), which generates inositol pyrophosphates, physiologically mediates numerous functions, such as insulin secretion and histone demethylation. IP6K1 deleted mice are small and males are sterile. Here, we report that IP6K1 physiologically associates with alpha-actinin, and localizes in the focal adhesion complex. IP6K1 deletion elicits a severe deficit of focal adhesion kinase (FAK) activity. IP6K1 deleted cells display substantial decreases of FAK phosphorylation, as well as impaired spreading and migration delay. Regulation of FAK by IP6K1 requires its kinase activity. Pharmacologic inhibition of IP6K by TNP recapitulates the phenotype of IP6K1 deletion. This study demonstrates that IP6K1 physiologically regulates focal adhesion kinase activity and cell migration via kinase dependent mechanisms.

Disclosures: C. Fu: None. J. Xu: None. A.M. Snowman: None. S.H. Snyder: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.21/F25

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: PKU Academy

CNPq

UNESC

Title: Hyperphenylalaninemia causes alterations in BDNF signaling in brain of rats

Authors: *P. F. SCHUCK[1], F. MALGARIN[2], G. SCAINI[2], L. M. GOMES[2], M. CARVALHO-SILVA[2], L. W. KIST[4], S. O. MARQUES[3], T. F. LUCIANO[3], T. P. MACAN[2], R. S. MARTINS[5], C. T. SOUZA[3], R. C. C. KUBRUSLY[5], M. R. BOGO[4], G. C. FERREIRA[6], E. L. STRECK[2]; [1]Lab. de Erros Inatos do Metabolismo, Univ. Do Extremo Sul Catarinense, Criciuma, Brazil; [2]Lab. de Erros Inatos do Metabolismo, [3]Lab. de Fisiologia e Bioquimica do Exercicio, Univ. do Extremo Sul Catarinense, Criciuma, Brazil; [4]Laboratório de Biologia Genômica e Mol., Pontificia Unidade Catolica do Rio Grande do Sul, Porto Alegre, Brazil; [5]Laboratório de Neurofarmacologia, Univ. Federal Fluminense, Rio de Janeiro, Brazil; [6]Inst. de Bioquimica Medica Leopoldo de Meis, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Hyperphenylalaninemia (HPA) is the hallmark of phenylketonuria (PKU), a genetic disorder caused by the deficiency of phenylalanine (Phe) hydroxylase. Brain injury is a clinical characteristic of PKU patients, although the pathophysiology of this damage is poorly understood. The aim of the present work was to investigate the brain-derived neurotrophic factor (BDNF) levels in brain of rats submitted to an experimental model of HPA. In this study, animals received a single subcutaneous administration of 0.9 % NaCl (control group) or 5.2 μmol/g Phe plus 0.9 μmol/g p-chlorophenylalanine (HPA group). One hour after the administration, the animals were euthanized by decapitation. The brain structures cerebral cortex, striatum and hippocampus were isolated and homogenized and BDNF levels, pro-BDNF, tropomyosin-related kinase B (TrkB) receptor and $p75^{NTR}$ relative messenger RNA levels, total and phosphorylated c-Jun N-terminal kinase (JNK), total and phosphorylated protein kinase C (PKC), tissue-type plasminogen activator (tPA) and p11 immunocontent were determined. It was observed that animals subjected to acute HPA presented decreased BDNF levels in cerebral cortex, hippocampus and striatum, while pro-BDNF mRNA levels were increased in striatum. Furthermore, phosphoPKC/PKC ratio was decreased in cerebral cortex and hippocampus of HPA group, probably due to the decrease of BDNF signaling. On the other hand, TrkB and $p75^{NTR}$ (BDNF and pro-BDNF receptors, respectively) mRNA expression, and tPA, p11 and phosphoJNK/JNK immunocontent were not altered by high Phe levels in any structure. Taken together, our results suggest that Phe induces alteration in BDNF homeostasis. Since this neurotrophic factor plays a fundamental role in brain development and plasticity, contributing to synaptogenesis, synaptic plasticity, cognitive functions and memory, it is tempting to speculate that BDNF alterations might contribute to the intellectual deficiency observed in PKU patients.

Disclosures: P.F. Schuck: None. F. Malgarin: None. G. Scaini: None. L.M. Gomes: None. M. Carvalho-Silva: None. L.W. Kist: None. S.O. Marques: None. T.F. Luciano: None. T.P. Macan: None. R.S. Martins: None. C.T. Souza: None. R.C.C. Kubrusly: None. M.R. Bogo: None. G.C. Ferreira: None. E.L. Streck: None.

Poster

214. Animal Models of Neurodevelopmental Disorders: Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 214.22/F26

Topic: A.07. Developmental Disorders

Support: NIH Grant EY021624

Title: Transcriptomic analysis of retinal pericytes exposed to high glucose and Notch signaling

Authors: *A. I. MACHUCA PARRA, J. D. LAM, L. A. KIM, J. F. ARBOLEDA-VELASQUEZ;
Ophthalmology, Schepens Eye Res. Inst., Boston, MA

Abstract: Notch signaling pathway is highly conserved and essential for controlling cell fate decision and tissue patterning during development. The Notch 3 receptor is mainly expressed in mural cells, which include pericytes and vascular smooth muscle cells. In humans, Notch 3 malfunction has been related to Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). These patients develop arteriopathy, chronic ischemic degeneration of neurons and glia, vascular smooth muscle cell degeneration and the appearance of granular osmiophilic deposits in vessels. Diabetic Retinopathy (DR) is a progressive degeneration of the neurovascular unit in the retina and the leading cause of permanent vision loss worldwide. Interestingly, CADASIL and DR both share a key pathological feature: the progressive loss of mural cells, which causes the destabilization of the vasculature and endothelial dysfunction. Since little is known about the gene expression of pericytes in CADASIL and DR, we prepared co-cultures of bovine retinal pericytes (BRPs) and human embryonic kidney cells (HEK) expressing the Delta1-GFP receptor with normal (5 mM) and high glucose (20 mM). Delta1-GFP expression is controlled by a tetracycline inducible promoter in our cell line. For BRPs-HEK co-cultures, we tested four conditions including: normal glucose with Tetracycline (1 μg/ml) and without Tetracycline (controls) and high glucose with and without Tetracycline. BRPs were isolated after coculture by FACS followed by total RNA isolation and transcriptomic analysis using RNA sequencing. Data was analyzed using Partek Flow software. Whole transcriptomic profiles were constructed for all four conditions. Principal component analysis (PCA) of the transcriptomes demonstrated similar expression profiles for control samples (high glucose without Tetracycline) while the profiles of the high glucose with Tetracycline samples were broadly dispersed, indicating significance gene expression changes. High glucose with Notch activation was associated with significantly elevated Notch-related genes including PTP4A3 (54,794 fold increase, $p= 2.11x10^{-4}$), SMTN (32,475 fold increase, $p= 4.14x10^{-5}$), and BAD (13,219 fold increase, $p= 7.91x10^{-7}$). These findings suggest a relationship

between high glucose and Notch signaling. This approach will help to identify molecular targets and signature Notch genes related to pericyte function and survival in CADASIL and DR.

Disclosures: A.I. Machuca Parra: None. J.D. Lam: None. L.A. Kim: None. J.F. Arboleda-Velasquez: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.01/F27

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

Support: NIH Grant R01-MH091424

Title: Regulatory control of microglial phagocytosis in the developing rat cerebellum.

Authors: *M. PEREZ-POUCHOULEN, S. J. YU, M. M. MCCARTHY;
Dept. of Pharmacol., Univ. of Maryland, SOM, Baltimore, MD

Abstract: Microglia are the resident immune cells of the central nervous system and play important roles during normal development and under injury or infectious conditions in adulthood. We recently showed microglia are non-uniformly distributed in the developing rat cerebellum and execute phagocytosis in an age and region dependent manner. Such microglial phagocytosis peaks from PN17 to PN19 in the granular layer, a timeframe when proliferation of granule cells is still occurring. Furthermore, we showed that microglial phagocytic cups engulf dead cells. Based on this, we sought to determine whether cell death regulates the phagocytic activity of microglia. To this end, we first injected both the cell death inhibitor BMN673 and vehicle (1µl, i.c.v.) from PN4 to PN6, when the highest cell death occurs in the cerebellum, and counted pyknotic cells on PN7 vermis stained with cresyl violet to confirm the cell death inhibitor works. We found a significant decreased of 26.35% in pyknotic cells in the granular layer, but not, in the molecular layer in animals treated with BMN673 compared to control. This confirms that BMN673 reduces cell death in the developing cerebellum, but in a regionally specific-manner. Subsequently, we injected through the cisterna magna 1µl of the cell death inhibitor BMN673 (5nM/µl) from PN12 to PN16 in male and female rats, obtained the cerebellums and processed them for Iba1 immunohistochemistry. We quantified the number of phagocytic cups exhibited by microglia as well as microglial morphology in the cerebellar cortex at PN17 as we previously described (Perez-Pouchoulen et al. eNeuro, 2015). The cerebellums from animals treated with BMN673 showed a 16.5% significant reduction in overall microglia density compared to control. We also looked at the phagocytic cups per phagocytic microglia

and found that animals treated with BMN673 had a significant lower number of phagocytic cups per phagocytic microglia than control animals (1.08 versus 1.18, respectively). Thus, it appears that inhibition of cell death during the second postnatal week reduces microglial phagocytosis during the third postnatal week. There were no sex differences in the effects of BMN673 treatment. We conclude that the phagocytic activity of cerebellar microglia, which peaks at PN17, is very tightly regulated and coupled to naturally occurring cell death during the prior week of development. NIH Grant R01-MH091424 to M.M.M.

Disclosures: M. Perez-Pouchoulen: None. S.J. Yu: None. M.M. McCarthy: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.02/F28

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

Support: NINDS Grant P30NS069266

NEI Grant R00EY019547

Title: Haploinsufficiency of the minor spliceosome-specific U11 snRNA in the mouse cortex results in enhanced motor performance.

Authors: *M. BAUMGARTNER[1], P. PERRINO[2], R. H. FITCH[2], R. N. KANADIA[1]; [1]Physiol. and Neurobio., [2]Psychological Sci., Univ. of Connecticut, Storrs, CT

Abstract: Eukaryotic gene expression requires splicing, the removal of introns from pre-mRNA transcripts, which is mediated by the spliceosome, a complex of small nuclear RNAs (snRNAs) and associated proteins. In most metazoans, there are two distinct spliceosomes: the major spliceosome, which removes the vast majority of introns (major introns), and the minor spliceosome, which splices <0.5% of introns (minor introns). Mutations that inactivate the minor spliceosome have been linked to the severe developmental disorder microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1) and Roifman syndrome, which commonly presents with dwarfism, microcephaly, intellectual disability, and hypotonia. Together, these disorders underscore the importance of minor splicing in brain development and function. To verify the causal link between minor spliceosome disruption and microcephaly, we generated a conditional knockout (cKO) for *Rnu11*, which encodes the U11 snRNA, a crucial component of the minor spliceosome. *Emx1*-cre-mediated removal of *Rnu11* in the developing cortex resulted in severe microcephaly at birth, caused by embryonic loss of self-amplifying neural progenitor

cells (NPCs). However, neuron-producing NPCs were not affected by U11 loss, as U11-null neurons were produced and survived postnatally. Despite their postnatal survival, these neurons displayed severe axon projection defects at P0, which were not observed in wild-type or heterozygous mice. Behavioral analyses of adult wild-type, heterozygous, and mutant mice revealed significant motor deficits and anxiety in the mutant mice. Unexpectedly, we also found that heterozygous mice displayed significant enhancement on the rotarod test, relative to the wild-type mice. Together, our findings indicate that minor splicing is not only essential for neuron development and function, but that 50% loss of U11 significantly modulates the function of neurons involved in motor coordination.

Disclosures: M. Baumgartner: None. P. Perrino: None. R.H. Fitch: None. R.N. Kanadia: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.03/F29

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

Support: RO1 NS080932

Title: Selective sensory-motor connections determined by dendritic and axonal positioning

Authors: *N. BALASKAS, T. M. JESSELL, D. NG;
biochemistry and molecular biophysics, Columbia Univ., new york, NY

Abstract: The selectivity with which pre-synaptic axons form connections with their post-synaptic targets is thought to rely on complementary recognition molecules. The prominence of this view has recently been challenged by emerging evidence that the settling position of neuronal cell bodies is a critical determinant of connection selectivity. This positional perspective, however, leaves open the question of whether the pattern of neuronal dendrites and axonal projections also contributes to the specificity of connections. To address this issue we focused on spinal motor circuits, mapping the orientation of dendrites in different motor pools as well as the trajectory of sensory axons. We find that lumbar motor pools exhibit diverse dendritic orientations, ranging from simple crescent-like patterns to configurations with radial morphology. We provide genetic evidence that in FoxP1 mutants, in which motor neurons lack pool identities, the dendritic diversity of limb-innervating motor neurons reverts to a common bipolar pattern that resembles the organization of hypaxial motor neurons. These results imply that the ground-state assignment of motor neuron dendritic arbors is bipolar and that radial orientation represents the apex of a dendritic hierarchy. The axons of muscle-defined sensory

neurons also exhibit distinct angular trajectories *en route* to their recipient motor pools. Dendritic and axonal orientation accurately predicts the identity of target motor pools, as well as the asymmetric dendritic domain of sensory-motor synapses. These findings argue for a significant role for dendritic and axonal positional cues in defining the assembly of sensory-motor circuits, simplifying the task of recognition.

Disclosures: N. Balaskas: None. T.M. Jessell: None. D. Ng: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.04/F30

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

Support: NIH/NICHD 5 K01-HD078484-02 (PI: Gillick)

Cerebral Palsy Foundation (PI: Gillick)

Foundation for Physical Therapy (PI: Gillick)

Sao Paulo Research Foundation - FAPESP (Lixandrão)

MnDRIVE (Rich)

Title: Cortical activation and excitability in congenital hemiparesis.

Authors: M. LIXANDRAO[1], C. PRUDENTE[2], B. MUELLER[2], T. RICH[2], M. CHEN[2], *B. T. GILLICK[2];
[1]Federal Univ. of Sao Carlos, Sao Carlos, Brazil; [2]Univ. Minnesota, Minneapolis, MN

Abstract: Background: Functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS) are non-invasive techniques that allow insights into the re-organization of the cortical motor system and resultant hand function after perinatal stroke.
Aims: To report cortical activation during paretic and non-paretic hand movements and excitability during TMS testing in children with perinatal hemiparesis.
Methods: Five children with perinatal stroke (3 male, age= 11.4±2.9 years) participated in MRI and TMS assessments. Structural T1-weighted images and functional T2-weighted images with blood oxygenation level dependent (BOLD) contrast were acquired. fMRI conditions included active movement and resting sessions with each hand. fMRI data processing and registration to individual T1 images were carried out using FSL (FMRIB's Software Library). Whole-brain

analysis was employed to contrast active blocks with baseline. Z statistic images were thresholded using clusters determined by Z greater than 2.3 and a (corrected) cluster significance threshold of p=0.05. TMS assessment included single-pulses delivered over the motor cortex using a Magstim 200 stimulator connected to a 70-mm figure of eight coil. The resting motor threshold (RMT) was determined for the first dorsal interosseos muscles to assess motor evoked potentials (MEP) responses.
Results: All children presented with a MEP response during TMS testing over the contralesional hemisphere (RMT= 56.4±11.1% maximal stimulator output (MSO)). During non-paretic hand movement, four children presented with significant contralesional activation in precentral gyrus. Three children presented with ipsilesional MEP response due to TMS (RMT= 66.6±11.1% MSO). During paretic hand movement, these three children presented with significant ipsilesional activation in precentral gyrus. In the two children who did not present with an ipsilesional MEP response, no significant activation was found in the ipsilesional precentral gyrus during paretic hand movement. However, these children presented with bilateral activation or ipsilateral activation in the occipital lobe during paretic hand movement.
Conclusion: The absence of ipsilesional precentral gyrus activation during paretic hand movements and the absence of ipsilesional MEP could be related to persistent or expanded ipsilateral corticospinal tract projections after perinatal stroke. Activity was also noted in occipital areas during paretic hand movement, potentially related to motor imagery, task planning or an alternative cortical pattern of reorganization.

Disclosures: M. Lixandrao: None. C. Prudente: None. B. Mueller: None. T. Rich: None. M. Chen: None. B.T. Gillick: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.05/F31

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

Support: Damon Runyon HHMI Fellowship

HHMI

NIH Grant R01-NS033245

Brain Research Foundation

Harold and Leila Mathers Foundation

Project A.L.S.

Title: Developmental origins of inhibitory interneuron diversity in limb and thoracic motor circuits

Authors: *L. B. SWEENEY[1], J. B. BIKOFF[2], M. BAEK[3], M. I. GABITTO[2], S. BRENNER-MORTON[2], C. DIAZ[2], J. S. DASEN[3], T. M. JESSELL[2], C. R. KINTNER[1];
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Abstract: Motor output varies along the rostrocaudal axis of the tetrapod spinal cord. At limb levels, a large number of motor pools are needed to control the alternation of flexor and extensor muscles and produce movement about a joint. At thoracic levels, a smaller number of pools supply muscle groups that support posture, inspiration and expiration. We have examined whether the difference in motor neuron and muscle number at limb and thoracic levels is associated with a similar distinction in interneuron diversity.
We used V1 spinal inhibitory interneurons as a means to explore interneuron diversity along the rostro-caudal axis of the mouse spinal cord. V1 interneurons regulate locomotor rhythm, and with V2b interneurons, control many aspects of flexor/extensor alternation. Differential expression of 19 cell type specific transcription factors divides the lumbar V1 population can into ~50 distinct subpopulations (Bikoff et al 2016, Gabitto et al 2016). These diverse V1 subpopulations fall into four major clades, with differential synaptic connectivity and electrophysiological properties, suggesting they define distinct microcircuits.
We have now examined the expression of these 19 transcription factors at thoracic levels, to define distinctions in V1 subset representation at thoracic and limb levels. Using a Bayesian framework to infer cell type identity, our analysis indicates that the four lumbar V1 clades and many of their subpopulations also exist at thoracic levels. This analysis also detects V1 interneuron subpopulations that are restricted to limb versus thoracic levels, and vice versa. The identification of such restricted V1 subpopulations provides an initial step in defining segment-specific spinal microcircuits.
The existence of rostro-caudally restricted V1 subpopulations led us to explore how V1 diversity is generated along an organism's rostrocaudal axis. Is V1 diversity influenced by cell-intrinsic transcriptional programs, cell-extrinsic cues from the surrounding cellular environment, or both? Using the transcription factors that define limb- and thoracic-specific V1 populations as markers, we find that limb- and thoracic-specific differences in V1 interneurons, like motor neurons, require the Hox gene, *HoxC9*. But in motor neuron deficient *Olig2* mutant mice, the rostrocaudal distribution of limb-specific V1s is largely unaffected, supporting a model in which early Hox patterning of the spinal cord specifies the rostro-caudal identity of V1 interneurons, independent of motor neurons. Future work aims to explore the connectivity and function of limb- and thoracic-specific V1 interneuron subpopulations.

Disclosures: L.B. Sweeney: None. J.B. Bikoff: None. M. Baek: None. M.I. Gabitto: None. S. Brenner-Morton: None. C. Diaz: None. J.S. Dasen: None. T.M. Jessell: None. C.R. Kintner: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.06/F32

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

Support: HHMI

NIH Grant R01-NS033245

NIH-NINDS Grant R01-NS047357

NIH Grant R01-NS078375

NIH Grant R21-NS079981

Department of Defense GR. 10235006

Brain Research Foundation

Title: Spinal inhibitory interneuron diversity and position delineate variant motor microcircuits

Authors: *J. B. BIKOFF[1], M. I. GABITTO[1], A. PAKMAN[2], E. DROBAC[3], A. F. RIVARD[5], T. A. MACHADO[1], A. MIRI[1], S. BRENNER-MORTON[1], E. FAMOJURE[1], C. DIAZ[1], L. F. ABBOTT[4], F. J. ALVAREZ[5], G. Z. MENTIS[3], L. PANINSKI[2], T. M. JESSELL[1];
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Abstract: Animals interact with the world through movement, transforming patterns of neural activity into the orderly contraction of muscles. The circuits directly responsible for movement reside in the spinal cord, where inhibitory interneurons play a crucial role in shaping patterned motor output. Yet the grain of identity of these inhibitory interneurons and the organizational logic through which they influence motor output have remained obscure.
We have used the genetic accessibility of mice to gain insight into the organization of inhibitory

circuits for limb motor control. We undertook a detailed analysis of transcriptional diversity within the V1 class of spinal inhibitory interneurons. This class represents the largest inhibitory population in the ventral spinal cord, and their elimination slows rhythmic locomotor output and perturbs flexor/extensor alternation. Our analysis identifies 19 transcription factors that fractionate the parental V1 population into highly diverse subsets[1]. Transcriptionally-defined V1 subsets exhibit distinct physiological signatures and highly structured spatial distributions, supporting the view that transcriptionally-defined subsets correspond to distinct cell types.
To define the extent of cell-type diversity within this inhibitory population, we developed a sparse Bayesian framework that uses transcription factor expression and positional information to derive a rigorous estimate of V1 interneuron diversity. Bayesian analysis infers the existence of ~50 candidate V1 cell types, each defined by 3-9 transcription factors, and more generally provides a means of delineating cell type heterogeneity in any mixed tissue[2].
Finally, we explored the connectivity of two subsets, $V1^{Sp8}$ and $V1^{R}$ (Renshaw) interneurons, with proprioceptive sensory neurons and motor neurons operating on hip, ankle, and foot muscles. Anatomical and electrophysiological analyses demonstrated that interneuron position appears to constrain patterns of input from proprioceptive sensory neurons and motor neurons, and thus determines microcircuit organization. Moreover, variant patterns of inhibitory circuitry operate on different hindlimb muscles, exemplified most clearly by the monosynaptic connectivity of hip but not ankle or foot sensory afferents onto $V1^{R}$ interneurons. Our findings reveal the existence of diverse V1 interneuron subtypes which form variant inhibitory microcircuits in response to biomechanical constraints operating on muscles controlling different limb joints.
[1]Bikoff, J.B. et al. (2016). *Cell* 165, 207-219.
[2]Gabitto, M.I., et al. (2016). *Cell* 165, 220-233.

Disclosures: J.B. Bikoff: None. M.I. Gabitto: None. A. Pakman: None. E. Drobac: None. A.F. Rivard: None. T.A. Machado: None. A. Miri: None. S. Brenner-Morton: None. E. Famojure: None. C. Diaz: None. L.F. Abbott: None. F.J. Alvarez: None. G.Z. Mentis: None. L. Paninski: None. T.M. Jessell: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.07/F33

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

Support: LABEX CORTEX (ANR-11-LABX-0042)

Title: Developmental changes in the macaque distribution of corticospinal neurons in the contro- and ipsilateral hemisphere

Authors: *A. R. RIBEIRO GOMES[1,3], E. OLIVIER[4], H. KILLACKEY[5], M. GUENOT[6], P. GIROUD[1,3], M. BERLAND[3], K. KNOBLAUCH[1,3], C. DEHAY[2,3], H. KENNEDY[1,3]; [1]Dept. of Integrative Neurosciences, [2]Dept. of Embryonic Stem Cell and Cortical Develop., Inserm U1208, Stem-Cell and Brain Res. Institu, Bron, France; [3]Claude Bernard University, Univ. of Lyon, Lyon, France; [4]Inst. of Neuroscience, Sch. of Medicine, Catholic Univ. of Louvain, Louvain, Belgium; [5]Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [6]Pierre Wertheimer Hospital, Hospices Civils de Lyon, Lyon, France

Abstract: In adult non-human primates direct cortical control over motor output is largely mediated by contralateral projections to spinal motor centers, and only a minority of all corticospinal neurons (CSN) project ipsilateral (Galea and Darian-Smith, 1994). Both contralateral and ipsilateral CSN are restricted to motor, somatosensory, cingulate and insular regions, although compared to the contralateral projection the tangential distribution of ipsilateral projecting neurons is much reduced. In the newborn macaque the areal distribution of contra and ipsilateral CSN is marginally more widespread than that observed in the adult (Galea et al. 1995).

An ongoing study of the team concerns the developmental changes in the areal distribution of CSN during in utero development. By injecting the retrograde tracer fast blue at cervical levels of the spinal cord of cynomolgus monkeys at embyronic day (E) 95 and E105 we have shown that the retrogradely labeled CSN in the contralateral hemisphere are restricted to layer 5, and that there is a greater extent of cortical territory projecting to the contralateral spinal cord during development than in newborn and adult, including projections from prefrontal, occipital and temporal cortices.

Here we report the distribution of ipsilateral projections to the cervical spinal cord during development. Preliminary results show that CSN projecting ispilaterally (1) are restricted to layer 5, and (2) their distribution is similar to that of the contralateral CSN. Ongoing analysis aims to quantify these projections across regions in order to allow direct comparison of areal weights of ipsi- and contralateral projections.

Overall, our results indicate that the difference between ipsi- and contralateral CSN projections is quantitative, rather than qualitative. This is especially relevant to studies of human development. These studies suggest developmental motor disorders related to lesions of the motor cortex could be the consequence of a maintained exuberant ipsilateral projection from the non-lesioned hemisphere (Eyre 2007).

Galea MP, Darian-Smith I (1994) Cereb Cortex 4:166-194
Galea MP, Darian-Smith I (1995) Cereb Cortex 5:518-540
Eyre (2007) Neurosc Behav Rev 31:1136-1149

Disclosures: A.R. Ribeiro Gomes: None. E. Olivier: None. H. Killackey: None. M. Guenot: None. P. Giroud: None. M. Berland: None. K. Knoblauch: None. C. Dehay: None. H. Kennedy: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.08/F34

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

Title: Labeling Dbx1 neurons and glia in the preBötzinger complex based on the timing and dose of tamoxifen administration in inducible Dbx1 Cre-driver transgenic mice

Authors: *C. A. MARTIN, A. KOTTICK, C. A. DEL NEGRO;
Applied Sci., The Col. of William & Mary, Williamsburg, VA

Abstract: The brainstem preBötzinger Complex (preBötC) generates the rhythm that underlies inspiratory breathing movements in mammals, and its core rhythmogenic interneurons are derived from neural progenitors that express the embryonic transcription factor Dbx1. Elucidating the cellular mechanisms of respiratory rhythm generation depends on the ability to fluorescently tag and manipulate Dbx1-derived preBötC neurons (i.e., Dbx1 neurons). To that end we employ tamoxifen-inducible Dbx1 Cre-driver mice with Cre-dependent reporters to map neurons as well as glia formed by Dbx1-expressing precursors. The timing and dose of tamoxifen administration during embryonic development critically influences fusion protein expression in Dbx1-derived cells. To better understand preBötC development, and ultimately optimize Cre-dependent fusion protein expression in Dbx1 preBötC neurons specifically, we examined the relationship between the timing of tamoxifen administration and the pattern of labeled neurons and glia in the preBötC. We varied the timing of tamoxifen administration between E7.5 and E11.5 corresponding to the window of Dbx1 expression during embryonic development, and then performed passive clearing (CLARITY) and confocal imaging of the preBötC using medullary slices from juvenile mice. Immunohistochemistry and morphometric criteria were used to define populations of Dbx1-derived neurons (immunoreactive for NeuN) and Dbx1-derived astroglia (immunoreactive for Sox9). Experiments using human synapsin driven adeno-associated virus (AAV) in the preBötC aided the morphological identification of neurons in this region. Using an ImageJ cell counting function to quantify Dbx1 neuronal and glial populations with induced fluorescence at different time points we found that glia are formed at a constant rate from E7.5 through E11.5 while neuron formation peaks at E9.5. These data can be employed to plan breeding and tamoxifen administration strategies that optimize intersectional mouse genetic approaches that label and manipulate Dbx1-dervied neurons (or glia) and study their roles in the respiratory brainstem.

Disclosures: C.A. Martin: None. A. Kottick: None. C.A. Del Negro: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.09/F35

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

Support: NIH Grant 1R25GM107760

Title: Expression of a genetically-encoded fluorescent voltage sensor, ASAP1, in neurons of the embryonic chick spinal cord

Authors: A. FRASSATO[1], L. DEROSSETT[1], S. FROMHERZ[2], P. R. PATRYLO[3], *A. A. SHARP[4];
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Abstract: Understanding the interactions of large numbers of neurons within various circuits is a fundamental goal in neuroscience. However, obtaining membrane potential recordings from large numbers of neurons in freely behaving animals is challenging, especially for embryonic systems. Nonetheless, numerous approaches to record from large ensembles of neurons have been developed, but many of these approaches, e.g. large electrode arrays, are not amenable to embryonic systems due to the relatively small size and fragility of embryonic tissues. The new generation of fluorescent reporter molecules with increased temporal dynamics and greater fluorescence may now be ready to be applied to embryonic systems. The goal of this study was to adapt the recently developed genetically-encoded fluorescent voltage sensor Accelerated Sensor of Action Potentials 1 (ASAP1, St-Pierre et al. 2014) for use in chick embryos. The chick embryo has been one of the primary model organisms for studying vertebrate sensorimotor development. The similarity of chick developmental timing to that of humans along with its bipedal nature and development outside of the mother provides numerous experimental advantages over rodent models. Transposon systems can be used to establish stable expression of heterologous proteins via electroporation. It has been shown that the Piggybac system can be used to allow for channelrhodopsin-2 expression in chick embryos and that the relatively transparent nature of embryonic tissue allows for light stimulation of motor neurons in order to regulate motility in mid-stage chick embryos (Sharp and Fromherz, 2011). Therefore, it was hypothesized that a similar approach could be used to introduce ASAP1 and to allow for optical voltage recording from neurons in both freely moving embryos and reduced preparations. Standard molecular procedures were used to exchange the open reading frame for ASAP1 from pcDNA3.1/Puro-CAG-ASAP-1 (AddGene) with the channelrhodopsin-2 open reading frame in the plasmid pPB-ChIEF-Tom from the aforementioned study in order to create pPB-ASAP-1. In order to induce ASAP1 expression in neurons of the spinal cord, pPB-ASAP-1 was

electroporated into the neural tube of embryonic day (E) 3 embryos. Fluorescence from the GFP-component of ASAP1 could be detected in living embryos on E4 and was observed to persist until at least E10. Imaging of tissue sections indicate that ASAP1 is likely expressed in the plasma membrane of neuronal somata, dendrites and axons. Functional imaging and electrophysiological experiments are underway to determine the potential applications of this approach to advance our understanding of neural development.

Disclosures: A. Frassato: None. L. DeRossett: None. S. Fromherz: None. P.R. Patrylo: None. A.A. Sharp: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.10/F36

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

Support: HHMI

Title: Analyzing ensemble activity in the developing zebrafish spinal cord

Authors: *Z. WEI, Y. WAN, P. KELLER, S. DRUCKMANN;
Janelia Res. Campus, Ashburn, VA

Abstract: Statistical analysis of the dynamics of ensembles of neurons is a question of long-standing interest in neuroscience. A central approach is the use of latent space methods where the detailed observed dynamics are assumed to have been generated by a phenomenological lower dimensional space where each of the dimensions corresponds to a mode of network activity. A major difficulty of these approaches is that they are underconstrained, i.e., there are many different descriptions of latent spaces that can capture a particular set of observed dynamics. Moreover, the ensemble activity may change over time on multiple different timescales. Here, we utilize a unique experimental paradigm, long timescale functional imaging in embryonic zebrafish spinal cord, to both inform specific biological questions and test the applicability of latent space models to describe complex ensemble dynamics over multiple timescales.
A hallmark of the developing zebrafish spinal cord is the emergence of patterned ensemble activity, but this dynamic process is only poorly understood. We analyze the underlying dynamic changes using cellular-resolution functional imaging data from multiple segments of the spinal network. We first compare neuronal correlations against time and observe that spinal cord neurons undergo a rapid transition from sporadic single-neuron activity at the early embryonic stage to ipsilaterally-correlated and contralaterally-anticorrelated activity late in development. In

order to probe the mechanism for such evolution, we develop a continuous-time factor analysis model that identifies sparse, yet anatomically-meaningful functional communities (FC) across time. With an infomax-like constraint on network structure, our model predicts that FCs are located exclusively ipsilaterally and reveals a seamlessly development of FCs as a function of time. Interestingly, we find that FCs first occur locally within small groups of neurons, and subsequently merge with other ipsilateral FCs through synchronization from anterior to posterior spinal cord, which finally forms a patterned network. At the single cell level, our model shows that within a FC, the activity of each neuron can be predicted from the others in the same FC. Our model can thus estimate the joining time of each cell to a FC, as its activity is essentially explained by others. Importantly, the joining time of cells shows an anterior to posterior gradient along the spinal cord. In summary, we show that latent space methods, appropriately tailored and complemented by specific analyses, can shed light on ensemble activity and its development over time in the embryonic zebrafish spinal cord.

Disclosures: **Z. Wei:** A. Employment/Salary (full or part-time): HHMI Janelia Research Campus. **Y. Wan:** A. Employment/Salary (full or part-time): HHMI Janelia Research Campus. **P. Keller:** A. Employment/Salary (full or part-time): HHMI Janelia Research Campus. **S. Druckmann:** A. Employment/Salary (full or part-time): HHMI Janelia Research Campus.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.11/F37

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

Support: MEXT24119002

Title: Information structure of proprioceptive feedback in infantile spontaneous movements

Authors: *H. KANAZAWA[1,2], Y. YAMADA[3], Y. KUNIYOSHI[1];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Res. Fellow of JSPS, Tokyo, Japan; [3]IBM Res. - Tokyo, Tokyo, Japan

Abstract: From early developmental phase, human infants exhibit complex and various types of spontaneous whole-body movements. Recent animal studies have shown that these spontaneous movements play an important role in early sensorimotor development. In addition, human studies have actively characterized infantile spontaneous movements in terms of limb trajectory and its coordination in order to deepen our understanding of how the spontaneous movements contribute to the sensorimotor development. Although motor output patterns have been well characterized,

little is known about the characteristics of sensory feedback evoked by spontaneous movements. In this study, we quantify proprioceptive feedback during infantile movements by combined use of experimental data of whole-body kinematics and a musculoskeletal body model of human infant. Specifically, we performed full-body motion capture of one infant (3-months-old) during spontaneous movements without external stimulation. We recorded three-dimensional positions of total 54 markers using 8 infrared cameras at 100 fps, and obtained joint angles for each frame. Combining these joint angles with an infantile musculoskeletal body model based on MRI/CT data, we estimated 88 muscle lengths of both legs during the spontaneous movements. We regarded the muscle lengths as proprioceptive signals, and investigated the characteristics of proprioceptive feedback. First, we calculated pair-wise correlations of muscle lengths between each two muscles. As a result, 39.5% of all possible pairs in the modeled muscles showed significant correlations during infantile spontaneous movements. Next, we categorized the muscles into 8 groups according to the level of the spinal cord from which they arise, and then compared the correlations of muscle length within and across the groups. Consequently, correlations within group were significantly higher than those across group (median correlation coefficients; 0.53 vs 0.22, $p < 0.001$, ranksum-test). By combined the measured kinematics and infantile musculoskeletal model, we quantified information structures of proprioceptive feedback in spontaneous movements of human infant. Our preliminary results indicate that infantile spontaneous movements can provide structured proprioceptive information reflecting spinal segments, although previous studied have reported less inter-limb correlations in motor patterns. Combined with analysis of motor outputs, our quantification of sensory feedback can help deepen our understanding of the relationship between spontaneous movements and sensorimotor development.

Disclosures: H. Kanazawa: None. Y. Yamada: None. Y. Kuniyoshi: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.12/F38

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

Support: JSPS KAKENHI Grant # 26430020 to HH

JSPS KAKENHI Grant # 16K10100 to SM

Title: Alteration of dendrite morphology and electric responsibility in the sensorymotor cortex on the developmental white matter injury model rat

Authors: *Y. UEDA[1], Y. BANDO[2], S. MISUMI[1], S. OGAWA[1], H. HIDA[1];
[1]Dept Neurophysiol & Brain Sci., Nagoya City Univ. Grad Sch. Med. Sci., Nagoya-Shi, Japan;
[2]Dept Funct Anat & Neurosci, Asahikawa Med. Univ., Asahikawa-shi, Japan

Abstract: Since the brain of preterm infant is vulnerable to hypoxia-ischemia (H-I), the preterm infant tends to contract the developmental white matter injury (DWMI), causing the neurodevelopmental deficits such as cerebral palsy, cognitive disorder, and attention deficit/ hyperactivity disorder. We previously reported a DWMI model by right common carotid artery occlusion followed by 6% hypoxia for 1 hour in postnatal day 3 (P3) rat. We found oligodendroglial but neuronal loss in this model, concomitant with mild hindlimb dysfunction with motor coordination deficit and the reduction in the hindlimb area of the sensorymotor cortex in adulthood (Misumi et al., Cell Transplant, 2016). However, it is unclear when these alterations starts and if the change of neuronal morphology contributes to these alterations. To answer these questions, we performed electrophysiological and histological experiments in our DWMI model rat. We first performed intracortical microstimulation (ICMS) at P35 to elucidate if the change of motor map has already started earlier than 10-week-old when we had already confirmed the change. Electric stimulation (duration, 200μsec; 30 bipolar pulses at 333Hz; 10μA-270μA) was given to the sensorymotor cortex (1.0-3.0 mm posterior and lateral from bregma) through glass-insulated tungsten electrode (Impedance, 750kΩ) and the response of muscle contraction was checked. The size of the hip-joint contracting portion tended to be smaller in the DWMI model. To elucidate this tendency to shrink the hip-joint portion in DWMI, we next focused dendritic morphology in the cortex as the number of neurons was kept in our model. We evaluated the motor cortex of which 1.0-3.0 mm posterior and lateral from bregma. The basal dendrites in the intermediate layer of the cortex were more developed in the ipsilateral H-I side. These data indicate that H-I insult at P3 causes the alterations of the dendritic formation and electrical responsiveness in the cortex, probably due to the change of sensory inputs and/or the trophic support from oligodendrocytes which development is disturbed in our DWMI model.

Disclosures: Y. Ueda: None. Y. Bando: None. S. Misumi: None. S. Ogawa: None. H. Hida: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.13/F39

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

Support: Conacyt CB-2010-01-0154645

Title: Novel efferent pathways from the mouse substantia nigra

Authors: *E. SOTO, J. A. MENDEZ;
Univ. Autonoma De San Luis Potosi, San Luis Potosi, Mexico

Abstract: Substantia nigra (SN) plays a central role in motor control. Whereas the substantia nigra pars compacta (SNc) supplies the dorsal striatum with dopamine, the substantia nigra pars reticulata is the main output nucleus of the basal ganglia. In addition to Dopaminergic and GABAergic neurons, SN contains purely Glutamatergic neurons as well as Dopaminergic neurons that corelease GABA or Glutamate. The output connections of Dopaminergic neurons of the substantia nigra are very well known. However, despite local projection evidence, little is known of output projections of the Glutamatergic neurons. In order to gain insight into novel Glutamatergic projection pathways from the substantia nigra, we performed pressure injections of the anterograde tracer Dextran Alexa-Fluor 546 into the SNc of adult p90 mice. 10 days later, the fluorescent signal was detected within several brain areas including the dorsal striatum. To confirm some of the projections, the retrograde tracer FluoroGold was injected and the signal was recovered in the substantia nigra. Then the identity of the projecting neurons was assessed by multiplex single cell RT-PCR against Tyrosine Hydroxylase (TH), Glutamate Decarboxylase (GAD1) and the vesicular Glutamate Transporter type 2 (VGluT2). In addition to the expected Dopaminergic striatonigral and nigrocampal pathways, we identified Glutamatergic neurons that project their axons from the substantia nigra to the following nuclei: putamen caudate, globus pallidus and the CA1 region of the hippocampus. Also, we detected the presence of GABAergic neurons from the SNc projecting to the putamen caudate, globus pallidus, the CA1 region of the hippocampus and the parafasicular nucleus of the thalamus. Remarkably, neurons of the Glutamatergic/GABAergic double phenotype were found to project from the SNc to the globus pallidus. Our results contribute to a better understanding of the circuitry of the substantia nigra, a so called Dopaminergic nucleus.

Disclosures: E. Soto: None. J.A. Mendez: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.14/F40

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

Title: Where handedness starts: Gene expression asymmetries in human fetal spinal cord

Authors: *J. SCHMITZ, S. OCKLENBURG, O. GUNTURKUN;
Dept. of Biopsychology, Ruhr-University, Bochum, Germany

Abstract: Handedness is the strongest and most widely investigated form of motor asymmetries in humans, but little is known about its underlying molecular mechanisms. Since about 85% of embryos exhibit more right arm than left arm movements already 10 weeks after gestation, fetal cortical gene expression asymmetries have been suggested as underlying functional hemispheric asymmetries. However, the motor cortex is not yet functionally linked to the spinal cord at this developmental stage, which indicates that early motor asymmetries are likely controlled by the spinal cord. Human spinal cord was collected from fetal tissue at 10, 12, and 14 gestational weeks following induced pregnancy terminations. Cervical and anterior thoracal left and right segments were separated along the midline. Total RNA was extracted from each half of the spinal cord. Next generation sequencing revealed gene expression profiles and genes with significant expression differences were examined for functional gene ontology (GO) groups. We then targeted candidate genes that had previously been associated with development of hemispheric asymmetries. The number of genes showing significant expression asymmetries, measured as $\log_2$(fold change) of at least 1.5, critically varied with the developmental stage. By far the most asymmetrically expressed genes were found at week 10 with 3.3% being asymmetrically expressed between the left and right spinal cord. With 0.05%, asymmetry was reduced in week 12 and almost absent in week 14 (0.01%). Accordingly, the number of significant GO groups was the highest in week 10 (123) and reduced in week 12 (41). In week 14, no GO group reached statistical significance. Out of the candidate genes, *FOXP2* displayed a significant rightward asymmetry at week 12. *BDNF-AS* was significantly stronger expressed in the left spinal cord at week 10. The findings are discussed with regard to existing models on handedness ontogenesis. As motor asymmetries start by gestational week 10, we suggest this developmental stage as the critical period for handedness formation. Expression asymmetries in genes relevant for CNS development might provoke differential development of neuronal circuits in the right arm and hand, for example in terms of larger motoneuronal somata in segments innervating the right than the left arm, which causes more developed motor behavior. Spinal cord and motor cortex are functionally connected from week 17. By then, the well-established behavioral asymmetry might lead to asymmetries in use-dependent neuronal plasticity processes in the motor cortex, ultimately leading to the cortical correlates of handedness.

Disclosures: J. Schmitz: None. S. Ocklenburg: None. O. Gunturkun: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.15/F41

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

Support: SRP/151318006

Title: Anatomic course and variation of femoral nerve in human fetuses

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Abstract: INTRODUCTION Femoral nerve is primary nerve innervating the anterior aspect of the thigh and the largest of the peripheral branches of the lumbar plexus. It emerges through the psoas major fibers, then passes underneath the inguinal ligament just lateral to the femoral artery as it enters the thigh. Variant origin, level of the divisions and branches measurements of the femoral nerve have a clinical important. The femoral nerve block is performed on the main trunk of the femoral nerve just below the inguinal ligament .The higher division of the femoral nerve in iliac fossa results in incomplete femoral nerve block. **MATERIAL AND METHODS** This study was performed on 30 fetuses (17 male and 13 female) with no gross anomalies from the fetus collection of Necmettin Erbakan University, Meram Medical Faculty Anatomy Department. We were used microdissection instruments, 0,01 mm precision digital caliper (stainless hardened), microsurgery microscope (Kaps Sam 62) and a camera (Canon D1000). We determined the course and variation of femoral nerve in human fetuses. Furthermore,we measured thickness and lenght of trunk and divisiones of the femoral nerve.The obtained data were evaluated by using SPSS 21.0 (Statistical Package for Social Sciences). Data were analyzed by both descriptive (mean value, standard deviation, maximum and minimum values, percentages) and quantitative statistical methods. Results were evaluated statistically in %95 confidence interval and differences were accepted significant if $p<0.01$. **RESULTS AND CONCLUSION** The localization of the division points femoral nerve divides into its branches were assessed under 3 categories as 1-above the inguinal ligament, 2- under the inguinal ligament, 3- at a level below the inguinal ligament. Femoral nerve divides into its branches above the inguinal ligament in 2 fetuses (6.7%), under the inguinal ligament in 17 fetuses (56.7%) and at a level below the inguinal ligament in 11 fetuses (36.7%). The right distance between femoral nerve and its dividing point was found 3.56±1.53 cm and 2.36±0.65 cm fort he left sides in males. These mesuraments wee determined 3.82±2.03 cm and 4.90±3.56 cm for females, respectively. When right and left parameters of all fetuses were compared, it was

observed that statistically difference was between thickness of anterior and posterior branches of femoral nerve and also distance between femoral artery and femoral nerve ($p<0.05$).

Disclosures: **M. Buyukmumcu:** A. Employment/Salary (full or part-time): Meram Medical school. **E. Cihan:** A. Employment/Salary (full or part-time): DEHA Rehabilitation Center. **A. Aydin kabakcı:** A. Employment/Salary (full or part-time): Meram Medical School. **D. Akin:** A. Employment/Salary (full or part-time): Meram Medical school. **S. Gungorer:** A. Employment/Salary (full or part-time): Meram Education and Research Hospital.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.16/F42

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

Support: JSPS KAKENHI Grant 26430064

JSPS DC1 201505680

Title: Identification of the interactor of CLAC-P/Collagen type XXV in motor nerve innervation of skeletal muscles

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Abstract: CLAC-P/Collagen type XXV is a trans-membrane collagen, which was originally identified as a component of senile plaques in Alzheimer's disease brains. Collagen type XXV is classified as a member of membrane-associated collagens with interrupted triple helices(MACIT), together with type XIII, XVII, and XXIII collagens. To examine the *in vivo* function of collagen type XXV, we generated *Col25a1* knock-out (KO) mice, in which motor axons properly elongated toward the target muscle, but failed to arborize within the muscle. Failure in intramuscular innervation of motor axons led to severe and selective loss of motor neurons in the spinal cord. Further analysis revealed that collagen type XXV expressed in skeletal muscles is indispensable for the proper elongation and arborization of motor neurons in the target muscle. However, it remains to be solved how collagen type XXV controls motor innervation. In this study, we examined receptor protein tyrosine phosphatase (RPTP) σ and δ, whose knock-out phenotype resembles that of *Col25a1* KO mice, as a candidate of the interactor

of collagen type XXV in this context. To investigate whether the two molecules bind each other, we performed cell surface binding assay, where HEK293 cells expressing RPTP were incubated with secreted form of collagen type XXV. We found that the secreted collagen type XXV specifically bound to the surface of RPTP-expressing cells. Deletion mutant analysis of RPTP revealed that the immunoglobulin-like domain of RPTP was essential for the binding to collagen type XXV. Furthermore, we have developed a co-culture preparation of spinal cord explants and HEK293 cells expressing collagen type XXV, with which we are able to assess the interaction between motor axons and collagen type XXV. Data on the specific interaction between collagen type XXV and RPTPs on the motor nerve will be presented.

Disclosures: H. Munezane: None. H. Oizumi: None. T. Yoshida: None. T. Wakabayashi: None. T. Iwatsubo: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.17/F43

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

Support: JST (Japan Science and Technology Agency) CREST

Title: Development of visuo-motor processing required to hit the fast ball over 150 km/h in baseball batting

Authors: *H. KOBAYASHI[1], M. SHINYA[1], H. OBATA[2], K. HAGIO[1], M. KUWATA[1], K. NAKAZAWA[1];
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Abstract: Baseball batting is a complex visuo-motor skill that requires a batter to produce a spatially and temporally accurate bat swing in as little as 400 ms, when a ball is pitched at 150 km/h. Although visuo-motor processing ability develops with age, it is unknown when a baseball batter acquires the specific visuo-motor ability to cope with the over 150 km/h fast ball. The present study aimed to verify development of the visuo-motor ability to deal with fast ball and whether reaction times would relate to the visuo-motor ability. We measured the time from the ball release to the moment of hit the ball (time to contact: TTC) during the official games in each generation (average level primary school: average level PS, top level primary school: top level PS, junior high school: JH, high school: HS and college: CL). We recorded the pitcher and batter in the same video picture with a high-speed camera (600 fps) to analyze TTC and the ball

velocity of each pitch with a radar gun. In reaction time tasks, 15 PS (age: 11.0 ± 0.6 years), 23 JH (age: 13.6 ± 0.5 years), 35 HS (age: 16.7 ± 0.4 years) and 53 CL (age: 20.6 ± 0.9 years) baseball players performed the button-press simple and Go/Nogo reaction time tasks. Participants were instructed to react as quickly as possible to the green LED (both tasks), but not to the red LED (Go/Nogo task). As we expected, average level PS pitchers were the lowest average ball velocity during the actual game than the pitchers in the other groups. Although the average TTC in the CL game was shortest among the groups (447.5 ± 31.2 ms), the top level PS (469.2 ± 46.6 ms) showed the shorter TTC than the average level PS (592.7 ± 57.2 ms), JH (549.2 ± 50.6 ms) and even HS (489.0 ± 37.5 ms). It was remarkable that the shortest TTC in the top level PS (410 ms) was comparable to that in the CL (400 ms). In reaction time tasks, mean simple reaction times of the PS group (216.3 ± 21.5 ms) were the significantly longer than the HS (182.9 ± 14.3 ms) and the CL (190.4 ± 24.5 ms) groups, but not different from the JH group (214.8 ± 29.3ms). Similarly, mean Go/Nogo reaction times of the PS group (293.8 ± 37.7 ms) were longest among the groups (JH: 270.0 ± 34.8 ms, HS: 237.7 ± 30.7 ms and CL: 239.1 ± 31.5 ms). The observed short TTC in the top PS players suggests that they have already obtained the visuo-motor processing required to hit the over 150 km/h ball in baseball batting. Together with the longer reaction time observed in PS players further imply that the neural mechanism underlying baseball batting is different from that underlying simple or choice reaction tasks. Overall, the present results strongly suggest that the neural function required to baseball batting develops rapidly before puberty.

Disclosures: H. Kobayashi: None. M. Shinya: None. H. Obata: None. K. Hagio: None. M. Kuwata: None. K. Nakazawa: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.18/F44

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

Support: Helse SørØst 2013022

Title: Transcription factor profiling of vestibulospinal neurons in mouse and chicken embryos reveals developmental subdivisions

Authors: *A. LUNDE[1], B. OKATY[2], S. DYMECKI[2], J. C. GLOVER[1];
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Abstract: Vestibulospinal neurons are organized into discrete groups that project from the brainstem to either the ipsilateral or the contralateral side of the spinal cord, enabling animals ranging from agnathans to humans to maintain proper balance and posture. Our previous studies in the mouse and chicken have mapped the developmental origin of the ipsilateral lateral vestibulospinal tract group (LVST) to hindbrain segment (rhombomere) 4, and of the contralateral medial vestibulospinal tract group (cMVST) to rhombomeres 4 and 5.
To characterize differential expression patterns of post-mitotic transcription factors in the LVST and cMVST, we performed RNAseq analysis on manually sorted retrogradely labeled neurons in embryonic day (E) 13.5 mice, and embryonic day (d) 7.5 chickens. Control tissue from the medial parts of rhombomere 4 and 5 was also analyzed to exclude pan-neuronal and rhombomere-generic transcription factors. Highly differentially expressed transcription factors were further investigated by immunohistochemistry at E13.5-15.5 in the mouse, and d7.5-9 in the chicken. Serial sectioning and confocal microscopy was used to generate 3D models of the LVST and cMVST groups with superimposed immunolabeling to assess the transcription factor expression patterns within the groups.
RNAseq analysis revealed well over 100 significantly differentially expressed transcription factors in both mouse and chicken. Of the top 100 upregulated transcription factors in LVST versus cMVST, compiled separately for mouse and chicken, roughly 10% were shared between the two species. For cMVST versus LVST, roughly 15% were shared. Immunohistochemical analysis of select transcription factors revealed that they are expressed in characteristic spatial domains, indicating a developmental subdivision of both the LVST and the cMVST.
These data provide new information about the transcription factors that specify the two main vestibulospinal neuron groups, as well as a first step towards unraveling the molecular heterogeneity of each group.

Disclosures: A. Lunde: None. B. Okaty: None. S. Dymecki: None. J.C. Glover: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.19/F45

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

Support: NSERC RGPIN/386290 -2010

Title: Sim1 transcription factor regulates the distinct neurogenesis profiles of spinal V3 INs and their subsequent functional separation

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Abstract: V3 interneurons (INs) in the spinal cord are a major group of excitatory commissural INs that are essential in establishing a robust and balanced locomotor rhythm during walking. In the early embryonic mouse spinal cord, V3 INs arise from the most ventral progenitor domain (P3) as marked by the post-mitotic expression of the basic helix-loop-helix transcription factor, Sim1. Subsequently, V3 INs form both dorsal and lateral migratory streams establishing separate subpopulations by postnatal day (P) 0 with distinct laminar distributions, axon projection profiles and membrane properties. In order to better understand the developmental mechanisms underlying the formation of different V3 subpopulations we investigated their respective temporal neurogenesis profiles. V3 INs were visualized by the expression of Td-tomato fluorescent protein using a Sim1Cre;Rosa26TdTom mouse line. Distinct dorsa-ventral subpopulations in the higher lumbar region were further divided into ascending and descending groups via biotin-dextran-amine retrograde labeling. The birthdates of V3 INs at P0 were determined by preempted 5-Ethynyl-2′-deoxyuridine (EdU) pulses between embryonic day (E) 9.5 and E12.5, respectively. Dorsal V3 INs are born between E9.5 and E10.5 while ventral V3 INs are born later between E10.5 to E12.5. In addition, preliminary data also suggests the dorsal and early born ventral V3 INs are predominately ascending projecting cells while later born ventral V3 INs are descending. Furthermore, using a Sim1 complete knockout mouse model, we have observed a potential delayed exit of the mutant V3 INs from their progenitor state and therefore a shifted neurogenesis profile to later embryonic stages. Subsequently, dorsal and ventral V3 subpopulations are no longer physiologically distinguishable at P0 in the Sim1 mutant. These results suggest embryonic Sim1 expression establishes a patterned V3 neurogenesis profile and downstream functional separation of V3 subpopulations.

Disclosures: D.A. Deska-Gauthier: None. J. Chopek: None. Y. Zhang: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.20/F46

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

Title: Conditional knockout of GluN2B in motoneurons < the corticomotoneuronal synapse elimination during development in rodents.

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Abstract: In *in vitro* organotypic co-culture of the cerebral cortex and spinal cord, the corticospinal (CS) neurons make synapses upon spinal neurons but later exuberant synapses in the ventral spinal cord are eliminated. This type of the CS synapse elimination was shown to be dependent upon spinal GluN2B subunit-containing NMDA receptors. We previously suggested that monosynaptic connections between CS neurons and the motoneurons innervating the forearm muscles (forearm MNs) in juvenile rodents were subsequently eliminated during development *in vivo*. However, it is not known whether the CS-MN synapse elimination *in vivo* is dependent upon GluN2B or not. In this study, we tested this possibility *in vivo* using Grin2b-flox mice and a genetically modified rabies virus.

To delete GluN2B gene specifically in the forearm MNs, the neonates of Grin2b-flox mice received intramuscular injections of adeno-associated virus (AAV) encoding Cre recombinase, which retrogradely infect the forearm MNs. To examine the monosynaptic connectivity between CS neurons and the forearm MNs in those animals, we employed monosynaptic tracing with the recombinant rabies virus. Three proteins were introduced into the forearm MNs of Grin2b-flox mice through retrograde infection of adeno-associated viruses (AAVs) from the neuromuscular junctions at postnatal day 1 (P1): (1) TVA, which is a receptor for EnvA that is engineered to be expressed in the recombinant rabies virus (i.e. EnvA-psudotyped rabies virus), (2) rabies virus glycoprotein which is required for transsynaptic spread of the rabies virus, and (3) Cre recombinase to delete GluN2B gene. Cre-mediated Grin2b targeting was confirmed by whole cell recordings from the AAV-infected MNs in the spinal cord slices prepared at P9-P10. Next, EnvA-pseudotyped- and glycoprotein-deleted rabies virus was directly injected into the forearm MN pool in the spinal cord (C5 to Th1 segments) at P18. Histological examination was carried out at P26. Labeled neurons were found in the cervical cord and brainstem similarly to the wild type mice but no labeled cell was detected in the cerebral cortex. These results indicate that the corticomotoneuronal synapses were eliminated normally from the GluN2B-deleted MNs. Thus, it is unlikely that the GluN2B in the MN is indispensable for the corticomotoneuronal synapse elimination.

Disclosures: N. Murabe: None. S. Fukuda: None. T. Ohno: None. N. Isoo: None. T. Mori: None. H. Mizukami: None. K. Ozawa: None. K. Sakimura: None. Y. Yoshimura: None. M. Sakurai: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.21/DP01 (Dynamic Poster)

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

Support: HHMI

Title: Emergence of patterned activity in the developing zebrafish spinal cord

Authors: *Y. WAN, Z. WEI, S. DRUCKMANN, P. KELLER;
HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Spontaneous, patterned neuronal activity has been closely linked to developmental mechanisms shaping the early nervous system and is suggested to play a key role in the fine-tuning of developing circuits. However, little is known about how patterned activity emerges *de novo* and what factors control this maturation process. Major limitations to this end have been (1) the lack of functional recordings at the whole-circuit level and throughout the period in which patterned activity emerges, and (2) the technical challenge of mapping the origin and roles of neurons participating in the functional maturation process back to neurogenesis, when neurons are first born. Here, we use Simultaneous Multi-view (SiMView) Light-sheet Microscopy to image the emergence of patterned activity in the developing spinal cord of embryonic zebrafish. We developed imaging assays and computational tools to record embryogenesis at the cellular level and systematically track cellular dynamics and lineage relationships in the developing spinal cord. By seamlessly transitioning from developmental imaging to high-speed volumetric functional imaging, we furthermore mapped calcium activity in all post-mitotic neurons for a large fraction of the developing spinal cord at a temporal resolution of 4 Hz. These data show that spinal cord neurons undergo a rapid transition from sporadic single-neuron activity to ipsi-laterally correlated and contra-laterally anti-correlated activity between 18 and 22 hours post fertilization. We developed a computational model to reconstruct the maturation process of this spinal cord circuit from our image data at the single-neuron level and characterize dynamic changes in functional connectivity as a function of time. We found that early functional communities are first established by spatially neighboring neurons, and neighboring communities subsequently merge by synchronization to form a patterned network. The time of recruitment of neurons to the spinal cord circuit follows, on average, a gradient from the anterior to the posterior spinal cord. Finally, we identified the cell types of active neurons using genetic markers and found that different types of neurons play different roles in circuit maturation. Ventral interneurons and motor neurons appear to serve as pioneers in the emergence of local functional communities, whereas dorsal commissural neurons may play a key role in establishing and maintaining the phase-locked state between left and right hemi-segments of the spinal cord.

Disclosures: Y. Wan: None. Z. Wei: None. S. Druckmann: None. P. Keller: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.22/F47

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

Support: NIHR-MRC UK studentship

Title: Sox14 is a definitive developmental marker for cerebellar nuclei neurons projecting to the inferior olive

Authors: H.-T. PREKOP[1], C. FERNANDES[1], L. ZAGORAIOU[2,3], T. M. JESSELL[3], *R. J. WINGATE[1], A. DELOGU[1];
[1]King's Col. London, London, United Kingdom; [2]BRFAA, Athens, Greece; [3]Columbia Univ., New York, NY

Abstract: The neuronal circuitry between the cerebellum and inferior olive (IO) is of crucial importance in motor function. However, the role of climbing fibre input from the IO is still under debate: The two main hypotheses associate the signal with motor learning by communicating error to drive synaptic plasticity, and with motor timing by phase-locking action potentials. The foundation of the latter hypothesis is the observation that neurons of the IO are characterised by intrinsic oscillatory properties and electrical coupling, which control synchronous firing of climbing fibres to excite and entrain large cohorts of Purkinje cells in an isochronic manner. Previous work has suggested that GABAergic nucleo-olivary afferents from the cerebellar nuclei modulate and determine the spatial distribution of electrotonic coupling within olivary populations, thereby creating a reciprocal firing loop between the two structures. Confirmation of this role has been hampered by the lack of an *in vivo* tool to target the nucleo-olivary neurons to assess the contribution of these cells in the circuitry. Our lab has defined Sox14 as a genetic marker for the nucleo-olivary neurons in the circuitry during development in mice. Using with a $Sox14^{GFP}$ knock-in mouse line, we identified cells expressing Sox14 in the nuclei of the cerebellum. Sox14 expression is initiated following the birth of these cells from E10.5-11.5 and is maintained until P21. Immunohistochemistry and *in situ* hybridization confirmed that these cells express both GABA and Gad1 mRNA consistent with an inhibitory neuronal identity. Using cre-dependent AAV viral tracing in $Sox14^{CRE}$ mice, we established that this genetically defined population specifically innervates the IO. Furthermore, we injected an AAV driving expression of diphtheria toxin A subunit to ablate cre-expressing cells and mCherry expression in all the infected cells (AAV-mCherry-flex-dtA). Following ablation of $Sox14^{+}$ cells, no other

cerebellar nuclei neurons, expressing mcherry, was observed to project to the inferior olive. Therefore, the cerebellar nucleo-olivary projection neurons are entirely derived from a Sox14 expressing inhibitory progenitor population. Preliminary behavioural assessment of mice in which Sox14 nuclear neurons have been specifically ablated suggest that targeted loss of nucleo-olivary neurons leads to deficits in motor learning.

Disclosures: H. Prekop: None. C. Fernandes: None. L. Zagoraiou: None. T.M. Jessell: None. R.J. Wingate: None. A. Delogu: None.

Poster

215. Sensorimotor Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 215.23/F48

Topic: D.04. Olfaction and Taste

Support: NIH/NIMH 1R01MH091348-01

Title: An *In vitro* model to study the role of the extracellular matrix in neurodevelopmental abnormalities in schizophrenia

Authors: *A. BOYER-BOITEAU[1], H. PANTAZOPOULOS[2,3], W. JANG[5], E. H. HOLBROOK[6], C.-G. HAHN[7], K. BORGMANN-WINTER[8], S. BUKHARI[2], S. BERRETTA[2,3,4]; [1]Translational Neurosci. Laboratory, Div. of Basic Neurosci., McLean Hosp., Belmont, MA; [2]Translational Neurosci. Laboratory, Div. of Basic Neurosci., Mclean Hosp., Belmont, MA; [3]Dept. of Psychiatry, [4]Program of Neurosci., Harvard Med. Sch., Boston, MA; [5]Dept. of Developmental, Mol. and Chem. Biol., Tufts Univ. Sch. of Med., Boston, MA; [6]Dept. of Otolaryngology, Massachusetts Eye and Ear, Harvard Med. Sch., Boston, MA; [7]Neuropsychiatric Signaling Program, Ctr. for Neurobio. and Behavior, [8]Dept. of Psychiatry, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: In the olfactory system, key aspects of brain development, such as neuron differentiation, and axon outgrowth occur robustly throughout life. The olfactory epithelium (OE) retains throughout adulthood the capacity for continuing neurogenesis, sustained by progenitor and stem cells. Maturing OE olfactory receptor neurons (ORN) send their axons into the subjacent lamina propria (LP), where they form odor-specific axon bundles and find their way to odor-specific olfactory glomeruli in the olfactory bulb (OB). Chondroitin sulfate proteoglycans (CSPGs), a main component of brain extracellular matrix (ECM), are suspected to play a key role in OE neuronal maturation and axon guidance. On going studies from our group indicate that CSPGs form ECM structures surrounding ORN axons accompanying them from the

OE through the OM and into odor-specific glomeruli in the (OB). This system is relevant to the pathophysiology of schizophrenia (SZ) and bipolar disorder (BD), neurodevelopmental disorders with significant olfactory identification deficits and CSPG abnormalities. The present in vitro study was designed to test the hypothesis that specialized cells within the OM, express and secrete CSPGs, in a differentiation stage-specific manner, and that this process may be altered in people with SZ. Stem cell lines were established using a protocol for isolating human nasal olfactory ectomesenchymal stem/progenitor cells. Starting with OM biopsies from control, SZ and BD subjects, primary cells were isolated, and spheres were generated to select for stem/progenitor cells and allowed to differentiate spontaneously. CSPGs were detected using immunocytochemistry and western blot with antibodies detecting distinct CSPGs and CS sulfation patterns. Our results show that OM stem/progenitor cells allowed to spontaneously differentiate in culture express and secrete CSPGs. This process was detected at early differentiation stages (day 1) and continued as cells matured, creating increasingly dense structures reminiscent of those observed in OM tissue. Differentiating cells were observed to align along these structures according clearly defined patterns. Preliminary results from comparison studies suggest that CSPGs labeled with the lectin *Wisteria Floribunda* agglutinin (WFA) and bearing the chondroitin sulfation pattern CS-6 may not be altered in SZ and BD subjects. Our results so far support the validity of this OM *in vitro* model for investigations on the role of CSPGs in axon guidance in the olfactory system. A disruption of this function in people with SZ or BD may result in miswiring of the connectivity between the OE and the OB, in turn contributing to olfactory deficits.

Disclosures: A. Boyer-Boiteau: None. H. Pantazopoulos: None. W. Jang: None. E.H. Holbrook: None. C. Hahn: None. K. Borgmann-Winter: None. S. Bukhari: None. S. Berretta: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.01/F49

Topic: A.09. Adolescent Development

Support: William T. Grant Foundation Grant 181941

Title: Resting state functional connectivity relates to response inhibition in adolescents

Authors: *S. M. TASHJIAN, D. GOLDENBERG, A. GALVAN;
Psychology Dept., UCLA, Los Angeles, CA

Abstract: Human neuroimaging literature has identified functional networks in the brain active in the absence of external stimuli, or when the brain is at rest. These networks are present across a variety of states, including during task performance. The strength of the correlation of spontaneous activity between regions of these networks can be a valuable tool for understanding individual differences in cognitive and behavioral abilities, and resting-state functional connectivity (rsFC) methods can elucidate links between the developing brain's functional organization and task performance.

Response inhibition, or suppression of a prepotent response to external stimuli, develops throughout adolescence and is a central component of impulse control. In the go/no-go (GNG) task, a standard measure of response inhibition, a motor response has to be selectively inhibited depending on whether a "go" or "no-go" cue is displayed. Several studies indicate that adolescents can perform with adult-like competence on response inhibition tasks. However, the brain regions recruited during task performance have been found to differ such that adolescents show greater recruitment of the prefrontal cortex compared to adults. These findings suggest for adolescents to achieve adult-like performance they must engage cognitive control circuitry to a greater extent. However, the extent to which functional connectivity of cognitive control circuitry is related to adolescent inhibitory control abilities is not well established.

The goal of this study was to examine the relation between adolescent behavioral performance on the GNG task and the strength of rsFC in cognitive control circuitry. Data for 48 adolescents (M_{age} = 16) were analyzed. Seed-based correlation analysis revealed a significant negative correlation between false alarms (responding "go" when a "no-go" cue is presented) and rsFC, $r(48)$=-.32, p=.029. Adolescents with greater rsFC between the superior frontal gyrus and the anterior cingulate cortex (ACC), caudate, cerebellum, medial prefrontal cortex, and dorsolateral prefrontal cortex (Z = 2.3, $p < .05$) were better able to inhibit their prepotent responses on the GNG task. These data support a role of the prefrontal cortex in adolescent response inhibition. Prior work establishes the role of the ACC in error monitoring and the caudate in response inhibition. Importantly, this study extends our understanding of the link between the stability of coordination in this circuitry and individual differences in behavioral response inhibition in adolescents.

Disclosures: S.M. Tashjian: None. D. Goldenberg: None. A. Galvan: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.02/F50

Topic: A.09. Adolescent Development

Support: ES014930

R01 ES013744

P30 ES023515

ES020364

Title: Perinatal lead exposure and white matter microstructure in children

Authors: ***M. HORTON**[1], P. CURTIN[2], C. GENNINGS[2], V. WANG[3], E. PROAL[4], L. SCHNAAS[5], M. TÉLLEZ ROJO[4], E. ROLDAN-VALADEZ[6], F. CASTELLANOS[7], C. TANG[3], R. WHITE[8], R. WRIGHT[3];
[1]Preventive Med., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Preventive Med., [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Natl. Inst. of Publ. Hlth. of Mexico (INSP), Mexico City, Mexico; [5]Natl. Inst. of Perinatology, Mexico City, Mexico; [6]Natl. Ctr. for Med. Instrumentation and Imaging Res. (Ci3M), Mexico City, Mexico; [7]New York Univ., New York, NY; [8]Boston Univ., Boston, MA

Abstract: Background
Perinatal lead (Pb) exposure is associated with adverse cognitive and behavioral outcomes that may be mediated by altered brain structure and function. Childhood Pb exposure has been associated with persistent impacts on adult white matter microstructure. The objective of this study was to assess the impact of perinatal Pb exposure on white matter microstructure in children using diffusion tensor imaging (DTI).
Methods
This study took place in the ELEMENT cohort in Mexico City. We randomly selected 20 subjects at age 6 years for a magnetic resonance imaging (MRI) pilot study. DTI images were acquired with a 3T Philips Achieva scanner using gradient echo planar imaging. A voxel-wise statistical analysis for diffusivity measures, including fractional anisotropy (FA), was performed along major white matter tracts. All 20 subjects had blood biomarkers of Pb collected during 2nd and 3rd trimesters and at delivery (umbilical cord blood). We examined correlations between Pb biomarkers and FA values. To capture associations between perinatal Pb exposure and within-brain variability at different time points, we focused on mean FA and standard deviation (SD) of FA across 48 template regions of interest (ROIs); the latter metric captures bidirectional effects that may discretely increase or decrease FA values. Linear regression models examined the association of 2nd, 3rd-trimester and cord blood Pb levels, FA and SD of FA from the ROIs.
Results
Pb levels in 2nd trimester blood were positively correlated with increased global FA after controlling for multiple comparisons ($p < 0.05$). Higher cord blood Pb was associated with increased FA ($\beta = 0.010$, $p = 0.05$) and increased variability of FA ($\beta = 0.0014$, $p = 0.07$).
Discussion
These pilot data suggest changes in white matter microstructure associated with perinatal Pb exposure. Pathological alterations can decrease or increase FA, thus our pilot findings may be consistent with neurotoxic effects of perinatal Pb exposure.

Disclosures: M. Horton: None. P. Curtin: None. C. Gennings: None. V. Wang: None. E. Proal: None. L. Schnaas: None. M. Téllez Rojo: None. E. Roldan-Valadez: None. F. Castellanos: None. C. Tang: None. R. White: None. R. Wright: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.03/F51

Topic: A.09. Adolescent Development

Support: NIH Grant MH086654

NIH Grant MH096773

NIH Grant MH099064

NIH Grant MH091238

NIH Grant U54MH091657

McDonnell Center for Systems Neuroscience

Title: Heritability of the human connectome

Authors: *O. MIRANDA DOMINGUEZ[1], E. FECZKO[1], J. T. NIGG[2], D. A. FAIR[3]; [1]Behavioral Neurosci., [2]Behavioral Neurosci. & Psychiatry, [3]Behavioral Neurosci. & Psychiatry & Advanced Imaging Res. Ctr., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Understanding the degree to which variance in the human connectome is shared among relatives is of high interest to the field. With the recent development of methods to reliably characterize individuals using fMRI data (i.e., "connectotyping" or functional fingerprinting (Miranda-Domínguez et al., 2014)), we can now ask to what extent the connectotype is familial or, in the case of twins, heritable. Following a machine-learning framework, we analyzed data from two well-characterized samples of child and adult sibling. The work revealed that the connectotype or "functional fingerprint" of the brain is familial and preserved in youth and adulthood. First, we show that the functional fingerprint enables the identification of an individual, even after several years. These findings are important as they suggest that despite developmental changes in connectivity patterns with age, the essence of the connectotype accounts for more individual subject variability than the developmental change. Second we show the correspondence of the connectotype across family members is consistent

with heritability. Using a machine learning algorithm we are able to classify the connectomes of sibling pairs at a high rate (74%). Correspondence is strongest for monozygotic twins (86%), followed by dizgygotic twins (77%), non-twin siblings (71%), and last, unrelated pairs. This heritability appears to be driven by high-order systems including the fronto-parietal, dorsal-attention, ventral-attention, cingulo-opercular, and default systems. Understanding the link between individual and familial connectomes is critical to future work characterizing individual differences in brain function.

References Miranda-Domínguez, Ó., Mills, B.D., Carpenter, S.D., Grant, K.A., Kroenke, C.D., Nigg, J.T., and Fair, D.A. (2014). Connectotyping: Model Based Fingerprinting of the Functional Connectome. PLoS One 9.

Disclosures: O. Miranda Dominguez: None. E. Feczko: None. J.T. Nigg: None. D.A. Fair: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.04/F52

Topic: A.09. Adolescent Development

Support: RO1 MH085953

U54 EB020403

T32MH073526

Title: High-resolution shape analysis reveals reciprocal patterns of subcortical alteration in 22q11.2 deletion and duplication carriers

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Abstract: 22q11.2 deletion syndrome (22qDel) results from a microdeletion on chromosome 22 and causes a range of physical and neurodevelopmental anomalies. Sixty percent of 22qDel patients meet criteria for a developmental neuropsychiatric disorder and roughly 30% develop

schizophrenia (SCZ). Recent evidence suggests that a reciprocal microduplication at the 22q11 locus (22qDup) may be protective against SCZ. Neuroanatomic variation underlying these genomic alterations has not yet been investigated. Here, using the ENIGMA Shape Analysis Pipeline, we reveal complex subregional differences between 22qDel, 22qDup, and healthy controls (CN).

T1-weighted brain MRI scans (22qDel=57, 22qDup=15, CN=49) were processed to derive two shape metrics of local thickness and surface area: 1) Radial distance (thickness), and 2) the Jacobian determinant (Jacobian) or surface dilation to a template, were measured across thousands of homologous points for bilateral accumbens, amygdala, caudate, hippocampus, putamen, pallidum, and thalamus shape models. A multiple linear regression was fitted at each surface point to assess Jacobian and thickness differences between groups after adjusting for age, sex, scanner, and intracranial volume. All results were corrected for multiple comparisons using standard FDR correction.

22qDel carriers displayed largely reduced shape metrics (local volume and surface area) compared to CN across all subcortical structures of interest. In contrast, 22qDup carriers showed largely increased volumes compared to 22qDel, and more subtle patterns of both increased and decreased shape measures compared to CN [Figure 1].

Our analysis identified complex patterns of increased and decreased local shape measures between groups, across many subcortical structures of interest. To our knowledge, this represents the first neuroimaging study of 22q11 gene dosage effects on brain structure, motivating future work to elucidate relationships between reciprocal genetic and anatomic variation at the 22q11 locus and neuropsychiatric phenotypes.

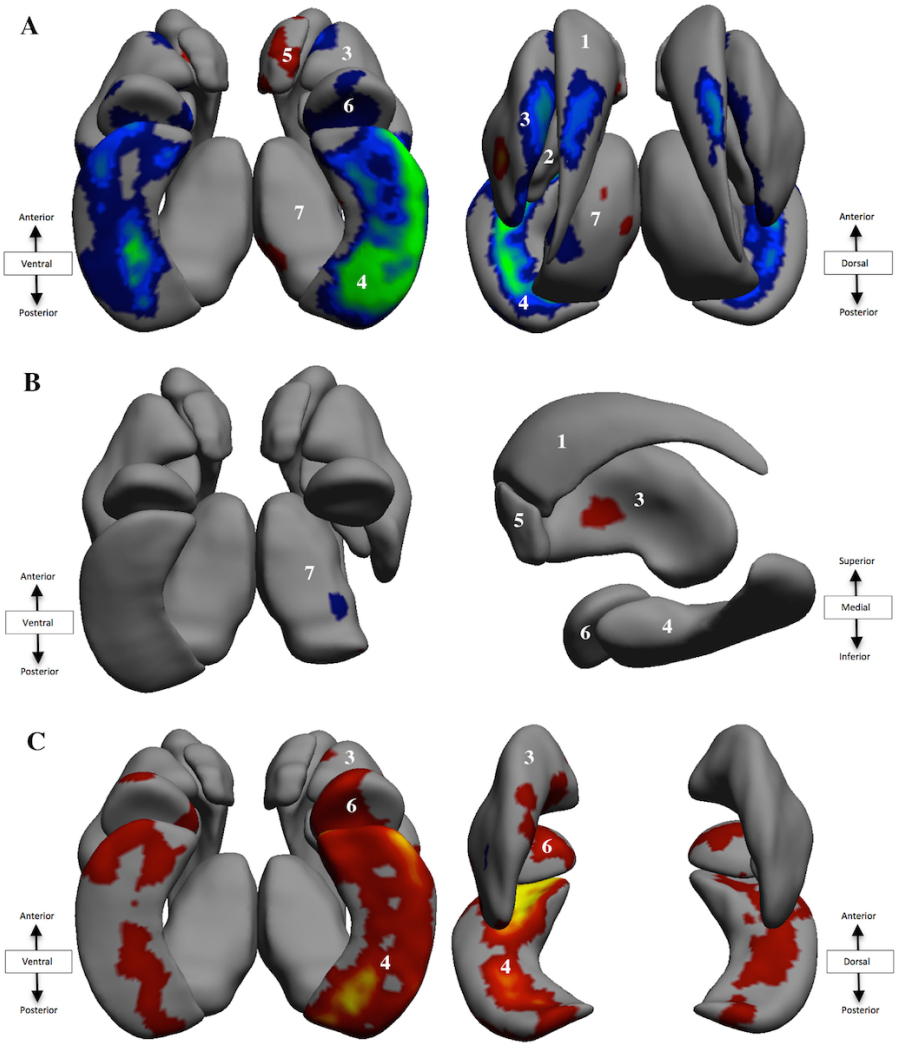

Figure 1. Maps showing β values plotted in significant regions after correction for multiple comparisons. **A. 22qDel vs. CN** Red/Yellow indicate positive β values or regions where 22qDel subjects have greater surface area/volume compared to CN. Blue/Green indicate negative β values or regions of reduced surface area/volume in 22qDel compared to CN. Left: ventral view; Right: dorsal view. **B. 22qDup vs. CN** Red/Yellow regions indicate greater surface area/volume in 22qDup subjects compared to CN. Blue/Green regions indicate reduced surface area/volume in 22qDup vs. controls. Left: ventral view (hippocampus removed; Right: medial view (thalamus and globus pallidus removed). **C. 22qDup vs. 22qDel** Red regions inidcate where 22qDup subjects have greater surface area/volume compared to 22qDel subjects. Left: ventral view; Right: dorsal/posterior view (with structures removed). *1) Caudate; 2) Globus Pallidus; 3 Putamen; 4) Hippocampus; 5) Nucleus Accumbens; 6) Amygdala; 7) Thalamus*.

Disclosures: C.R. Ching: None. B.A. Gutman: None. D. Sun: None. R.K. Jonas: None. A. Lin: None. L. Kushan: None. P.M. Thompson: None. C.E. Bearden: None. T. ENIGMA 22q11.2 Working Group: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.05/F53

Topic: A.09. Adolescent Development

Support: NIH/NIAAA 1R01AA019983-01

NIH/NIAAA 3R01AA019983-02S1

NIH/NCATS 1KL2RR031974-01

NIH/NICHD 2P30HD040677-11

Title: Awareness of alcohol advertising and limbic-frontal activations during an emotional counting stroop task in adolescents

Authors: *K. VIACAVA[1], S. EL DAMATY[2], B. W. STEVENS[2], J. LEISER[2], E. J. ROSE[3], D. H. FISHBEIN[3], J. W. VANMETER[2];
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Abstract: Awareness of alcohol advertising is a proximal factor that may also increase risk in adolescents for engaging in drinking behaviors (McClure et al., 2013). Although it is argued that effects of repeated exposure to alcohol advertising are independent of awareness, there is still no consensus on this topic. Thus, the aim of this study was to investigate if and to what extent different levels of awareness of alcohol advertising might covary with attentional bias (AB) to alcohol-related cues in mediating potential exposure effects. Participants were recruited as part of the Adolescent Development Study (ADS, funded by NIAAA), a prospective longitudinal neuroimaging study of alcohol initiation and escalation. A subset of 70 adolescents enrolled in the ADS performed an Emotional Counting Stroop (ecStroop) task during fMRI. Thirty-one participants from this sample (mean age = 14.85 yrs; 17 females) completed both the Media Exposure Questionnaire (MEQ) and the Advertising Awareness Questionnaire (AAQ). We examined neuronal activations from the subjects that did not complete the questionnaires (N = 39) to obtain a bias-free set of region(s) of interest (ROIs) to use in a subsequent analysis to determine whether awareness to alcohol advertising mediates the relationship between exposure and AB. Results revealed that awareness of alcohol ads positively mediated the relationship between alcohol ad exposure and AB towards alcohol-related words ($F(2,30) = 7.03$, $p < .01$, $R^2 = .33$); as awareness to alcohol ads increased, so did the association between exposure and AB. The association between awareness and AB ($r = .53$; $p < .01$) was slightly stronger than the one between exposure and AB ($r = .48$; $p < .01$), which supports the heuristic marketing receptivity

model (McClure et al., 2013) (i.e., the influence of marketing on behavior may involve both distal (exposure) and proximal (awareness) factors). Awareness of alcohol ads mediated the regulation of superior temporal gyrus (STG) over affective responses toward alcohol ads ($F(2,19) = 6.99$, $p < .01$, $R^2 = .45$). There were positive correlations between affective responses to alcohol ads and the insula ($r = .50$, $p = .02$), mid-cingulate ($r = .69$, $p < .01$) and STG ($r = .47$, $p = .03$), implying that as receptivity to alcohol ads increases, greater neural effort was devoted to process the emotional content captured in alcohol advertising. Altogether, these results substantiate the idea that awareness of alcohol advertising is more proximal to behavior than exposure and that both might increase receptivity to alcohol consumption in adolescents.
Keywords: alcohol advertising, awareness, exposure, attentional bias, adolescents, fMRI

Disclosures: K. Viacava: None. S. El Damaty: None. B.W. Stevens: None. J. Leiser: None. E.J. Rose: None. D.H. Fishbein: None. J.W. VanMeter: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.06/G1

Topic: A.09. Adolescent Development

Support: ROIMH07703 (TDS)

R21MH106799 (DSB,TDS)

R01MH107235 (RCG)

RC2MH089983 (REG)

K01MH102609 (DRR)

R01NS085211 (RTS)

Center for Biomedical Image Computing and Analysis (RTS, TDS)

Title: Modular evolution of structural brain networks in adolescence supports executive function and is impacted by socioeconomic status

Authors: *G. BAUM[1,2], R. CIRIC[2], D. R. ROALF[2], T. M. MOORE[2], A. E. KAHN[3], R. F. BETZEL[3], M. QUARMLEY[2], P. A. COOK[4], R. T. SHINOHARA[5], K. RUPAREL[2], R. C. GUR[2], R. E. GUR[2], D. S. BASSETT[3], T. D. SATTERTHWAITE[2];

[2]Psychiatry, [3]Bioengineering, [4]Radiology, [5]Biostatistics and Epidemiology, [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Human brain maturation during adolescence is characterized by complex changes that support behavioral flexibility and enhanced executive function. Recent work has demonstrated that functional brain modules become increasingly differentiated during youth, but it remains unclear whether structural brain networks undergo an analogous reconfiguration. Here, we evaluated the development of structural brain networks in a sample of 882 participants (ages 8-22 years) who received diffusion tensor imaging as part of the Philadelphia Neurodevelopmental Cohort. We hypothesized that structural brain networks would become increasingly modular with age, that such modular structure would support improvements in executive function, and that environmental adversity associated with low socioeconomic status (SES) would impact this process. Applying tractography methods and community detection procedures, we observed that structural brain networks become increasingly modular with age ($p < 0.0001$): within-module connectivity increased, whereas between-module connectivity decreased. Furthermore, as adolescence progressed, the community structure of structural networks became more similar to that found in adults. Increased network segregation was reflected on a regional level by widespread declines in the participation coefficient, which were especially prominent in regions within the default mode network. The only exception to this was the anterior prefrontal cortex, where conversely the participation coefficient increased with age. Notably, even when controlling for age, increased modular segregation was correlated with improved executive efficiency in a cognitive battery ($p < 0.0001$). In contrast, lower SES (as summarized by a previous factor analysis) was associated with impaired development of structural modules ($p < 0.0001$). These findings are buttressed by convergent results from supplementary analyses that used alternate edge measures (fractional anisotropy versus streamline connectivity), varied modularity resolution parameters, and use of an independent partition derived from functional brain networks. Taken together, these data delineate a developmental process whereby structural brain networks become increasingly segregated in order to support executive function, and emphasize that this process may be impacted by environmental adversity. This work provides an empirical basis for additional studies investigating how environmental factors may impact development of the brain's structural connectome and be associated with risk of neuropsychiatric symptoms.

Disclosures: G. Baum: None. R. Ciric: None. D.R. Roalf: None. T.M. Moore: None. A.E. Kahn: None. R.F. Betzel: None. M. Quarmley: None. P.A. Cook: None. R.T. Shinohara: None. K. Ruparel: None. R.C. Gur: None. R.E. Gur: None. D.S. Bassett: None. T.D. Satterthwaite: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.07/G2

Topic: A.09. Adolescent Development

Title: Puberty associated with decreased striatal intrinsic functional connectivity in 14 yo adolescents

Authors: *M. ERNST[1], A. GORKA, 20892[2], B. BENSON[2], T. LAGO[2], H. LEMAITRE[3], E. ARTIGES[4], M.-L. MARTINOT[5], J.-L. MARTINOT[6];
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Abstract: Adolescence, the transition from childhood to adulthood, is marked by steep changes in social and risky behaviors, as well as enhanced vulnerability to psychiatric disorders. Importantly, the effects of puberty on the ontogeny of brain organization remain unclear. Striatal intrinsic functional connectivity (iFC) using resting state fMRI was examined in 387 adolescents who participated in the IMAGEN Study. Data were collected from 5 sites across Europe. Data processing and analyses were conducted with Analysis of Functional NeuroImages (AFNI). Eighty participants were removed from the analysis due to excessive motion, leaving 307 adolescents (14.5 +/- 1.3 yo; 2.6 +/- 1.4 Puberty Development Score (PDS); 52% male). Multiple regression analyses were used to examine the effects and interactions of puberty, age, sex, and site on striatal iFC. Using a voxel-wise approach, puberty was negatively correlated with ventral striatum (VS) iFC to ventromedial prefrontal cortex (vmPFC), and with dorsal striatum (DS) iFC to thalamus and insula. As an initial interpretation, these negative correlations of puberty with striatal iFC to regions involved in attention, risk-taking behavior and value coding, suggest a role of pubertal maturation in the rise of risky behaviors and impulsive decision-making in adolescence.

Disclosures: M. Ernst: None. A. Gorka: None. B. Benson: None. T. Lago: None. H. Lemaitre: None. E. Artiges: None. M. Martinot: None. J. Martinot: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.08/G3

Topic: A.09. Adolescent Development

Support: NSF GRFP grant DGE1144152

Title: Developmental emergence of frontostriatal connectivity mediates flexible upregulation of cognitive control under high stakes

Authors: *C. INSEL, E. K. KASTMAN, S. F. SASSE, M. C. GARRAD, L. H. SOMERVILLE; Psychology, Harvard Univ., Cambridge, MA

Abstract: Development of frontostriatal circuit function is thought to result in unique integration of reward and cognitive control. In high stakes situations, adults often strategically improve cognitive control when performance matters. But it remains unclear whether adolescents can integrate frontostriatal systems to flexibly upregulate goal directed cognitive control. The aim of this study was to test whether adolescents could selectively enhance flexible cognitive control when high incentives were at stake. We examined whether the frontostriatal mechanisms subserving motivation-cognition interactions would exhibit a unique functional profile paralleling a developmental shift in performance. 88 participants aged 13-21 underwent fMRI during an incentivized go/no-go task with low and high stakes conditions. First, a high (+$1.00/-$0.50) or low (+$0.20/-$0.10) stakes cue was shown. Next, a series of go's (required response) and no-go's (required no response) were presented. There was a significant age by stakes interaction whereby adults improved during high stakes but adolescents did not. This effect was mediated by developmental changes in functional connectivity between the ventral striatum and vlPFC that was selectively enhanced during high stakes with age. This indicates that the ability to capitalize on stakes may be an especially late developing feature of goal directed behavior. The maturation of of frontostriatal connectivity may set the stage for successful goal directed cognitive control.

Disclosures: C. Insel: None. E.K. Kastman: None. S.F. Sasse: None. M.C. Garrad: None. L.H. Somerville: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.09/G4

Topic: A.09. Adolescent Development

Title: Earlier alcohol use and lower neuropsychological performance in adolescents from public compared to private schools

Authors: *A. R. WILLHELM[1], K. R. VIACAVA[2], J. W. VANMETER[3], R. M. M. DE ALMEIDA[2];
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Abstract: Adolescence and puberty are characterized by the development of physical, cognitive and emotional abilities that provide the necessary skills for autonomy later in life (Spear, 2000). However, this period is also marked by impulsive and risk-taking behaviors that may increase the initiation and escalation of alcohol and drugs use, setting the stage for addiction (Spear, 2013). The school's social environment might play a role in this process, as it impacts the onset of alcohol consumption (Koch & McGeary, 2005). Investigating the relationship between alcohol drinking in adolescence and type of school (public and private) is relevant given the accumulated findings of alcohol use on both cognitive skills (Renna, 2008) and academic achievement (Balsa, Giuliano, & French, 2011). Here we show a mediation model of age in the relationship between type of school and alcohol use in adolescence. Participants were 190 adolescents (10-16 years, mean age = 13.76, 112 females) selected from four public and two private schools located in Porto Alegre - RS, Southernmost Brazil. Neuropsychological measures, such as impulsivity (Barratt Impulsiveness Scale - BIS for youth), inhibitory control (Go/No-go Task), processing speed and attentional control (Five Digits Test), as well as IQ (Wechsler Abbreviated Scale of Intelligence - WASI) were assessed. Results revealed that approximately 60% of the sample had started drinking alcohol and 17% used illegal drugs. The proportion of individuals consuming alcohol increased with age such that 87% of adolescents between 15 and 16 years old had consumed alcohol. Age positively mediated the relationship between type of school and alcohol use, indicating that adolescents from public schools started drinking earlier than those from private schools ($p < .001$). In accordance with the adolescence neurodevelopmental model (Spear, 2013), we found a significant increase in neuropsychological performance as a function of age ($p < .01$). There were also significant differences in neuropsychological performance between types of school, particularly in impulsivity ($p < .05$) and inhibitory control ($p < .01$), such that better neuropsychological performance was found in students from private schools. In conclusion, adolescents from public schools initiated alcohol

use earlier and showed lower neuropsychological performance than same-aged peers from private schools.

Disclosures: A.R. Willhelm: None. K.R. Viacava: None. J.W. VanMeter: None. R.M.M. de Almeida: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.10/G5

Topic: A.09. Adolescent Development

Title: The family environment predicts children's fMRI-measured responses to emotional stimuli

Authors: *S. K. TOLIA[1], M. NOBILE[2], M. RE[2], P. PANDHER[1], K. RAMASESHAN[1], V. A. DIWADKAR[1], P. BRAMBILLA[3];
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Abstract: Introduction
A child's familial environment affects behavioral measures of cognitive and emotional development, and is likely to exert effects on functional brain responses to emotional context. Yet these effects have not been characterized. Here, we examined the effects of intra-family care based on the Parental Bonding Instrument (PBI) (Parker et al., 1979) on fMRI responses while children attended to continuously presented emotional faces of varying valence. The study focused on attention to negatively or positively valence because each class evokes obligatory but distinct emotional demands.
Methods
36 consenting or assenting participants (mean age:15.0 yrs.) rated the intra-family environment using the PBI. We focused on the "care" (as opposed to the "overprotection") dimension, deriving a common care metric using ratings for both parents. fMRI data were collected (3T, Siemens) using a novel emotional continuous performance task (Soloff et al., 2015). We manipulated contextual attention to rapidly presented (1 s) negative or positive faces in an extended block of trials (30 s). Emotional context (+ve or –ve) defined whether a letter ("A" or "X") on a face was a target or distracter (an overlaid "X" was a target only if the facial emotion was consistent with the context). By gating the cognitive response to evaluated emotional context, we sought to induce obligatory emotion regulation. fMRI data were processed (SPM8) using typical methods. First level contrasts were created to identify relative differences in

activation (Negative ≠ Positive). Contrasts were forwarded to second level regression analysis: values from the PBI were employed as a single regressor of interest (p<.05, cluster level).

Results

PBI predicted *increased* fMRI responses to negatively valenced contexts across multiple regions associated with attention, interoception and affective control. These included the precuneus, inferior and superior parietal lobules, the angular gyrus, the superior and middle temporal gyrus, the insula and the dorso-lateral and orbitofrontal cortices.

Conclusion

Intra-family care is highly predictive of functional brain activity when children are attending to negatively valenced faces. The increased activation in regions associated with emotion regulation, face processing and control (Weibert et al., 2015) may reflect a normative effect of increased care within the family environment on the brain's regulatory emotional responses. These results have implications for understanding of familial influence on cognitive development and responses, and suggest that psycho-social factors may exert substantial effects on functional brain responses.

Disclosures: S.K. Tolia: None. M. Nobile: None. M. Re: None. P. Pandher: None. K. Ramaseshan: None. V.A. Diwadkar: None. P. Brambilla: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.11/G6

Topic: A.09. Adolescent Development

Support: Danish medical research council 0602-02099B

Lundbeckfonden R32-A3161

Danish Medical Research Council 09-060166

Title: Developing consistency in reaction time associated with stable individual differences in motor system microstructure - a longitudinal DTI study of children and adolescents

Authors: *K. S. MADSEN[1,2], T. L. JERNIGAN[3], C. REUTER[3], W. K. THOMPSON[4], W. F. C. BAARÉ[1];

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San Diego, La Jolla, CA; [4]Inst. of Biol. Psychiatry, Mental Hlth. Ctr. Sct. Hans, Roskilde, Denmark

Abstract: The corticospinal tracts (CST) and striatum continue to develop throughout childhood and adolescence, and indices of their maturation can be obtained using diffusion tensor imaging (DTI), with mean diffusivity (MD) decreasing with age. We observed previously that faster and more consistent reaction times (RT) on a visuomotor task were associated with lower MD in the CST and striatum after controlling for age in 7-13 year old children. It is unknown whether these associations are mediated by individual differences in the phase of maturation of the CST and striatum in children of similar age, or by more stable individual differences in the architecture of the motor system. Here, we examined this question.

Eighty-eight healthy participants aged 7-19 years underwent longitudinal structural MRI, including DTI on a 3T scanner, (2-11 visits, 708 visits) and a 5-choice RT task (Fig. 1a). Mean RT and RT coefficient of variance (RTcv) were estimated. ROIs were made in the CST, caudate nuclei, and putamen. Mean ROI MD were then extracted bilaterally. Within-subject age-related changes in RT and ROI MD were estimated using generalized additive mixed models (GAMMs). Multivariate functional principal component analysis (mFPCA) of the mean RT, RTcv and ROI MD as a function of age was also conducted (Fig. 1b), allowing for correlation of component scores across measurement domains.

Significant age-related decreases were observed for mean RT and RTcv ($ps<10^{-11}$) as well as for MD in all ROIs ($ps<10^{-15}$). The results from the mFPCA are reported in Fig. 1b. The age-related changes in mean RT and RTcv were correlated with the maturational trajectories of CST MD, and for RTcv also to putamen MD (Fig. 1c). Further, individual differences in both RT performance and ROI MD appeared to be stable over time. Together this suggests that children with better RT performance have lower MD in the motor system, while children with poorer performance higher MD in the motor system, and that this relationship remains stable within the investigated age range in spite of superimposed biological changes associated with maturation.

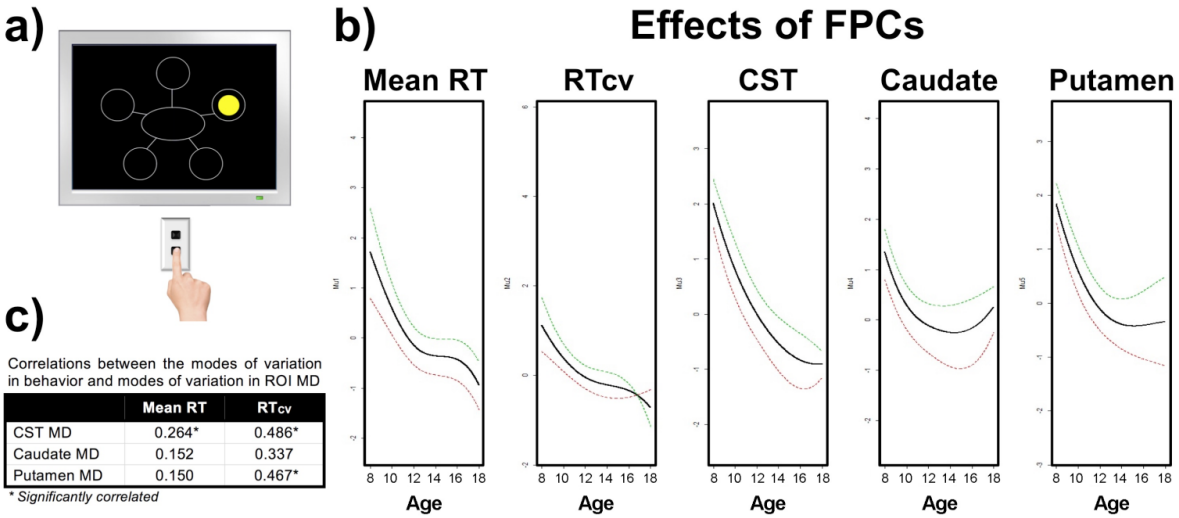

Figure 1. a) Set-up of the 5-choice reaction time (RT) task in CANTAB. Participants are instructed to press a button on a pad. After a random delay, a yellow dot appears in one of five circles. Participants must release the button as quickly as possible and touch the yellow dot. The button release time was used to estimate mean RT and RTcv. b) Results from the mFPCA of the behavioural performance and mean diffusivity (MD) of the ROIs as a function of age. From left to right, the plots display the mean fits (black lines) of the standardized mean RT, RTcv, corticospinal tract (CST) MD, caudate nuclei MD and putamen MD against age. The dashed green and red lines represent respectively the +1 and -1 standard deviation of the first principal component scores for each domain. c) Correlations between the principal component scores of the behavioral measures (mean RT and RTcv) and ROI MD (CST, caudate and putamen). An * indicates a significant relationship between two scores.

Disclosures: K.S. Madsen: None. T.L. Jernigan: None. C. Reuter: None. W.K. Thompson: None. W.F.C. Baaré: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.12/G7

Topic: A.09. Adolescent Development

Support: NIH Grant R00HD065832

NIH Grant R01MH094343

NIH Grant P41EB015922

NIH Grant U54EB020406

Title: Hippocampal shape analysis in typical development

Authors: *K. M. LYNCH, L. ZHAO, A. TOGA, K. CLARK;
Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA

Abstract: Introduction: The hippocampus is a subcortical structure critical for learning and memory. While several studies have described hippocampal changes in typical aging, few studies have addressed the normal developmental trajectory of the structure in early childhood and adolescence. The purpose of this study is to examine the cross-sectional age-related changes and sex differences in hippocampal shape in a cohort of typically developing children.
Methods: In total, 770 subjects (3-21 years) were scanned through the Pediatric Imaging, Neurocognition, and Genetics (PING) multi-site project (11 +/- 4.9 years; 356 Female). Hippocampal volumes extracted from T1-weighted MRI images were converted to a triangulated mesh and registered to the average hippocampus surface using Metric Optimization for Computation Anatomy (MOCA). Hippocampal radial thickness measures were mapped onto the average surface using Laplace-Beltrami eigen-projections. Age-related changes in hippocampal shape were analyzed using linear mixed effects models and random field theory.
Results: Significant age-related changes (controlling for sex) and sex differences are shown in Figure 1. Specifically, the bilateral hippocampi exhibit the largest age-related changes between 3 and 7 years (corrected p=.0001). Additionally, sex differences were most pronounced during adolescence. A significant age*sex interaction in overall size was noted in the left hippocampus, with males exhibiting an increase in size with age while the structure remains stable in females (p=0.046).

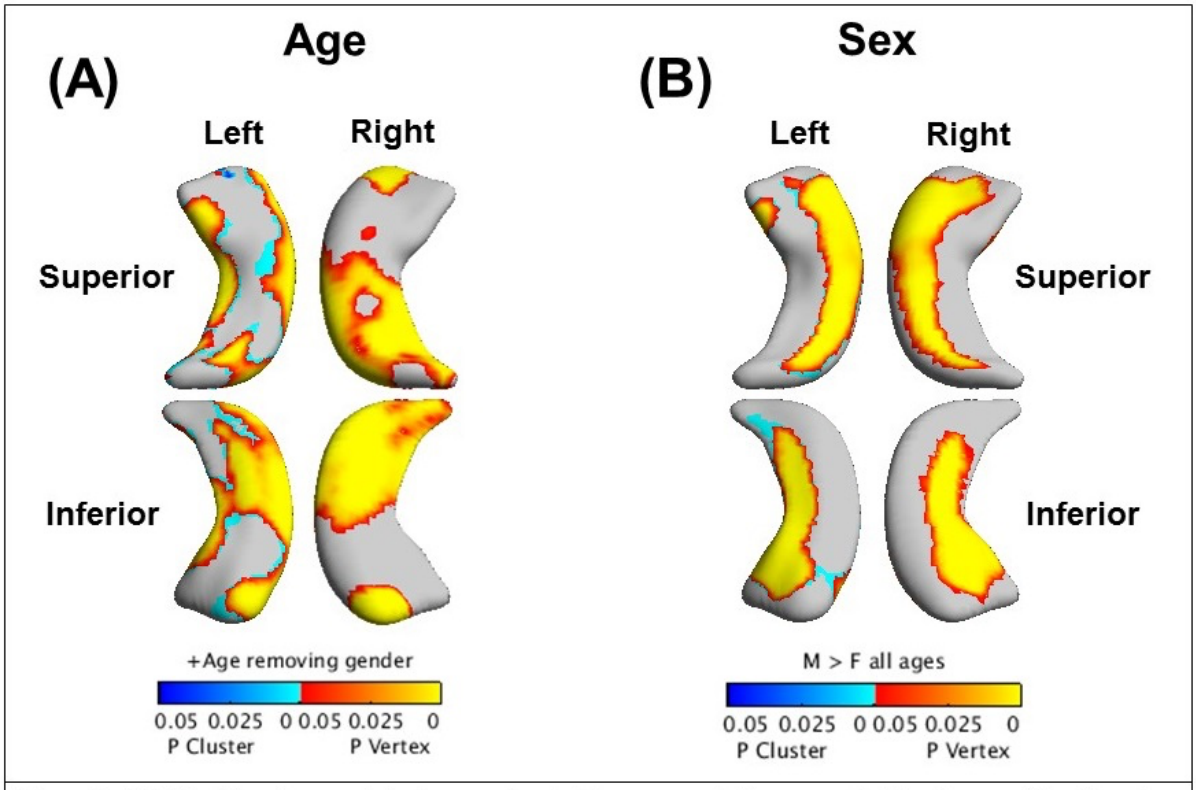

Figure 1: (A) Significant age-related expansion in hippocampal shape, controlling for sex. Significant regions include the CA1 and CA2-3 in the left hippocampus and hippocampal tail and posterior CA1 in the right hippocampus. (B) Significant sex differences in hippocampal shape among all ages. Note the bilateral expansion of CA1 and subiculum in males versus females.

Conclusion: Our results show that hippocampal shape changes significantly during childhood and adolescence, with the greatest changes occurring in before puberty. Meanwhile, sex differences in the hippocampus do not emerge until adolescence, suggesting that hippocampal shape may be under hormonal control. These results show that shape analysis is a powerful tool for detecting specific, regional developmental changes in the hippocampus.

Disclosures: K.M. Lynch: None. L. Zhao: None. A. Toga: None. K. Clark: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.13/G8

Topic: A.09. Adolescent Development

Support: Mind, Brain, & Behavior Interfaculty Initiative at Harvard University

NIDA R03 DA037405

S10RR023401

S10RR023043

Title: Adaptive adjustment in cognitive control over reward in adolescence

Authors: *J. Y. DAVIDOW[1], K. R. A. VAN DIJK[1,2], J. SNYDER[3], C. VIDAL[1], M. A. SHERIDAN[3], L. H. SOMERVILLE[1];
[1]Dept. of Psychology and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Dept. of Radiology, Massachusetts Gen. Hosp., Charlestown, MA; [3]Dept. of Psychology and Neurosci., Univ. of North Carolina, Chapel Hill, NC

Abstract: Theories of the mechanisms underlying increases in risk-seeking behavior during adolescence suggest a dynamic interplay in the ability to exercise cognitive control for appetitive cues. Prior work has evaluated control for stimuli whose appetitive qualities are central to the stimulus itself, finding that adolescents can be impaired at exerting control over appetitive stimuli. A critical open question is whether adolescent control limitations persist for cues that were previously rewarding, but no longer signal reward. We tested whether a history of reward association is sufficient to perturb adolescents' cognitive control. Inhibitory cognitive control for cues with and without previous reward association was assessed with the Conditioned Appetitive Response Inhibition Task (CARIT) during fMRI. A final sample of 130 participants 8-26 years old performed the inhibitory control task containing two no-go cues, prior to which one cue underwent operant conditioning to form a reward association, while the other was never associated with reward. Behaviorally, we found an interaction between age and previous reward conditioning, such that beginning in early adolescence proportionally more errors were made to the previously rewarded stimulus. Adolescents recruited regions within the striatum less than children and adults when successfully withholding a response for the previously rewarded cue compared to the neutral cue, consistent with signals in primate midbrain at the omission of a cued reward, and adolescent fMRI studies showing reduced striatal signals to relatively smaller rewards. Thus, in the present study, though the intrusion of reward on cognitive control emerged during adolescence, this interference was attenuated in adolescents in the absence of continued reinforcement, whereas it persisted for adults. This suggests a potential adaptive mechanism in adolescence, whereby initial strong reward-related responses could be tempered by rapid adaptation.

Disclosures: J.Y. Davidow: None. K.R.A. Van Dijk: None. J. Snyder: None. C. Vidal: None. M.A. Sheridan: None. L.H. Somerville: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.14/G9

Topic: A.09. Adolescent Development

Support: William T Grant Foundation Grant 181941

William T Grant Foundation Grant 183805

National Science Foundation GRFP

Title: Association among sleep, neural circuitry, and pubertal hormones during adolescence: Implications for risky decision-making

Authors: *D. GOLDENBERG, S. M. TASHJIAN, A. GALVAN;
Psychology, UCLA, Los Angeles, CA

Abstract: Adolescence is characterized by dramatic increases in pubertal hormones, profound changes in sleep habits, and significant remodeling of motivational and regulatory neural systems. Separate lines of research have linked these neurobiological changes during adolescence with increased rates of risky decision-making. Importantly, emerging evidence suggests these neurobiological changes are intricately related to one another. Wake-sleep cycles likely change during adolescence in a manner that is hormone-dependent (Hagenauer & Lee, 2013) and poor sleep quality in adolescents is related to reduced functional connectivity between regulatory (e.g. DLPFC) and reward-related regions (e.g. ventral striatum, insula) during risky decision-making (Telzer et al., 2013). Given the potential link between these key neurobiological changes, the purpose of the current study was to examine the associations between sleep quality, pubertal hormone levels, and neural response during risky decision-making in a sample of adolescents. Fifty-five adolescents (ages 14-18 years; 52% female) provided salivary assays analyzed for pubertal hormone levels (testosterone, estradiol, and DHEA). Sleep quality and duration were assessed subjectively (self-report) and objectively (actigraphy measurements) over a two-week period. Participants underwent functional magnetic resonance imaging (fMRI) while performing a decision-making task designed to contain conditions of response inhibition and risky choice in pursuit of reward. This is important because poor sleep during adolescence likely impairs recruitment of regions involved in cognitive control (e.g. prefrontal cortex) in addition to eliciting increased neural response in reward-related regions (e.g. ventral striatum), both of which may result in greater risky behavior. A repeated-measures ANOVA revealed significant differences in mean reaction time by trial type [$F(2.85, 88.21)=34.16$, $p < .01$], suggesting differential processing by condition. Main effects during risky choice revealed significant activation in reward-related regions (e.g. ventral striatum) while cautious choice elicited

response in regulatory-regions (e.g. DLPFC). Both cautious choice and response inhibition elicited activation in the DLPFC, with a lesser extent of activation during inhibition. Only cautious choice elicited activation in the insula. Functional connectivity analyses will be conducted and correlated with measurements of sleep quality. Moderation analyses will assess the influence of pubertal hormones on the relationship between sleep and functional connectivity during risky decision-making.

Disclosures: D. Goldenberg: None. S.M. Tashjian: None. A. Galvan: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.15/G10

Topic: A.09. Adolescent Development

Title: I want it now! The role of pubertal testosterone in impatience of adolescent boys

Authors: *C. LAUBE, R. LORENZ, W. VAN DEN BOS;
Max Planck Inst. For Human Develop., Berlin, Germany

Abstract: The onset of adolescence is associated with an increase in transgressive behaviors, from juvenile delinquency to substance use and unprotected sex, which are often attributed to increased impulsiveness. This increase is assumed to result from differential maturation of a subcortical affective brain network and a cortical control network. More recently it has been hypothesized that pubertal hormones may specifically modulate the function of the subcortical regions, and increasing reward seeking behavior. However, much is still unknown about how pubertal hormones impact brain and behavior. Here we focus on the role of testosterone in impulsiveness in adolescent males. In an behavioral study (*N*=72, ages 11-14) we found dissociable effects of age and pubertal testosterone on impatient behavior on an intertemporal choice task. That is, increased sensitivity to immediate rewards was specifically related to an increase in pubertal testosterone, whereas a general reduction of impatience was related to increased age. To gain further insight into these mechanisms, we designed a follow-up study in which we aim to investigate the different effects of age vs. pubertal testosterone on brain function and structure (*N* = 75, ages 10-15, and *N*=25, ages 20-30) on several delay discounting paradigms in and outside the MRI scanner. Preliminary results reveal that adolescents showed less activity in the insula in presence of immediate rewards compared to adults, and insula activity was also related to individual differences in pubertal status. We will discuss these results against the background of current neurodevelopmental models of adolescent brain development and will present specific effects related to testosterone. As a conclusion, our results highlight the

importance of studying pubertal development and emphasize the necessity to also integrate measures of pubertal development (and not exclusively age) when trying to understand adolescent impulsivity.

Disclosures: C. Laube: None. R. Lorenz: None. W. van den Bos: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.16/G11

Topic: A.09. Adolescent Development

Support: John D. and Catherine T. MacArthur Foundation

T32 Predoctoral Fellowship Award (NPI/NIH T32DA024635)

Title: The 'triple threat': Influence of peers, excitement, and reward on cognitive capacity in adolescents

Authors: *K. S. BREINER[1], A. LI[3], A. O. COHEN[3], L. STEINBERG[4], R. J. BONNIE[5], E. S. SCOTT[6], K. A. TAYLOR-THOMPSON[7], M. D. RUDOLPH[8], J. CHEIN[4], J. A. RICHESON[9,10], D. V. DELLARCO[3], D. A. FAIR[8], B. J. CASEY[3,10], A. GALVAN[2,11];
[1]Psychology/ Psychiatry & Biobehavioral Sci., [2]Psychology, UCLA, Los Angeles, CA; [3]Psychiatry, Sackler Inst. for Developmental Psychology, Weill Cornell Med. Col., New York, NY; [4]Psychology, Temple Univ., Philadelphia, PA; [5]Sch. of Law, Univ. of Virginia, Charlottesville, VA; [6]Columbia Law Sch., Columbia Univ., New York, NY; [7]Sch. of Law, New York Univ., New York, NY; [8]Biobehavioral Neurosci. & Psychiatry, Oregon Hlth. & Sci. Univ., Portland, OR; [9]Psychology, Northwestern Univ., Evanston, IL; [10]Psychology, Yale Univ., New Haven, CT; [11]Brain Res. Institute, UCLA, Los Angeles, CA

Abstract: Adolescence marks a time of increased risky and impulsive behavior when the capacity for cognitive control is susceptible to socioemotional influences[1]. Adolescents are more risky in their choices when among peers[2] and more impulsive than adults to social cues[1,3]. Thus, social cues can trigger impulsive actions, overriding goal-directed behavior during adolescence. This adolescent specific effect in behavior is paralleled by greater activity in reward related circuitry[3-4]. Yet, when this capacity matures is still unclear. Moreover, studies examining the effects of incentives and peers typically examine these factors independently, although they rarely are in real-life.
The goal of the current study was to examine changes in the capacity for self control with age

under conditions that may better reflect real world situations for teens. We tested the impact of the perceived presence or absence of a peer when performing an impulse control task[5] that consisted of positive social cues under a state of excitement. A diverse sample of 171 participants ages 13 to 25 years were tested. We found that under excitement when detecting smiling faces in the presence of peers, adolescents showed diminished cognitive control relative to when alone, as indexed by d-prime $F(1, 66) = 9.19$, $p < .005$ and to other age groups $F(2, 163) = 4.62$, $p < .01$). This pattern of performance was paralleled by greater activity in the orbitofrontal cortex (MNI coordinates: x=6.5, y=36.5, z=-20.5, 44 voxels; $p < .02$, corrected) in teens relative to adults for the peer condition $F(2, 163) = 5.53$, $p = .005$.
These data suggest the combined "triple threat" to self control of peers, excitement and positive cues may lead to a greater anticipation of reward and increased impulsivity during adolescence but not in other age groups. This research has important implications for law and policy regarding diminished cognitive capacity during the adolescent years in emotionally charged situations.

1. Cohen, A.O., et al. 2016. When is an adolescent an adult? Assessing cognitive control in emotional and nonemotional contexts. *Psychol Sci*, *1*.
2. Gardner, M., Steinberg, L. 2005. Peer influence on risk taking, risk preference, and risky decision making in adolescence and adulthood: An experimental study. *Dev Psychol, 41*.
3. Somerville, L.H., et al. 2011. Frontostriatal maturation predicts cognitive control failure to appetitive cues in adolescence. *J Cogn Neurosci, 41*.
4. Chein, J., et al. 2011. Peers increase adolescent risk taking by enhancing activity in the brain's reward circuitry. *Dev Sci, 14*.
5. Cohen, A.O., et al. 2016. The impact of emotional states on cognitive control circuitry and function. *J Cogn Neurosci, 28*.

Disclosures: K.S. Breiner: None. A. Li: None. A.O. Cohen: None. L. Steinberg: None. R.J. Bonnie: None. E.S. Scott: None. K.A. Taylor-Thompson: None. M.D. Rudolph: None. J. Chein: None. J.A. Richeson: None. D.V. Dellarco: None. D.A. Fair: None. B.J. Casey: None. A. Galvan: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.17/G12

Topic: A.09. Adolescent Development

Support: NSRDP Grant 2016-10063239

Title: Resting-state functional connectivity can differentiate children and adults: An EEG study

Authors: S. KANG[1,2], D. KIM[2], *S.-H. JIN[3];
[1]Seoul Natl. Univ. Col. of Nursing, Seoul, Korea, Republic of; [2]Natl. Standard Reference Data Ctr. for Korean EEG, Seoul, Korea, Republic of; [3]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Objective: To evaluate whether the support vector machine (SVM)-based group classifier based on electroencephalography (EEG) in the eyes-closed resting-state can distinguish children and adults. **Methods:** We compared resting-state functional connectivity between 150 children (age range = 8 to 11) and 123 adults (age range = 24 to 50). Imaginary coherence values between the 19 EEG electrodes were calculated. The most informative connectivity with statistical significance (Bonferroni corrected, $p < .00001$) across leave-one-out group comparisons were sorted in a descending manner and became candidate feature sets for support vector machine (SVM) learning to identify the optimal group classifier. **Results:** The optimal SVM group classifier with the top 8 ranked features in the alpha frequency band distinguished children and adults with a mean accuracy of 79.3% (sensitivity = 77.1%; specificity = 81.5%; positive predictive value = 81.1%; negative predictive value = 78.5%). Adults had enhance functional connectivity between frontal and parietal (Fz-P4, F4-P4) or central regions (Fp1-C4, Fp2-C4), between frontal regions (F7-F3, F7-Fz, Fp1-F8), and between central and parietal regions (Cz-P4). Interestingly, all functional connections composing the optimal SVM model were observed in the alpha frequency band among the conventional 4 frequency bands. **Conclusion:** We showed the potential of electrophysiological resting-state functional connectivity, which reflect differences of functional brain networks between children and adults mainly in the alpha frequency band, as a possible EEG biomarker that differentiates children and adults.

Disclosures: S. Kang: None. D. Kim: None. S. Jin: None.

Poster

216. Adolescents: Human Imaging I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 216.18/G13

Topic: H.02. Human Cognition and Behavior

Support: NIH grant 5R37AG-006265-25

Title: Age associated differences in resting-state network topology predict differences in task-evoked activity

Authors: *M. Y. CHAN[1], F. ALHAZMI[1], N. K. SAVALIA[1], D. C. PARK[1,2], P. F. AGRES[1], G. S. WIG[1,2];
[1]Ctr. for Vital Longevity and Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Dallas, TX; [2]Dept. of Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: The organization and information processing of human brain areas can be examined using multiple features (e.g., resting-state and task-based connectivity, task-evoked activity, morphometry). It is hypothesized that individual differences in these features are related to one another. While a number of recent studies have described the spatial overlap between resting-state functional correlation (RSFC) network topology and task-evoked activity, it is unclear whether individual variations in a brain area's RSFC topology may inform its activity during task execution. To pursue this question, we analyzed RSFC patterns using graph theory to characterize the network topology of brain areas, and then examined how individual differences in RSFC topology were related to differences in task-evoked activity (blood-oxygen-level dependent; BOLD) collected from two independent tasks with differential stimulus and task processing demands (i.e., a visual classification task and a semantic categorization task).
Data from the Dallas Lifespan Brain Study were analyzed, first focusing on a sample of healthy young adults (N=64; 20-34y) to understand how the connectional topology of a given brain node (brain area) as defined by RSFC relates to its activity during task. We found that relative to other nodes of the same system, nodes with connections largely limited to other nodes of their own functional system (i.e., exhibiting a lower participation coefficient [PC]) elicited greater BOLD activity than nodes with many connections to nodes in other systems (i.e., higher PC). Importantly, individual differences in 'activation selectivity' were primarily observed in those functional systems under heavy processing demands during the corresponding task. We next examined healthy participants across the adult lifespan (N=238; 20-89y) in order to compare brain networks that exhibited age-related differences in resting-state functional topology (Chan et al., 2014). With advanced age, decreased distinctions were observed in node topology in terms of connections within and between systems. Furthermore, brain networks with less differentiated topology (i.e., older adults) exhibited decreased activation selectivity across nodes. Together, the results provided evidence that the functional topological properties observed at rest are important for describing the functional activity of brain areas during active tasks (or vice versa) across participants. In doing so, these results also provided a possible network-based explanation for previous reports of the 'dedifferentiation' in brain activity observed in aging.

Disclosures: M.Y. Chan: None. F. Alhazmi: None. N.K. Savalia: None. D.C. Park: None. P.F. Agres: None. G.S. Wig: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.01/G14

Topic: B.05. Transporters

Title: SLC7A10 - a novel candidate for human hyperekplexia

Authors: *C. VILLMANN[1], S. ATAK[1], R. J. HARVEY[2], P. DREHMANN[1];
[1]Univ. Wuerzburg, Wuerzburg, Germany; [2]UCL Sch. of Pharm., London, United Kingdom

Abstract: Human hyperekplexia (Startle disease) is a rare neuromotor disorder. Characteristic symptoms are enhanced startle responses following unexpected sensory or tactile stimuli. Several affected genes including the *GLRA1* as the most common gene, *GLRB*, *SLC6A5* as well as *ARHGEF9* and *GPHN* have been identified as the underlying causes for the observed pathology. Mutations in these genes can be assigned to about 20% of patients leaving the underlying pathology for 80% of patients diagnosed for startle disease unknown. We screened the genomic DNA from 50 hyperekplexia patients for mutations in Asc-1. The Asc-1 transporter is a plasma membrane antiporter with high affinity for neutral small amino acids such as glycine, L-serine, D-serine, alanine and cysteine. All 11 exons including flanking intronic sequences have been analyzed by sequencing. In our screening we discovered three exonic single nucleotide polymorphisms generating one missense mutation in exon 7 c.919G>A; G307R and two synonymous mutations c.1158G>A; T386T in exon 9 and c.1374G>T; T458T in exon 10. In addition, we detected several intronic nucleotide variations that might have an input on splicing or binding of enhancer/silencer elements. Furthermore, we detected 5'UTR and 3'UTR variations that may influence gene expression by changes in the translation efficiency, stability and transport of the mRNA. The missense mutation G307R was cloned into an expression vector for analysis of the transporter function in transfected cell lines. To do so, HEK293 cells were transfected with the mutated Asc-1 G307R together with h4F2hc. The latter represents an accessory subunit of the transporter required for transport of Asc-1 to the surface. Cells carrying both transporter subunits were incubated with [^{3}H]-glycine to determine uptake of labeled ligand in comparison to cells transfected with wild-type Asc-1. As a control, D-isoleucine incubation was used which is an inhibitor of Asc-1. The functional analysis of the mutated variant is under investigation. A previous knockout study of the Asc-1 (alanine-serine-cysteine) transporter in mice suggested Asc-1 as a novel gene for startle disease. Our data show that the human *SLC7A10* (Asc-1) indeed represents a novel candidate gene for human hyperekplexia.

Disclosures: C. Villmann: None. S. Atak: None. R.J. Harvey: None. P. Drehmann: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.02/G15

Topic: B.14. Neuro-Oncology

Support: R01NS082851

R01NS052634

Title: Ferroptosis and the molecular regulation of SLC7A11 in glioma

Authors: *R. A. UMANS[1], J. MARTIN[2], E. THOMPSON[2], H. SONTHEIMER[2];
[2]Ctr. for Glial Biol. in Health, Disease, and Cancer, [1]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Ferroptosis, a non-apoptotic, iron-catalyzed cell death pathway, is mediated by reactive oxygen species and has been implicated in various forms of cancer. Ferroptosis studies have shown that the tumor suppressor gene, *p53*, negatively regulates the solute carrier *SLC7A11*. *SLC7A11* encodes the catalytic subunit for System XC, a cysteine/glutamate antiporter. *SLC7A11* is overexpressed in almost half of gliomas and is a marker of poor clinical prognosis. Coincidentally, *p53* is also mutated at a similar percentage in glioma. To date, ferroptosis has not been investigated in glioma but would further elucidate how high *SLC7A11* gliomas are more lethal *in vivo*. We hypothesize that *p53* negatively regulates *SLC7A11* in glioma and that high *SLC7A11* results in a higher concentration of antioxidant, protecting these cells from ferroptosis-induced oxidative cell death. To address this hypothesis, we have induced ferroptosis with the drug erastin in continuous and xenoline cultures with different *p53* and *SLC7A11* statuses. Additionally, we have performed biochemical assays for the roles of *p53* and *SLC7A11* in ferroptosis as well as chemically and genetically modulated the *p53* status in our cell lines. With these ferroptosis assays in vitro, we demonstrated sensitivity to ferroptosis in cell lines that have wild-type *p53* and lower *SLC7A11* expression status. Furthermore, wild-type *p53* cells can be protected from ferroptosis with a glutathione analog as well as conversely making mutant *p53* cells sensitive to ferroptosis by reactivating *p53*. Understanding the molecular regulation of *SLC7A11* in gliomagenesis would reveal potential therapeutic targets that may be protective for tumor progression. These therapies may also be used in conjunction with already approved *SLC7A11* inhibitors to more intently target the glioma cell population that promotes the bleak outcome in this CNS disease.

Disclosures: R.A. Umans: None. J. Martin: None. E. Thompson: None. H. Sontheimer: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.03/G16

Topic: B.05. Transporters

Support: NIH/NINDS NS087068

AHA predoctoral fellowship (15PRE25310013)

AHA postdoctoral fellowship (14POST20480080)

Title: Functional characterization of the mitochondrial na^{+}-ca^{2+} exchanger (nclx) in the peripheral nervous system using nclx knock-out mice

Authors: *J. RYSTED, Z. LIN, A. GNANASEKARAN, Y. USACHEV;
Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: In neurons, mitochondria efficiently buffer Ca^{2+} influx during excitation, and then release Ca^{2+} back into the cytosol, which helps shape $[Ca^{2+}]_i$ transients and regulates many Ca^{2+}-dependent neuronal functions such as excitability, neurotransmission, gene expression and neuronal survival. Past research has identified solute carrier from family 8 member B1 (SLC8B1, also known as NCLX) as the mitochondrial Na^{+}-Ca^{2+} exchanger. NCLX has been proposed to be one of the mechanisms involved in mitochondrial Ca^{2+} extrusion found in the inner mitochondrial membrane. However, the role of NCLX in neurons remains largely unknown. Using RT-PCR, we found that NCLX is expressed throughout the brain, and is also present in dorsal root ganglia (DRG) neurons. By simultaneously monitoring Ca^{2+} concentration in the cytosol ($[Ca^{2+}]_{cyt}$) and mitochondria ($[Ca^{2+}]_{mt}$) of cultured DRG neurons, we found that NCLX knockout (KO; Jackson Lab, C57BL/6J background) did not significantly alter the amplitude of depolarization-induced $[Ca^{2+}]_{cyt}$ or $[Ca^{2+}]_{mt}$ elevation as compared to WT animals. However, $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mt}$ recovery to baseline in response to moderate and strong depolarization (30 mM, 50 mM KCl respectively, both for 30 s) was slowed by 2-3 fold, but not halted, in NCLX KO as compared to WT animals. In contrast, when $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{mt}$ elevations were elicited by trains of action potentials (5-8 Hz, 4-8 s) using extracellular field stimulation no significant difference was found between NCLX KO and WT DRG neurons in either recovery kinetics or amplitudes. In control experiments, we found that the pharmacological inhibitor of mitochondrial Na^{+}-Ca^{2+} exchanger, CGP37157 (3 and 10 μM) markedly slowed the recovery of $[Ca^{2+}]_{mt}$ to the baseline in both WT and NCLX KO. In addition, replacement of Na^{+} with Li^{+} did not significantly alter the ability of mitochondria to extrude Ca^{2+} from the mitochondrial matrix in DRG neurons. Collectively, our data suggest that NCLX contributes to Ca^{2+} extrusion from mitochondria in DRG neurons following large depolarization-induced $[Ca^{2+}]_i$ transients, but not

smaller ones evoked by electrical field stimulation. Our findings also point to the existence of additional, distinct from NCLX transporter(s) that mediate Ca^{2+} efflux from mitochondria in a CGP37157-sensitive manner in DRG neurons.

Disclosures: J. Rysted: None. Z. Lin: None. A. Gnanasekaran: None. Y. Usachev: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.04/G17

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: The Swedish Research Council

The Swedish Brain Foundation

The Swedish Society for Medical Research

The Novo Nordisk foundation

Åhlens foundation

Engkvist Foundation

Thurings Foundation for metabolic research

Title: Identification of a novel osmoregulatory membrane transporter in D. Melanogaster

Authors: *M. M. ERIKSSON[1], E. PERLAND[2], E. LEKHOLM[2], M. WILLIAMS[2], R. FREDRIKSSON[1];
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Abstract: Membrane bound transporters are vital for cell homeostasis since they translocate molecules over cellular membranes. Disturbances in membrane transporters are involved in several diseases and are therefore crucial to study; it is highly possible that knew drug targets will be discovered and causes of diseases identified among the so far unstudied transporters. We have identified a possible sugar transporter in *D.melanogaster* belonging to the major facilitatory superfamily Pfam clan, with homologues in rodent and humans. With the use of the UAS/Gal4 system, we visualize its expression in the malphigian tubuli, the flies' equivalent to our kidney, and hind gut, suggesting an involvement in osmoregulation. Ubiquitous knock down of the *CG4928* protein in fruit flies leads to a constantly increasing edema, which eventually kills the

flies. In the edema, there was approximately 40% increased sodium concentration, while the potassium concentrations were unaffected. Furthermore, flies lacking the CG4928 protein have decreased starvation resistance ($p>0.001$) as seen in a starvation test. By measuring the mRNA levels of *CG4928* in wild type flies, reduced expression was detected within 3-9h after food removal. Interestingly, the knock down flies have normal food intake, hence suggesting the phenotypical changes are due to metabolic disruption rather than food consumption. To understand what changes occur in the CG4928 knock down flies, RNA sequencing analysis was performed on knock down flies' vs controls, and it revealed changed expression of genes involved in sugar metabolism and sugar homeostasis. Hence it is plausible that CG4928 is a novel sugar transporter. This was elucidated further by studying the gene expression in flies raised on different diets.
We have identified a novel membrane bound transporter in fruit flies that is involved in osmoregulation over the malphigian tubule and the hind gut. Furthermore, we provide data claiming *CG4829* to be a novel sugar transporter.

Disclosures: M.M. Eriksson: None. E. Perland: None. E. Lekholm: None. M. Williams: None. R. Fredriksson: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.05/G18

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: The Swedish Research Council

The Swedish Brain Foundation

The Swedish Society for Medical Research

The Novo Nordisk foundation

Åhlens foundation

Olle Engkvist Foundation

Thurings Foundation for metabolic research

Title: Characterization of solute carrier transporters in the brain

Authors: *S. BAGCHI, V. ARAPI, R. FREDRIKSSON;
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Abstract: Glutamate is a key regulator in the brain and imbalance in the glutamine-glutamate cycle has been shown to cause numerous neuropsychiatric conditions, including anxiety, bipolar disorder and mood disorders. Drug targets within the glutamate system for neuropsychiatric diseases are currently lacking. We aim to functionally characterize transporters of solute carrier family 38, also named as sodium-coupled neutral amino acid transporters (SNATs) that are possibly involved in glutamine and glutamate transport during glutamine-glutamate cycle and can be potential drug targets. These transporters include 11 members that are expressed throughout the brain. SNAT6, one of the transporters within this family has already been reported to be exclusively expressed in the excitatory neurons and is localized at the synaptic membrane (Bagchi *et. al.* 2014, PLOS One). Due to its presence at the synaptic plasma membrane and clear interaction with SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complex protein Snap25 and vesicular protein Synaptotagmin (Bagchi *et. al.* 2014, PLOS One), SNAT6 is a strong candidate for glutamine transport in the glutamine-glutamate cycle at the active zone where the synaptic vesicles are fusing to the synaptic membrane to release neurotransmitters at the synapses. We are using high-resolution Total internal reflection fluorescence (TIRF) microscopy and single molecule imaging to visualize SNAT6 in respect to secretory vesicles at the synaptic membrane. Our group is developing transgenic mice where SNAT6 is knocked out conditionally and we will then use material (brain tissue, spinal cord and other organs where transporters of interest are expressed as well as primary cell cultures from embryos) from them to confirm localization and specify further functions of these transporters with immuno-staining, Proximity Ligation Assay and uptake assays with quantum dot conjugated amino acids. This will enable us to put the puzzle pieces together and get a more complete picture of glutamine-glutamate system.

Disclosures: S. Bagchi: None. V. Arapi: None. R. Fredriksson: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.06/G19

Topic: B.05. Transporters

Support: SIP20160638

SIP20150710

Title: Hipoglucemic plant Ibervillea sonorae extracts affect the Glucose Transporters expression GLUT1 and GLUT3 in glial and granular cells.

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Abstract: Mexico heads the first places with major number of persons sick with diabetes. National survey of Health and Nutrition reported that 20 percent of the diagnosed cases do not have access to any treatment, a 85.6 percent is treated by oral agents, whereas 6.2 percent do not use pharmacological therapy and that 13 percent uses insulin treatment or in combination. In Mexico, about 5000 species of plants have medicinal attributes. Ibervillea sonorae Greene belongs to Cucurbitaceae family and it is native to arid areas of northern of Mexico. Pharmacological studies showed that plant roots display hypoglycemic, antiinflammatory, antioxidant, antimicrobial and antifungal activities. A recent study conducted on human preadipocyte cells showed that aqueous root extracts from I. sonorae stimulate glucose uptake by a PI3K independent pathway. This evidence supports the antidiabetic properties attributed to I. sonorae root. This work, it is centered on the primary system of transport of glucose, on specific GLUT 1 and GLUT 3, due to the fact that its expression, regulation and activity play a basic role in the homeostasis neuronal. Roots of I. sonorae are washed with distilled water and dried at 40°C. The powder was extracted with 50 percent ethanol or water stirring occasionally. The extract was filtered and concentrated under reduced pressure on rotavapor and concentrated extract was spray-drying. To get of fractions of extracts of I. sonorae, five g of extract were separated by exclusion chromatography. One sought to determine the activity of watery and ethanol extracts of I. sonorae in the expression of the above mentioned carriers in an in vitro system using cells gliales of Bergmann (BGC) and neuronal granullar cells, for it technologies were used of western blot in solid phase and captures of glucose in cultures and hereby to evaluate the expression to level membranal. Glucose levels increase in capture assay and the GUT1 at maximum expression (572 percent at 4 hours). In case of the granullar cells, the same trends were observed in the capture and the expression of GLUT3 (371.22 percent at 4 h). The effect of fractions of I. sonorae showed a differential effect in the Glucose Transport in CBG. We conclude that the GLUT1 and GLUT3 expression and the glucose capture support a directly proportional relation under stimuli of I. sonorae to 0.1 percent w/v. The ethanolic extract promoted the capture of glucose in more than the acuose extract to the same concentration. I. sonorae promoted in major measure the carriers' expression of glucose in astrocytes that in neurons.

Disclosures: M.G. Ramírez-Sotelo: None. S. Rosales-Sosa: None. M. Oliver-Slavador: None. I.Y. Arciniega-Carreón: None. A.I. Cabrera-Llanos: None. A. Ortega: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.07/G20

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: The Swedish Reserach Concil

The Swedish Brain Foundation

The Swedish Society for Medical Reserach

The Novo Nordisk Foundation

Ahlens foundation

Thurings Foundation

Bergvalls Foundation

Title: Functional characterization of an amino acid transporter situated in the Golgi apparatus

Authors: *K. NORDENANKAR, F. LINDBERG, R. FREDRIKSSON;
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Abstract: Solute carriers (SLCs) are the largest class of membrane bound transporters proteins. Many of them are neuronal amino acid transporters with important functions in cellular processes, including role as transporters for amino acids between cells and for neurotransmitter cycling. The SLC38 family includes eleven members and are also called sodium-coupled neutral amino acid transporters (SNATs). Glutamine appears as a favored substrate for most of the members throughout the SNAT family. Glutamine is the most abundant amino acid in the human body and is involved in more physiological processes than any other amino acid such as providing ATP for intracellular protein turn over, nutrient transport through the plasma membrane, cell growth and migration as well as maintenance of cell integrity. Here we now report the functional characterization of the SNAT10 transporter by using gene targeting. We have produced a conditional knock out mouse for SNAT10, based on the 'knockout-first' design, a strategy that combines the advantages of both a reporter-tagged and a conditional mutation. By utilizing different behavioral paradigms, including rotarod, beam walking, elevated plus maze and marble burying test we are investigating the basal motor, cognitive and emotional behavior of the mice. We also characterized the localization of the protein, using immunohistochemistry on mice brain at different developmental stages and embryonic cell cultures.

Disclosures: K. Nordenankar: None. F. Lindberg: None. R. Fredriksson: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.08/G21

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: GACR 16-03913Y

Title: Potentiation of presynaptic glutamate release by pregnanolone sulfate: mechanisms and implications for neuroprotection

Authors: *T. SMEJKALOVA, D. CHUDA, L. VYKLICKY;
Inst. of Physiology, CAS, Praha, Czech Republic

Abstract: Tonic NMDAR activation by elevated extracellular glutamate mediates excitotoxicity, but phasic NMDAR activation by synaptically released glutamate can be neuroprotective. Thus, optimal neuroprotection may be achieved by blocking tonically but not synaptically activated NMDARs. Recently, we have shown that endogenous neurosteroid pregnanolone sulfate (PA-S) has better selectivity for inhibiting tonically over synaptically activated NMDARs than memantine, an NMDAR inhibitor with known neuroprotective effects. At the same time, PA-S, unlike memantine, potentiates presynaptic glutamate release. The mechanism of the presynaptic potentiation by PA-S, and the consequences of increased synaptic glutamate release for PA-S neuroprotection are unknown. To characterize the mechanism of the presynaptic potentiation by PA-S, we compared presynaptic effects of PA-S and a structurally similar neurosteroid pregnenolone sulfate (PE-S), which has been shown to increase mEPSC frequency in primary hippocampal cultures via a pertussis toxin (PTX)-sensitive mechanism. Our recordings of mEPSCs in hippocampal microisland cultures reveal a striking variability in the PE-S effect, not previously appreciated from recordings in mass cultures. PE-S (30 μM) increases mEPSC frequency in autaptic neurons 0- to 50-fold (average normalized mEPSC frequency in PE-S is 8.92 ± 5.49, $n = 9$; $p < 0.05$). As expected, pretreatment of cells with PTX (500 ng/ml for 24 h) blocks the PE-S effect (normalized mEPSC frequency in PE-S in PTX-treated cells is 1.35 ± 0.27, $n = 6$, $p = 0.16$). In contrast, PA-S (100 μM) consistently increases mEPSC frequency and this effect is insensitive to PTX (normalized mEPSC frequency in PA-S is 3.20 ± 0.42 in control cells, $n = 6$, $p < 0.05$; and 4.53 ± 1.11 in PTX-treated cells, $n = 4$, $p < 0.05$). We conclude that PA-S and PE-S potentiate glutamate release by different mechanisms. Increased synaptic glutamate release in PA-S may contribute to toxic glutamate buildup and thus reduce PA-S neuroprotective potential. We compared neuroprotection against *in-vitro* hypoxia by 200 μM PA-S vs.10 μM memantine; these concentrations cause similar tonic NMDAR inhibition, but memantine has no known presynaptic effect. Using primary hippocampal cultures stained with propidium iodide and Hoechst 33342, we find $37 \pm 4\%$ neuronal death after 3 h hypoxia, in

contrast to only 11 ± 4% neuronal death after hypoxia with 200 µM PA-S, and 15 ± 3% neuronal death after hypoxia with 10 µM memantine (n = 5, ANOVA $p < 0.05$). These results show no evidence of a toxic effect of the glutamate release potentiation by PA-S and confirm that neurosteroids are a promising class of NMDAR inhibitors with clinical potential.

Disclosures: T. Smejkalova: None. D. Chuda: None. L. Vyklicky: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.09/G22

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: KAKENHI 26350498

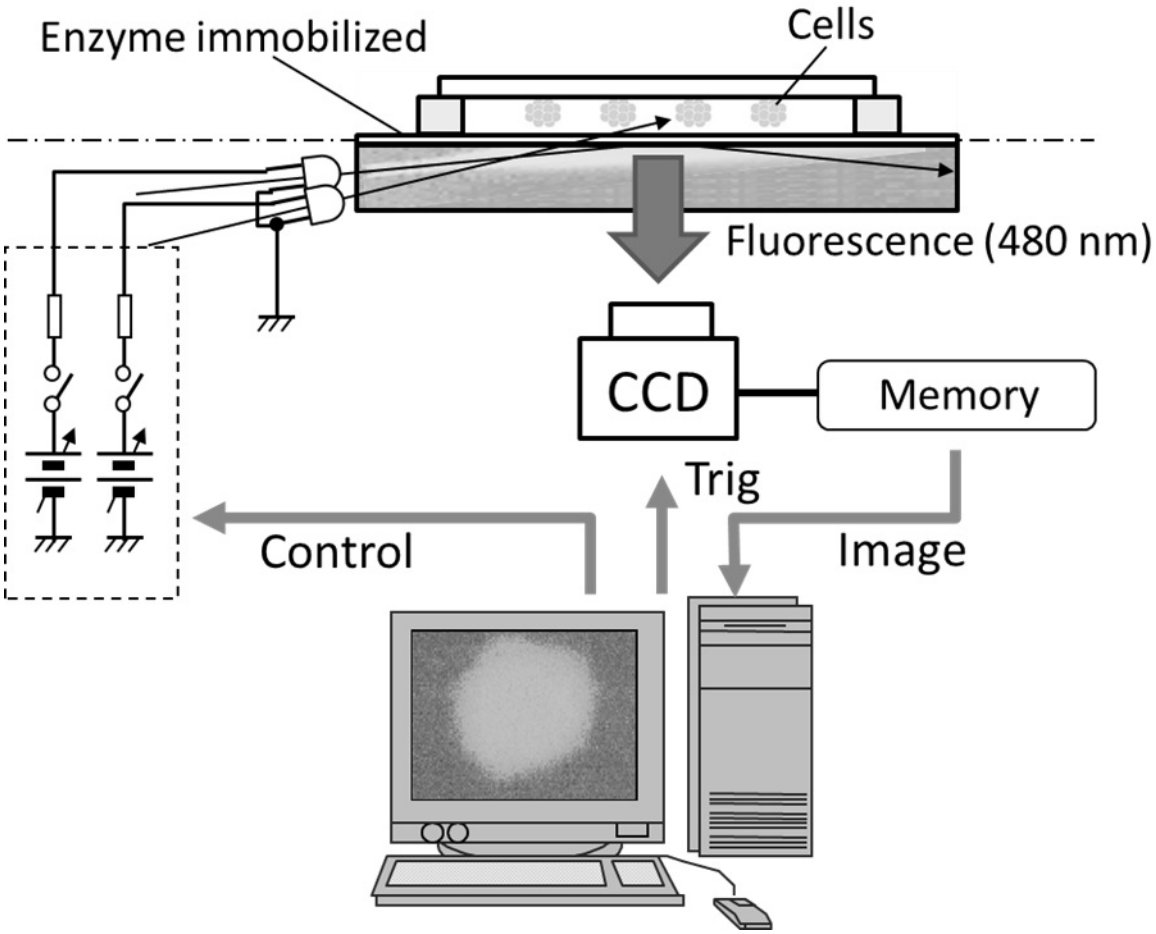
Health Labour Sciences Research Grant

Title: Visualization of neurotransmitter released from cultured granule cells and the neuroshere cells using enzyme-linked photo-assay combined with ICA

Authors: H. MABUCHI[1], N. TAKAHASHI[1], K. SATO[2], Y. SEKINO[2], N. HOZUMI[1], *S. YOSHIDA[1];
[1]Toyohashi Univ. Technol., Toyohashi, Japan; [2]Natl. Inst. of Hlth. Sci., Tokyo, Japan

Abstract: The detection of neurotransmitter release gives us a lot of knowledge about neuronal conditions and differentiations. In order to observe the spatio-temporal transmitter release in cerebellar cortex, we have developed the enzyme-linked photo- assay system for glutamate and GABA, with an immobilized specific enzyme on a quartz surface and the CMOS camera. Using this device, we have succeeded in visualizing region-specific transmitter releases in both the developing and juvenile cerebellar slices. However it has been unclear whether this system could apply the detection of the transmitter release from cultured cells, because background autofluorescence derived from mitochondria in individual cells might be more significant than the target fluorescence. In order to discriminate the target signal from background fluorescence, we proposed a new simple device for visualizing the neurotransmitter release. In this device, we irradiated the samples with two UV-LED lights aligned with different angles, and analyzed the data by using the independent component analysis (ICA). The mixture ratios of the target and background signals of two images derived from two UV lights, were different, so that the target signal could be separated by the ICA. Using this improved system, we observed the stimulation-evoked releases in developing cerebellar slices distinctly. So, we next investigated whether this

new system can detect glutamate release at single-cell level using cultured rat cerebellar granule cells, or the cells differentiated from rat neurosphere. Granule cells were derived from neonatal rat cerebellar external layer and cultured for a week in high K^+ conditioned medium. Cultured neural stem/progenitor cells derived from rat embryo were proliferated in a medium containing bFGF and differentiated by removing bFGF and EGF for several weeks. Both differentiated cultured cells were stimulated with 10 μM AMPA, and stimulation-induced glutamate release was detected. We suggest that the new photo- assay device would become useful to detect cell-level neurotransmitter release.



Disclosures: H. Mabuchi: None. N. Takahashi: None. K. Sato: None. Y. Sekino: None. N. Hozumi: None. S. Yoshida: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.10/G23

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS0066037

Title: The concentration of extracellular glutamate in the nucleus accumbens

Authors: *D. CHIU, C. E. JAHR;
OHSU, Vollum Inst., Portland, OR

Abstract: Glutamate is present in the extracellular space surrounding neurons and glia, and the concentration of extracellular glutamate has implications for glutamate receptor availability and function. Predicting these effects has been difficult, however, as measurements of extracellular glutamate by different techniques have yielded estimates ranging over 3 orders of magnitude, from 25 nM to >10 μM. One possible explanation for this discrepancy is the existence of a glutamate gradient across the extracellular space, maintained by glutamate transporters (EAATs), with micromolar glutamate in the non-synaptic extracellular space, and nanomolar glutamate in synaptic clefts. In this study we used electrophysiology and two-photon calcium imaging to investigate the concentration and distribution of extracellular glutamate in rat nucleus accumbens (NAc).

In voltage clamp recordings from medium spiny neurons (MSNs) in acute slices, 100 μM D-AP5 blocked a standing current at +40 mV, indicating that NMDARs were activated by extracellular glutamate. The inhibited current corresponded to activation by ~30 nM glutamate, similar to the value obtained by measurements made in hippocampal slices. Using two-photon calcium imaging of dendritic spines, we observed spontaneously occurring, D-AP5-sensitive calcium excursions that were infrequent, consistent with activation of single NMDARs by nanomolar glutamate.

To test if there is compartmentalization of extracellular glutamate, we inhibited EAATs with TBOA. In 100 μM TBOA, with the barrier to diffusion between compartments removed, the rate of observed events increased more than two-fold. Two findings suggest that the increase in synaptic NMDAR activation reflects exposure to nanomolar and not micromolar glutamate. First, the rate of events also doubled in the presence of 5 μM NMDA, which is commensurate with ~100 nM glutamate. Second, the current evoked by 100 μM TBOA at +40 mV was smaller than the current evoked by 10 μM NMDA, indicating that, even with glutamate transport inhibited, the average concentration at all NMDARs is less than 200 nM.

These results indicate that extracellular glutamate in the NAc is in the low nanomolar range, and

are not consistent with the presence of micromolar glutamate in any compartment of the extracellular space.

Disclosures: D. Chiu: None. C.E. Jahr: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.11/G24

Topic: B.06. Neurotransmitter Release

Support: Roche Innovation Center Basel

Title: Synapse specific physiology of somatostatin interneurons is driven by Elfn1 mediated presynaptic recruitment of mGluR7 and GluR6-containing kainate receptors.

Authors: *T. J. STACHNIAK[1], E. L. SYLWESTRAK[2,1,3], B. J. HALL[1], A. GHOSH[4,1]; [1]Roche Innovation Ctr. Basel, Basel, Switzerland; [2]Bioengineering, Stanford Univ., Stanford, CA; [3]Biozentrum, Univ. of Basel, Basel, Switzerland; [4]E-Scape Bio, San Francisco, CA

Abstract: Excitatory synapses onto somatostatin (SOM) interneurons are highly facilitating. Here we show that this facilitation requires assembly of a synaptic protein complex containing Elfn1, metabotropic glutamate receptor 7 (mGluR7), and glutamate receptor 6 subunit (GluR6)-containing kainate receptors. The striking facilitation seen in cortical layer 2/3 (L2/3) and the CA1 region of hippocampus is lost in Elfn1 knockout mice. Notably, synapses onto layer 5 (L5) cortical SOM interneurons that facilitate sub-optimally are less affected in Elfn1 knockout, compared to synapses onto L2/3 or hippocampal interneurons. Application of the GluR6 selective antagonist NS-102 reduces facilitation in L2/3 but not in L5, whereas both synapse types are sensitive to mGluR7 inhibition. Thus we identify GluR6 containing presynaptic kainate receptors as necessary to generate the characteristic facilitation typical of L2/3. Further, the strong synaptic facilitation visible in L2/3 can be attributed to two separate mechanisms: the actions of mGluR7 produce early facilitation within a spike train, while late facilitation in a spike train is primarily GluR6 dependent. As the proteomic content determines synaptic properties in L2/3 and L5, we have identified both an early, constitutive synaptic component and a late, discretionary synaptic component which expand the repertoire of synaptic physiological responses available through a stepwise construction of the synaptic proteome.

Disclosures: T.J. Stachniak: A. Employment/Salary (full or part-time): F. Hoffman La Roche. **E.L. Sylwestrak:** A. Employment/Salary (full or part-time): F. Hoffmann La Roche. **B.J. Hall:**

A. Employment/Salary (full or part-time): F. Hoffmann La Roche. **A. Ghosh:** A. Employment/Salary (full or part-time): F. Hoffmann La Roche, E-Scape Bio.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.12/G25

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant MH104506

NIH Grant MH078823

Taylor Family Institute for Innovative Psychiatric Research

Title: vGluT3+ cells in the mouse hippocampus exhibit diverse physiological properties

Authors: *C. A. BURLESON, A. BENZ, S. MENNERICK;
Dept of Psychiatry, Washington Univ. in St. Louis, Saint Louis, MO

Abstract: An essential component of neuron identity is neurotransmitter phenotype, which in turn is defined in part by the vesicular neurotransmitter transporter(s) expressed by the cell. The vesicular glutamate transporter-3 (vGluT3) has been noted for its unique distribution in the rodent brain; vGluT3 is often found in cells co-expressing markers for other neurotransmitter phenotypes. Furthermore, vGluT3 can be found in several cell populations lacking neuronal markers. The vGluT3 expression of the rodent hippocampus exemplifies this heterogeneity; in addition to being found in a neuronal population of CCK+ GABAergic basket cells, vGluT3 has been reported in cells lacking neuronal markers. While this unusual molecular phenotype of basket cells has been noted by several labs, little is known about the physiological characteristics of these cells, and presence of vGLUT3 has proven controversial. Here, we use a mouse line expressing the fluorescent protein tdTomato under control of VGLUT3 (VGLUT3-tdTomato) to study vGluT3+ hippocampal cells in acute brain slices. We first confirmed the presence of vGluT3 in tdTomato cells via immunohistochemistry. Then, based on membrane electrical properties, location, and morphology, we identified three unique cell types expressing vGluT3: 1. basket cell-like spiking neurons predominantly in the mossy fiber pathway of CA3 and stratum radiatum of CA1; 2. glia-like non-spiking, low input resistance cells with negative resting membrane potentials relative to the neuronal population predominantly found in the hilus of the dentate gyrus; and 3. high input resistance cells that responded to current injection with low amplitude “spikelets”, found throughout several hippocampal regions. Furthermore, the spiking

neurons exhibited different membrane properties in different hippocampal subregions . CA3/CA2 spiking neurons exhibited stronger excitability, including higher input resistances and faster spiking rates in response to current injection, compared to those found at the CA1/subiculum border. Most notably, CA3 neurons but not CA1 neurons exhibited depolarizing sag, typically attributed to h-type current, upon negative current injection. Taken together, our work demonstrates the unique distribution of vGluT3 in the hippocampus and begins to dissect the novel roles vGluT3+ cells can play in the hippocampal circuitry.

Disclosures: C.A. Burleson: None. A. Benz: None. S. Mennerick: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.13/G26

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant T32 NS061764-06

NIH Grant R01 NS076885

Title: Ambient glutamate tonically excites cortical interneurons via NMDA receptor activation during early postnatal development: implications for neonatal epileptogenesis

Authors: *E. HANSON, C. DULLA;
Neurosci., Tufts Univ., Boston, MA

Abstract: Astrocytic glutamate transport changes significantly through postnatal development and is highly responsive to injury. Effective glutamate transport is important for regulating concentrations of ambient glutamate, yet surprisingly little is known about both the developmental regulation of ambient glutamate and the effect of neonatal injury on ambient glutamate-mediated tonic excitation. To better understand the role of tonic excitatory signaling in normal cortical development and epileptogenesis we have examined (1) how ambient glutamate is developmentally regulated in the postnatal cortex, (2) cell-type specific tonic excitation mediated by NMDA receptors, and (3) disruptions in ambient glutamate regulation during injury-induced epileptogenesis. First, we used whole-cell voltage clamp electrophysiology to measure relative concentrations of ambient glutamate in the developing cortex of C57 mice. We found that relative concentrations of ambient glutamate are sharply and transiently elevated at P7-P8. To investigate the consequences of elevated ambient glutamate, we used whole-cell current clamp electrophysiology to measure the resting membrane potential and firing properties

of cortical interneurons (INs) and PCs. We found that INs but not PCs are tonically depolarized by NMDAR-mediated currents. Furthermore, in the same cells, blockade of these currents with APV decreased IN but increased PC action potential firing. These results suggest a difference in the voltage dependence of the NMDAR-mediated tonic current between INs and PCs, potentially due to differential expression of NR2C and NR2D NDMAR subunits. We then utilized the neonatal freeze lesion (FL) model to study glutamate regulation during the development of hyperexcitability. Induction of a FL at P0 is followed by a two week latent period during which a microgyrus develops in the cortex under the injury site. By P14 the paramicrogyral zone (PMZ) is hyperexcitable. Intriguingly, immunofluorescence revealed increased expression of the glutamate transporters GLT-1 and GLAST in PMZ during the FL latent period. Using whole-cell voltage clamp electrophysiology, we found that the transient elevation in ambient glutamate is abolished in the PMZ, but maintained in contralateral cortex. Furthermore, preliminary data suggest that the reduction in ambient glutamate corresponds to a decrease in tonic excitation of cortical INs. The consequences for acute network activity and long-term connectivity remain to be investigated, however, these results suggest that reduced tonic excitation of INs is a potential mechanism driving the development of hyperexcitability in the FL cortex.

Disclosures: E. Hanson: None. C. Dulla: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.14/G27

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA037779

NIH Grant DA036420

NIH Grant G12MD007586

Title: Understanding the interplay of proline and glutamate metabolism in neurons

Authors: *J. PANDHARE[1], S. DASH[1], M. BALASUBRAMANIAM[2], F. VILLALTA[3], C. DASH[4];
[1]Sch. of Grad. Studies and Research, Ctr. for AIDS Hlth. Disparities Rese, [2]Ctr. for AIDS Hlth. Disparities Rese, [3]Dept. of Microbiology and Immunology, Ctr. for AIDS Hlth. Disparities Rese, [4]Dept. of Biochem. and Cancer Biology, Ctr. for AIDS Hlth. Disparities Rese, Meharry Med. Col., Nashville, TN

Abstract: Glutamate is the most abundant excitatory neurotransmitter and plays key roles in normal brain functions, including cognition, memory and learning. It is a nonessential amino acid and does not cross the blood brain barrier. Thus it must be synthesized in neurons from local precursors. Glutamate can be synthesized from glucose through glycolysis and the Krebs cycle, however glutamine is the most recognized precursor for the biosynthesis of glutamate in the synaptic terminals. Alternatively, a possible route for glutamate biosynthesis in the brain is from proline via delta-1-pyrroline-5-carboxylate (P5C) via its tautomer, glutamic-gamma-semialdehyde (GSA). This biosynthetic pathway is sequentially catalyzed by the enzymes proline oxidase/dehydrogenase (POX/PRODH) and P5C dehydrogenase (P5CDH). Moreover, proline metabolism is unique, since P5C, the immediate product of proline catabolism, also serves as the precursor for proline biosynthesis. P5C can be generated from glutamate via the enzymatic activities of P5C synthase (P5CS) and P5C reductase (P5CR). Furthermore, glutamine can also be converted to proline via glutamate. However, the role of these metabolic interconversions in neuronal function is not clearly understood. Therefore, we are investigating the functional link between proline metabolic pathway and glutamine-glutamate metabolism in neurons. To test this we selectively supplemented glutamine to the HT-22 rat hippocampal neurons and measured the effects on the proline metabolic pathway. Our preliminary results revealed that the enzymes of the proline catabolic arm, POX and P5CDH, were minimally affected by glutamine supplementation. In contrast, the enzymes involved in proline biosynthesis from glutamate, P5CS and the two isoforms of P5CR, were induced. We also detected higher intracellular levels of proline in these cells. These results strongly suggest that glutamine is directed toward the biosynthesis of proline most likely via glutamate. Currently, we are conducting experiments to determine the effects of increased biosynthesis of proline in neuronal function.

Disclosures: J. Pandhare: None. S. Dash: None. M. Balasubramaniam: None. F. Villalta: None. C. Dash: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.15/G28

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Lundbeck Corporate

Title: Stable isotope labeled L-kynurenine (KYN) metabolism investigated using *In vivo* microdialysis coupled with a novel high content LC/MS/MS method

Authors: D. P. BUDAC[1], D. SONG[1], M. CAJINA[1], A. LEE[1], B. M. CAMPBELL[1], G. LI[1], C. SÁNCHEZ[1], *C. FORRAY[1], V. S. PALAMARCHOUK[2], G. N. SMAGIN[1,2];
[1]Lundbeck USA, Paramus, NJ; [2]PsychoGenics Inc, Tarrytown, NY

Abstract: Degradation of the amino acid tryptophan along the kynurenine pathway (KP) yields several neuroactive intermediates regulated by enzymes localized in astrocytes and microglial cells. The limited ability of many KYN-derived metabolites to cross the blood brain barrier suggests that CNS concentrations of these metabolites is largely regulated by local enzyme activity. KYN itself is actively transported into the brain by large neutral amino acids transporter. The fate of KYN in the brain is poorly understood and was investigated using a stable labeled analogue of KYN ([$^{13}C_6$]L-KYN) and in vivo microdialysis A sensitive LC/MS/MS method was developed and utilized to provide a time course for stable isotope labeled metabolite formation. Mice and rats were implanted with guide cannula placed into the striatum. One week after surgery, a microdialysis probe was inserted for the microdialysis experiment. Microdialysis samples were collected for 6 hrs after i.p. administration of 5 mg/kg of [$^{13}C_6$]L-KYN and analyzed for labeled and unlabeled *L*-kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid (QUIN), using LC/MS/MS method. In mice, [$^{13}C_6$]L-KYN metabolism showed a stronger kynurenine 3-monooxygenase (KMO) branch response with increased incorporation of ^{13}C label in 3-HK, 3-HAA, and QUIN. [$^{13}C_6$]L-KYN metabolism in rats progressed predominantly down the kynurenine aminotransferase (KAT) pathway as suggested by the higher amounts of labeled KYNA. The results demonstrate the utility of LC/MS/MS analysis of stable isotope labeled KP metabolite from in vivo microdialysis samples since this type of technique can be utilized to delineate the role of central and peripheral KP metabolites in diseases involving dysregulation of kynurenines metabolites. Further studies of brain-penetrable modulators of KP may be beneficial for the treatment of these diseases.

Disclosures: **D.P. Budac:** A. Employment/Salary (full or part-time): Lundbeck. **D. Song:** A. Employment/Salary (full or part-time): Lundbeck. **M. Cajina:** A. Employment/Salary (full or part-time): Lundbeck. **A. Lee:** A. Employment/Salary (full or part-time): Lundbeck. **B.M. Campbell:** A. Employment/Salary (full or part-time): Lundbeck. **G. Li:** A. Employment/Salary (full or part-time): Lundbeck. **C. Sánchez:** A. Employment/Salary (full or part-time): Lundbeck. **C. Forray:** A. Employment/Salary (full or part-time): Lundbeck. **V.S. Palamarchouk:** A. Employment/Salary (full or part-time): Psychogenics. **G.N. Smagin:** A. Employment/Salary (full or part-time): Lundbeck.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.16/G29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Corporate Lundbeck

Title: *In vivo* characterization of CNS penetrant KMO inhibitor using microdialysis coupled with a novel high content LC/MS/MS method

Authors: G. SMAGIN[1], D. P. BUDAC[1], M. CAJINA[1], A. LEE[1], B. CAMPBELL[1], G. LI[1], *C. SANCHEZ[2], C. FORRAY[1], V. S. PALAMARCHOUK[3], D. SONG[1];
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Abstract: Degradation of the amino acid tryptophan (TRP) along the kynurenine pathway (KP) yields several neuroactive intermediates regulated by enzymes localized in astrocytes and microglial cells. Impairment of KP metabolism is functionally significant in a variety of diseases that affect the brain. It is hypothesized that CNS penetrant small molecules which inhibit kynurenine 3-monooxygenase (KMO) normalize central kynurenine dysregulation and will be beneficial for CNS disorders. We identified a CNS penetrant KMO inhibitor Compound A and characterized its effect on central and peripheral KP metabolites after systemic administration. We demonstrated that by extending the analyte capacity of an LC/MS/MS method, dedicated to KP metabolite, to include drug molecules we could simultaneously monitor KP metabolites and drug compounds in one injection. Utilizing this technique alongside in vivo microdialysis allows us to perform direct comparisons of drug levels and their impact on KP metabolites over a time course.
Mice were implanted with the guide cannula placed into the striatum. One week after surgery, a microdialysis probe was inserted for the microdialysis experiment. Microdialysis samples were collected for 6 hrs after 30 and 100 mg/kg administration of Compound A and analyzed for Compound A, L-kynurenine (KYN), kynurenic acid (KYNA), 3-hydrohykynurenine (3-HK) and quinolinic acid (QUIN), using LC/MS/MS Terminal brain and plasma samples were taken for analysis of KP metabolites and compound exposure.
Administration of Compound A decreased concentrations 3-HK to 60% of baseline which co-incided with the increased concentration of Compound A in dialysis samples. Initial decrease in 3-HK concentrations was followed by a two-fold increase after 6 hrs. Concentrations of KYN, KYNA and QUIN in microdialysates collected from the brain increased 75-fold, 350-fold and 2-fold respectively, compared to vehicle control. The increase lasted through the six hour observation period. KYN and KYNA levels were also elevated in terminal brain and plasma

samples collected at the 6 hour mark. The results demonstrate that CNS penetrant KMO inhibitors while affecting both central and peripheral KP metabolites provide an avenue to understanding the biological role of these metabolites in brain disorders. Further studies of brain-penetrating modulators of KP may provide treatment options for such disorders.

Disclosures: **G. Smagin:** A. Employment/Salary (full or part-time): Lundbeck, PsychoGenics. **D.P. Budac:** A. Employment/Salary (full or part-time): Lundbeck. **M. Cajina:** A. Employment/Salary (full or part-time): Lundbeck. **A. Lee:** A. Employment/Salary (full or part-time): Lundbeck. **B. Campbell:** A. Employment/Salary (full or part-time): Lundbeck. **G. Li:** A. Employment/Salary (full or part-time): Lundbeck. **C. Sanchez:** A. Employment/Salary (full or part-time): Lundbeck. **C. Forray:** A. Employment/Salary (full or part-time): Lundbeck. **V.S. Palamarchouk:** A. Employment/Salary (full or part-time): Psychogenics. **D. Song:** A. Employment/Salary (full or part-time): PsychoGenics.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.17/G30

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grants R37-HD059288

NIH grants R01-NS069629

Title: Verification of the cross immunoreactivity of A60, a mouse monoclonal antibody against neuronal nuclear protein

Authors: ***M. SHANPING**[1], X. GUOXIANG[2];
[1]Renmin Hosp. of Wuhan Univ., Hubei, China; [2]Dept. of Anesthesiol. and Critical Care Medicine, Children's Hosp. of Philadelphia, Philadelphia, American Samoa

Abstract: A60, the mouse monoclonal antibody against the neuronal nuclear protein (NeuN), is the most widely used neuronal marker in neuroscience research and neuropathological assays. Previous studies identified fragments of A60-immunoprecipitated protein as Synapsin I (Syn I), suggesting the antibody will demonstrate cross immunoreactivity. However, the likelihood of cross reactivity has never been verified by immunohistochemical techniques. Using our established tissue processing and immunofluorescent staining protocols, we found that A60 consistently labelled mossy fiber terminals in hippocampal area CA3. These A60-positive mossy fiber terminals could also be labelled by Syn I antibody. After treating brain slices with saponin

in order to better preserve various membrane and/or vesicular proteins for immunostaining, we observed that A60 could also label additional synapses in various brain areas. Therefore, we used A60 together with a rabbit monoclonal NeuN antibody to confirm the existence of this cross reactivity. We showed that the putative band positive for A60 and Syn I could not detected by the rabbit anti-NeuN in Western blotting. As efficient as Millipore A60 to recognize neuronal nuclei and cell bodies specifically, the rabbit NeuN antibody demonstrated no labelling of synaptic structures in immunofluorescent staining. The present study successfully verified the cross reactivity present in immunohistochemistry, cautioning that A60 may not be the ideal biomarker to verify neuronal identity due to its cross immunoreactivity. In contrast, the rabbit monoclonal NeuN antibody used in this study may be a better candidate to substitute for A60.

Disclosures: M. Shanping: None. X. Guoxiang: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.18/G31

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: MEXT Grant-in-Aid for Scientific Research

Title: Inhibition of large neutral amino acid transporters suppresses kynurenic acid production via inhibition of kynurenine uptake in rodent brain

Authors: A. SEKINE, Y. KUROKI, T. URATA, N. MORI, *T. FUKUWATARI;
Dept. of Nutr., The Univ. of Shiga Prefecture, Hikone, Japan

Abstract: The tryptophan metabolite, kynurenic acid (KYNA), is a preferential antagonist of the α7 nicotinic acetylcholine receptor and *N*-methyl-D-aspartic acid receptor at endogenous brain concentrations. Recent studies have suggested that increases of brain KYNA levels are involved in psychiatric disorders such as schizophrenia and depression, and regulation of KYNA production has become a new target for treatment of these diseases. Kynurenine (KYN), the immediate precursor of KYNA, is transported into astrocytes via large neutral amino acid transporters (LATs). We have recently reported that several LATs substrate amino acids (leucine, isoleucine, phenylalanine, methionine and tyrosine) reduce KYN uptake and KYNA production in rat brain *in vitro* (*SpringerPuls* 2015;4:48). In the present study, the effect of LATs regulation on KYN uptake and KYNA production was investigated *in vitro* and *in vivo* using an LATs inhibitor, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH). In the *in vitro* study, cortical slices of rat brain were incubated with a physiological concentration of KYN and 3

μmol/L-3 mmol/L BCH. BCH inhibited KYNA production and KYN uptake in a dose-dependent manner, and their IC_{50} values were 90.7 and 97.4 μmol/L, respectively. In the *in vivo* study, mice were administered KYN (50 mg/kg BW) orally and BCH (200 mg/kg BW) intravenously. Administration of KYN increased brain KYN and KYNA levels compared with the mice treated with vehicle, whereas additional administration of BCH suppressed KYN-induced elevations in KYN and KYNA levels to 50% and 70% in the brain, respectively. These results suggest that inhibition of LATs prevented the increase of KYNA production via blockade of KYN uptake in the brain *in vitro* and *in vivo*. LATs can be a target to modulate brain function by regulation of KYNA production in the brain.

Disclosures: A. Sekine: None. Y. Kuroki: None. T. Urata: None. N. Mori: None. T. Fukuwatari: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.19/G32

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR

NSERC

Title: Synapse maturation and stabilization of axonal branches is promoted by D-serine in the developing visual system of the Xenopus tadpole.

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

Abstract: N-methyl-D-aspartate receptor (NMDAR) activation is essential for establishing and maintaining precise neural circuit refinement. The gliotransmitter D-serine is a co-agonist for NMDARs and modulates synaptic transmission and plasticity mediated by this receptor. Here we investigated the role D-serine plays in modulating synaptic transmission and axonal remodeling in the developing visual system of the Xenopus tadpole. We find that D-serine is an endogenous co-agonist of the NMDAR that is normally present below saturating levels. Using enzymatic biosensors we find that D-serine release in the optic tectum can be driven by glutamatergic activation and find that facilitating NMDAR activation by chronically elevating levels of D-serine promotes the maturation of functional synapses. In particular, we find that tadpoles raised

in D-serine (100 μM) for 2 days have a higher frequency of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) and higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. Moreover, animals raised in D-serine have significantly lower paired-pulse facilitation, consistent with a developmental increase in release probability. In contrast, acute wash-on of D-serine does not change the frequency or amplitude of mEPSCs or the probability of release at retinotectal synapses, indicating that D-serine does not facilitate release simply by acting directly on presynaptic neurons. Decreasing levels of endogenous D-serine, with a local injection of RgDAAO, reduces NMDAR-mediated synaptic transmission and results in deficits in synaptic maturation over 24 h. To examine the effects of D-serine on retinotectal axonal development, *in vivo* 2-photon images of single-transfected GFP-expressing retinal ganglion cell axons were collected daily over 4 days to assess growth and branch elaboration, and at shorter (10 min) intervals to assess branch dynamics. We find that increasing available D-serine results in the hyperstabilization of retinal axon branches. Axonal arbors remain less complex compared to control axons over 4 days of treatment with D-serine. Together, these findings reveal an important role for D-serine in promoting NMDAR-mediated synaptic maturation and stabilization of axonal branches.

Disclosures: M. Van Horn: None. A. Strasser: None. L. Pollegioni: None. E. Ruthazer: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.20/G33

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Investigating the effects of D-alanine on pituitary intracellular signalling: a comparison with D-serine

Authors: *K. NAIDOO, P. W. J. BURNET;
Psychiatry, Univ. of Oxford, Oxford, United Kingdom

Abstract: Background: The D-amino acid, D-alanine, is a N-Methyl-D-Aspartate (NMDAR) co-agonist and a strychnine-sensitive glycine receptor (GlyR) agonist, and is sequestered in adrenocorticotropic hormone (ACTH)-containing cells of the rodent pituitary, and may influence neural function through the modulation of stress hormones such as corticosterone. An exacerbated response of the hypothalamo-pituitary-adrenal (HPA) axis to stress is a common feature of major psychiatric disorders. However, the role of D-alanine in the modulation of the HPA axis is not known. Previous studies have shown that ACTH secretion from pituitary cells involves CREB and ERK1/2 signalling. We have, therefore, investigated the influence of D-

alanine on the levels of signalling proteins in cultures of the AtT20 mouse pituitary tumour cell-line. For comparison, the effect of D-serine, a co-agonist of central NMDARs but not GlyRs, was also examined. **Methods:** In one experiment, cultures of AtT-20 mouse pituitary cell line (2 X 10^5 cells per well) were incubated with control solution (culture media), D-alanine or D-serine (100, 200 and 500μM) for 24 hours. In a second study, cells were incubated with D-serine or D-alanine (500μM) for 24hrs, and then stimulated with CRH (100nM) for 5 and 15mins. At the end of each experiment, cells were lysed and protein extracted using standard procedures. All samples were analysed with western blotting, using specific antibodies against total CREB and ERK1/2, their phosphorylated (active) counterparts (pCREB, pERK1/2), and b-actin. All data were expressed as ratios (pCREB/CREB, pERK/ERK, CREB/β-actin, ERK/β-actin), and subjected to one-way ANOVA statistics. **Results:** Compared to controls, the levels of CREB/β-actin were significantly greater in AtT20 cells incubated with 500μM of D-alanine (+34%, p=0.011) or D-serine (+28%, p= 0.028). The levels of pCREB/CREB were also significantly greater in cells incubated with D-alanine (+25%, p=0.042), but not D-serine. The concentrations of pERK/ERK and ERK/β-actin are currently being assessed in these samples. The stimulation of cells with CRH in the absence or presence of D-alanine did not alter pERK/ERK or ERK/β-actin levels. Studies are now underway to evaluate CREB signalling in these samples, and in the pituitaries, hypothalamus and adrenals of rats administered with D-alanine or D-serine. Our data thus far, show that both D-amino acids stimulate intracellular CREB signalling in a pituitary cell-line, although D-alanine was with greater effect. Since CREB mediates ACTH secretion, manipulation of D-alanine levels in the pituitary may be therapeutically beneficial in stress-mediated disorders.

Disclosures: K. Naidoo: None. P.W.J. Burnet: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.21/G34

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS030549

NKTH-OTKA, Grant CNK77793, K83251

ERC-2011-ADG-294313, SERRACO

TAMOP 4.2.4. A/1-11-1-2012-0001

János Bolyai Research Scolarship

Title: The ascending projections from the median raphe region are mainly glutamatergic in the mouse forebrain

Authors: *A. SZONYI[1], M. I. MAYER[1], C. CSEREP[1], V. T. TAKACS[1], M. WATANABE[2], T. F. FREUND[1], G. NYIRI[1];
[1]Lab. of Cerebral Cortex Res., Inst. of Exptl. Med. - HAS, Budapest, Hungary; [2]Dept. of Anat. and Embryology, Hokkaido Univ., Sapporo, Japan

Abstract: The median raphe region (MRR) is thought to be serotonergic and plays an important role in the regulation of many cognitive functions. In the hippocampus (HIPP), the MRR exerts a fast excitatory control, partially through glutamatergic transmission, on a subpopulation of GABAergic interneurons that are key regulators of local network activity. However, not all receptors of this connection in the HIPP and in synapses established by MRR in other brain areas are known. Using combined anterograde tracing and immunogold methods, we show that the GluN2A subunit of the NMDA receptor is present in the synapses established by MRR not only in the HIPP, but also in the medial septum (MS) and in the medial prefrontal cortex (mPFC) of the mouse. We estimated similar amounts of NMDA receptors in these synapses established by the MRR and in local adjacent excitatory synapses. Using retrograde tracing and confocal laser scanning microscopy, we found that the majority of the projecting cells of the mouse MRR contain the vesicular glutamate transporter type 3 (vGluT3). Furthermore, using double retrograde tracing, we found that single cells of the MRR can innervate the HIPP and mPFC or the MS and mPFC simultaneously, and these double-projecting cells are also predominantly vGluT3-positive. Our results indicate that the majority of the output of the MRR is glutamatergic and acts through NMDA receptor-containing synapses. This suggests that key forebrain areas receive precisely targeted excitatory input from the MRR, which is able to synchronously modify activity in those regions via individual MRR cells with dual projections.

Disclosures: A. Szonyi: None. M.I. Mayer: None. C. Cserep: None. V.T. Takacs: None. M. Watanabe: None. T.F. Freund: None. G. Nyiri: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.22/G35

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: ERC-2011-ADG-294313, SERRACO

NIH Grant NS030549

TAMOP 4.2.4. A/1-11-1-2012-0001

Janos Bolyai Research Scholarship

Title: Cellular architecture and transmitter phenotypes of neurons of the mouse median raphe region

Authors: *K. E. SÓS, M. I. MAYER, C. CSEREP, F. S. TAKACS, A. SZONYI, T. F. FREUND, G. NYIRI;
Lab. of Cerebral Cortex Res., Inst. of Exptl. Medicine, HAS, Budapest, Hungary

Abstract: The median raphe region (MRR, which consist of MR and paramedian raphe regions) plays a crucial role in regulating cortical as well as subcortical network activity and behavior, while its malfunctioning may lead to disorders, such as schizophrenia, major depression, or anxiety. Mouse MRR neurons are classically identified on the basis of their serotonin (5-HT), vesicular glutamate transporter type 3 (VGLUT3), and gamma-aminobutyric acid (GABA) contents; however, the exact cellular composition of MRR regarding transmitter phenotypes is still unknown. Using an unbiased stereological method, we found that in the MR, 8.5 % of the neurons were 5-HT, 26 % were VGLUT3, and 12.8 % were 5-HT and VGLUT3 positive; whereas 37.2 % of the neurons were GABAergic, and 14.4 % were triple negative. In the whole MRR, 2.1 % of the neurons were 5-HT, 7 % were VGLUT3, and 3.6 % were 5-HT and VGLUT3 positive; whereas 61 % of the neurons were GABAergic. Surprisingly, 25.4 % of the neurons were triple negative and were only positive for the neuronal marker NeuN. PET-1/ ePET-Cre transgenic mouse lines are widely used to specifically manipulate only 5-HT containing neurons. Interestingly, however, using the ePET-Cre transgenic mice, we found that far more VGLUT3 positive cells expressed ePET than 5-HT positive cells, and about 38 % of the ePET cells contained only VGLUT3, while more than 30 % of 5-HT cells were ePET negative. These data should facilitate the reinterpretation of PET-1/ePET related data in the literature and the identification of the functional role of a putatively new type of triple-negative neuron in the MRR.

Disclosures: K.E. Sós: None. M.I. Mayer: None. C. Cserep: None. F.S. Takacs: None. A. Szonyi: None. T.F. Freund: None. G. Nyiri: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.23/G36

Topic: B.05. Transporters

Title: Constitutive phosphomimetic KCC2 inhibition in developing mice causes GABA-dependent network excitability and autistic-like behavior

Authors: *L. I. PISELLA[1], I. KHALILOV[1], D. DIABIRA[1], J. ZHANG[2,3], J. DUAN[3,4], I. MEDINA[1], K. T. KAHLE[4], J.-L. GAIARSA[1,2];
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Abstract: Models of epilepsy, intellectual disability, and autism spectrum disorders (ASDs) exhibit an altered GABAergic excitatory-inhibitory (E-I) developmental sequence due to dysregulated neuronal Cl- homeostasis. A post-natal decrease of neuronal $[Cl^-]_i$, establishing fast synaptic inhibition, is mediated by the Cl^-- extruding K+-Cl− cotransporter KCC2. KCC2 is inactive in immature neurons due in part to its phosphorylation at Thr^{906} and Thr^{1007}. During development, Thr^{906}/Thr^{1007} phosphorylation significantly decreases in parallel with an increase in KCC2 activity and a lowering of neuronal $[Cl^-]_I$. To determine the functional importance of regulated Thr^{906}/Thr^{1007} phosphorylation during development *in vivo*, we assessed the neuronal network properties and neurobehavior of KCC2 mice expressing one allele harboring the missense mutations Glu^{906} and Glu^{1007} "(*KCC2*$^{e/+}$)" that mimick constitutive KCC2 Thr^{906}/Thr^{1007} phopshorylation. Field potential and patch-clamp recordings from CA3 pyramidal neurons on acute slices showed that GABAergic responses remained excitatory in *KCC2*$^{e/+}$ mice up to postnatal day 20, indicating a significant delay in the GABAergic E-I sequence. CA3 pyramidal cells from *KCC2*$^{e/+}$ animals also displayed significantly increased seizure-like activity in response to application of 5 µM 4-amidopyridine, and a decreased ratio of spontaneous GABA-driven to glutamate-driven post-synaptic responses. *KCC2*$^{e/+}$pups (P10) emitted more ultra-sound vocalizations than WT littermates when separated from their mother, a characteristic autistic-like behavior. These data demonstrate the importance of regulated phosphorylation of KCC2 at Thr^{906}/Thr^{1007} for Cl^--dependent GABA signaling and neurodevelopment, and suggest alterations in this mechanism may contribute to the synaptic and behavioral excitability of ASDs.

Disclosures: L.I. Pisella: None. I. Khalilov: None. D. Diabira: None. J. Zhang: None. J. Duan: None. I. Medina: None. K.T. Kahle: None. J. Gaiarsa: None.

Poster

217. Amino Acid and Other Transporters

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 217.24/G37

Topic: B.05. Transporters

Title: $GABA_A$ receptor reversal potential as a reporter for cation-chloride co-transporter activity in Xenopus oocytes

Authors: *F. KNOFLACH[1], M.-C. HERNANDEZ[1], M. SAXE[1], S. BERTRAND[2], D. BERTRAND[2];
[1]F. Hoffmann-La Roche Ltd, Basel, Switzerland; [2]HiQScreen Sàrl, Geneva, Switzerland

Abstract: Potentiation of the γ-amino-butyric acid receptor type A ($GABA_A$) receptor is the widely recognized mechanism of therapeutically effective drugs like diazepam. However, less is known about the mechanism controlling intracellular chloride concentration, which determines the inhibitory vs excitatory activity of $GABA_A$ receptors. Development of compounds modulating inhibitory neurotransmitter responses requires a better understanding of the exact contribution of the sodium, potassium, and chloride co-transporters, also referred to as CCCs (cation-chloride co-transporters) in establishing and maintaining chloride homeostasis.
While monitoring of the intracellular chloride concentration by ion selective electrodes was shown to be amenable to large cells, this highly invasive technique requires penetration of at least two electrodes. We have assessed an alternative approach consisting in the measurement of the reversal potential of a chloride permeable channel that should allow quantification of the chloride gradient existing across the cell membrane. mRNA encoding for the human NKCC1 or NKCC2 co-transporters was co-injected in *Xenopus* oocytes with mRNAs encoding for the α5, β3 and γ2 $GABA_A$ receptor subunits. Oocyte membrane potential recordings were conducted using an automated system (HiClamp, MultiChannelSystems). Reduction of the extracellular chloride concentration caused a right shift of the reversal potential in line with the theoretical value calculated using the Nernst equation. However, incubation of the oocytes in a gluconate-containing chloride-free medium, caused a leftward shift of the $GABA_A$ reversal potential likely due to a lowering of the intracellular chloride concentration. Upon restoration of the normal chloride medium conditions, the $GABA_A$ reversal potential progressively returned to its initial value, indicative of active inward chloride transport. When the oocytes were incubated in presence of a transporter inhibitor, such as bumetanide, a marked slowing down of the return of the reversal potential towards its normal value was observed, illustrating the contribution of the exogenously expressed human CCCs. In conclusion, the described method provides a direct measurement of CCC activity which is not dependent on radioactive techniques e.g. rubidium flux.

Disclosures: **F. Knoflach:** A. Employment/Salary (full or part-time): F. Hoffmann-La Roche AG. **M. Hernandez:** A. Employment/Salary (full or part-time): F. Hoffmann-La Roche AG. **M. Saxe:** A. Employment/Salary (full or part-time): F. Hoffmann-La Roche AG. **S. Bertrand:** A. Employment/Salary (full or part-time): HiQScreen Sàrl. **D. Bertrand:** A. Employment/Salary (full or part-time): HiQScreen Sàrl.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.01/G38

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: JSPS KAKENHI 15K10556

Title: Delineate the serotonin innervation in octopus arm by immunohistochemistry

Authors: *J.-P. BELLIER[1], Y. XIE[3], S. M. FAROUK[4], Y. SAKAUE[2], I. TOOYAMA[1], H. KIMURA[1];
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Abstract: The arm of the octopus is a sophisticated structure in that it is composed of a tridimensional array of muscles and a massive sensory system. Its nervous system is the most advanced among invertebrates, and contains 500 millions neurons of which two-thirds are encompassed in its eight arms. To some extent, the octopus arm nervous system appears autonomic, able for instance to generate stereotypic motor activities. However, knowledge concerning neurochemical anatomy including neurotransmitters remains poorly described in the arm of the octopus.
We performed an immunohistochemical study aiming at identifying serotonin (5-HT)-containing structures and their networks in the octopus arm. The specificity of 5-HT antiserum was validated by absorption test. 5-HT immunoreactivity occurred exclusively in nerve cell bodies and fibers. 5-HT-positive cell bodies are found only in the cell layer of the brachial ganglion, whereas 5-HT-positive nerve fibers were distributed widely throughout the arm. While a few smooth or varicose nerve fibers running in the cerebrobrachial tract are likely derived from the central brain, most of other fibers appear to arise from 5-HT-positive cells in the brachial ganglion. These 5-HT-positive nerves join, as minor participants, nerve bundles connecting different nerve centers of the arms, including the intramuscular nerve cords, anastomotic tracts and the ganglia of the sucker. In addition, 5-HT-positive fibers appeared to terminate on sensory cells in the sucker epithelium that are immunostained for the peripheral type of choline acetyltransferase. The result suggests that intrinsic 5-HT nerves of the arm are involved in sensory transmission. A schematic representation of the serotoninergic pathway in the octopus arm will be presented.

Disclosures: J. Bellier: None. Y. Xie: None. S.M. Farouk: None. Y. Sakaue: None. I. Tooyama: None. H. Kimura: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.02/G39

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: TIFR Intramural Grant

Title: Serotonin modulates mitochondrial biogenesis and function in cortical neurons

Authors: *S. E. FANIBUNDA[1], A. SOOD[1], A. D. B. VAIDYA[2], U. SEETHARAM-KOLTHUR[1], V. A. VAIDYA[1];
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Abstract: Serotonin is an evolutionarily ancient molecule that modulates neuronal differentiation, growth and synaptic plasticity. Within neurons, mitochondria also play an important role in influencing specific neuronal functions such as synaptic plasticity, neurotransmission, neuronal excitability and cell death. However, the relationship between serotonin and mitochondrial physiology in neurons is currently poorly understood and explored. We hypothesized that serotonin may impinge on mitochondrial biogenesis and function. In cortical cultures, serotonin evokes a dose dependent increase in mitochondrial biogenesis and content, measured using mtDNA levels, mRNA and protein expression of specific mitochondrial markers and mitotracker staining. Further, serotonin also influences mitochondrial function, increasing ATP production, as scored by biochemical assays that measure cellular ATP content. An increase in mitochondrial output may arise through an increase in biogenesis or through an increase in OxPhos efficiency, and currently experiments are underway to explore these possibilities. We have mechanistically identified the specific serotonin receptors, that mediate the effects of serotonin on mitochondrial biogenesis, using serotonergic receptor specific agonists and antagonists. While the 5HT2A receptor agonist DOI mimics the effects of serotonin, also increasing mtDNA and ATP production, pretreatment with a 5HT2A receptor selective antagonist MDL100,907 prevents the effects of serotonin on mitochondrial biogenesis and function. Downstream of the 5HT2A receptor, the major signalling pathways, that contribute to the effects of serotonin on mitochondrial biogenesis are the phospholipase C and MAP kinase pathways. In contrast, specific inhibitors of the PI3-kinase Akt signalling pathway, did not alter the effects of serotonin on mitochondrial biogenesis. Further, studies using a SIRT1 inhibitor EX-527, implicate SIRT1 in contributing to the effects of serotonin on mitochondrial biogenesis and function. The increase in mtDNA and ATP evoked by serotonin, may mechanistically be mediated by the recruitment of PGC1α, as evidenced by an increased transcription of PGC1α and increased PGC1α levels. Experiments are currently underway, to mechanistically understand the

role of SIRT1 and PGC1α, as key downstream mediators of the effects of serotonin on mitochondrial biogenesis in cortical neurons. These effects of serotonin on mitochondrial physiology may be relevant to the effects serotonin has on aging, growth, plasticity, and neuronal metabolism.

Disclosures: S.E. Fanibunda: None. A. Sood: None. A.D.B. Vaidya: None. U. Seetharam-Kolthur: None. V.A. Vaidya: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.03/G40

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH P01 HD036379

Harvard Stem Cell Institute Seed Grant

NARSAD Distinguished Investigator Grant from the Brain and Behavior Foundation

Blavatnik Biomedical Accelerator at Harvard University Pilot Award

Title: Transcriptomic and functional diversity of serotonin neuron subtypes.

Authors: *B. W. OKATY, M. E. FRERET, B. D. ROOD, V. NIEDERKOFLER, T. E. ASHER, R. DOSUMU-JOHNSON, R. D. BRUST, S. M. DYMECKI;
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Abstract: Serotonergic (5HT) neurons modulate numerous behavioral, cognitive, and physiological processes and are implicated in a variety of clinical disorders. Though often viewed as a single neuron type based on neurochemical identity, phenotypic diversity within the 5HT neuron system has been observed across several parameters – such as neuropeptide expression, electrophysiology, and synaptic connectivity - suggesting the existence of specialized subtypes of 5HT neurons that differentially regulate biological functions. Given that cell phenotypes are strongly determined by cell type-specific gene regulation, global gene expression profiling, or transcriptomics, has emerged as an indispensible tool for classifying neuron subtypes and identifying the molecular underpinnings of their cellular properties. We recently combined intersectional genetic fate mapping, neuron sorting, and genome-wide RNA-Seq to deconstruct the mouse 5HT system at multiple levels of granularity—from anatomy, to genetic sublineages, to single neurons. We found that 5HT neuron transcriptomes cluster into groups

defined by a combination of lineage and anatomy, and that 5HT neuron subtypes with distinct transcriptomes display corresponding differences in electrophysiological properties and neuropeptide receptivity, as well as differential involvement in behaviors. These lineage and anatomy defined subtypes represent a first tier of system organization, and we are now further refining these subclassifications through multi-scale intersectional genetic experiments, combining pairwise driver genes suggested by our RNA-seq data set and performing RNA-seq, electrophysiology, and functional mapping experiments. Through these ongoing studies we continue to characterize the transcriptomic and functional diversity of 5HT neurons, and have uncovered previously undescribed 5HT neuron subtypes expressing unique combinations of co-regulated genes, showing differential responses to signaling molecules, and mapping to restricted sets of functions, such as aggression and social interest.

Disclosures: B.W. Okaty: None. M.E. Freret: None. B.D. Rood: None. V. Niederkofler: None. T.E. Asher: None. R. Dosumu-Johnson: None. R.D. Brust: None. S.M. Dymecki: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.04/G41

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Brain & Behavior Research Foundation (NARSAD Young Investigator Award 2014)

Rosen Family Research Scholar Award

MQ Research Fellowship

Alfred P. Sloan Foundation Fellowship in Neuroscience (2016)

Title: Histone serotonylation, a novel mechanism of neuroepigenetic plasticity.

Authors: *L. FARRELLY[1], R. THOMPSON[2], S. ZHAO[3], A. LEPACK[1], Y. LU[1], H. ZEBROSKI III[4], O. BERTON[1], H. MOLINA[4], H. LI[3], T. MUIR[2], I. MAZE[1];
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Abstract: Monoamines, such as serotonin, dopamine, etc, play a critical role in neuronal plasticity, with alterations implicated in the development and treatment of numerous brain disorders. Although vesicular packaging of monoamines is essential for neurotransmission, recent data have demonstrated the additional presence of reserve pools of extravesicular

monoamines in the nucleus of monoaminergic neurons; it remains unclear, however, whether nuclear monoamines may play roles independent of neurotransmission. Serotonin has previously been shown to form covalent bonds with certain cytoplasmic proteins via transamidation by the tissue transglutaminase 2 enzyme, a process known as serotonylation. As this modification alters the signaling properties of its substrates, we hypothesized that nuclear proteins may similarly be modified to control distinct aspects of their function. Here, we describe histone proteins as novel substrates for monoaminylation in vivo and attempt to delineate the biophysical, biochemical and molecular functions of these novel modifications in the context of neuronal development and plasticity. Utilizing a unique combination of biochemical, genome-wide and functional neurobiological approaches, our data indicate that H3 serotonylation acts to facilitate binding of adjacent H3 methylation binding proteins, thereby promoting neuronal genic activation and the facilitation of neural development. In sum, our data provide the first direct evidence that hydrophobic monoamines in brain contribute directly to neuronal gene expression via a novel, neurotransmission-independent epigenetic mechanism; such phenomena will likely have broad implications within the field of neuroscience and beyond.

Disclosures: L. Farrelly: None. R. Thompson: None. S. Zhao: None. A. Lepack: None. Y. Lu: None. H. Zebroski III: None. O. Berton: None. H. Molina: None. H. Li: None. T. Muir: None. I. Maze: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.05/G42

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIDA Avenir

NARSAD

Title: Potential roles for histone dopaminylation in cocaine induced transcriptional and behavioral plasticity

Authors: *A. LEPACK[1], L. FARRELLY[1], A. C. W. SMITH[1], Y. LU[1], R. THOMPSON[2], R. O'CONNOR[1], S. ZHAO[3], Z.-J. WANG[4], H. LI[3], D. M. DIETZ[5], T. MUIR[2], P. J. KENNY[1], I. MAZE[1];
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Abstract: Drug addiction is a debilitating disease that affects about 25 million Americans–ages 12 and up–with treatment costs averaging >190 billion dollars annually in the United States alone. Drug abuse is characterized by loss of control over drug intake, as well as persistent drug-seeking behaviors despite negative consequences to both the drug abuser and those directly affected by their behavior.
Persistent changes in neuronal gene expression are known to promote physiological alterations implicated in drug addiction. More recently, cell-type and brain region specific 'epigenetic' mechanisms have also been demonstrated to regulate transcriptional programs contributing to addiction-like behaviors; however, our understanding of how these mechanisms mediate life-long addiction remains limited. Dopaminergic neurotransmission in the central nervous system plays a critical role in psychostimulant-induced neural plasticity, with alterations in dopamine production/function being implicated in both the development and treatment of substance abuse disorders. Independent of its role in neurotransmission, we recently identified histone proteins as robust substrates for dopamine transamidation—so-called dopaminylation—*in vivo*. Our data indicate that histone H3 dopaminylation acts to enhance binding of adjacent histone posttranslational modification (PTM) interacting proteins ('readers') and likely plays a direct and critical role in dopamine associated neuronal transcription. Furthermore, our data demonstrate that chronic withdrawal from volitional administration of extended access to cocaine in rodents results in high levels of dopamine accumulation in the nucleus of dopamine producing neurons in the ventral tegmental area, as well as altered expression of TGM2, the H3 dopaminylase. Taken together, these data suggest that persistent states of addiction may result from increased genomic enrichment of H3 dopaminylation, potentiation of aberrant transcriptional plasticity and increased drug seeking behaviors. Using a combination of chemical biology, genome-wide and behavioral approaches, we are fully characterizing the functions of histone dopaminylation, both in the context of normal neuronal function and in rodent models of drug abuse.

Disclosures: A. Lepack: None. L. Farrelly: None. A.C.W. Smith: None. Y. Lu: None. R. Thompson: None. R. O'Connor: None. S. Zhao: None. Z. Wang: None. H. Li: None. D.M. Dietz: None. T. Muir: None. P.J. Kenny: None. I. Maze: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.06/G43

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: P50AA010761

Title: GIRK-mediated inhibition of lateral orbitofrontal cortex neurons by monoamines is lost following chronic exposure to ethanol

Authors: *S. NIMITVILAI, M. F. LOPEZ, P. J. MULHOLLAND, J. J. WOODWARD; Dept. of Neurosci. and Addiction Sci. Div., Med. Univ. of South Carolina, Charleston, SC

Abstract: Alcoholism is associated with changes in brain reward and control systems including the prefrontal cortex. In prefrontal areas, the orbitofrontal cortex (OFC) has been shown to be particularly sensitive to acute ethanol and has been suggested to play an important role in the development of alcohol abuse disorders. Recent findings from this laboratory demonstrate that both acute and chronic exposure to ethanol alters the intrinsic excitability of OFC neurons and that ethanol-dependent mice are impaired in a reversal learning task that requires the OFC. The OFC is extensively innervated by monoamine neurotransmitters including dopamine (DA), norepinephrine (NE) and serotonin (5HT), and drugs that target monoamine receptors have been used to treat a number of psychiatric diseases, including alcohol abuse disorders. In this study, we characterized the effect of monoamines on lateral OFC (lOFC) neuronal excitability in naïve mice and those treated with repeated cycles of chronic intermittent ethanol (CIE) exposure. DA, NE and 5HT all produced a dose-dependent (1, 10 and 50 μM) decrease in spike firing of lOFC neurons that was mediated via $G_{i\alpha}$-coupled D2, α2-adrenergic or $5HT_{1A}$ receptors, respectively. Lower concentrations of DA (1 and 100 nM) that had no effect on spiking under control conditions inhibited firing in the presence of the monoamine uptake inhibitor nomifensine. Inhibition of firing by DA or the D2 agonist quinpirole, but not by NE or 5HT, was prevented by the $GABA_A$ receptor antagonist picrotoxin and a picrotoxin-sensitive tonic current was enhanced by DA or the D1 agonist SKF81297, but not by quinpirole. In addition, sIPSC amplitude was increased by quinpirole, but not by dopamine. Monoamine inhibition of firing was suppressed by the GIRK channel blocker barium that itself enhanced spiking while the GIRK channel activator ML297 inhibited current-evoked spiking. In neurons from CIE-treated mice, spike frequency was nearly doubled and these neurons were largely resistant to the inhibitory effect of monoamines for up to at least 7 days of withdrawal. ML297 also had no or little effect on spiking in neurons from CIE-treated animals, despite no obvious change in GIRK1, GIRK2 or Gi/o protein expression. The results of these studies suggest that monoamines, acting via GIRK channels, are important modulators of the intrinsic excitability of lOFC neurons and that this modulation is significantly disrupted following chronic exposure to alcohol. Dysfunction of one or more of these neuromodulators may contribute to impaired OFC function associated with various neuropsychiatric diseases including alcohol dependence. Supported by P50AA010761.

Disclosures: S. Nimitvilai: None. M.F. Lopez: None. P.J. Mulholland: None. J.J. Woodward: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.07/G44

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: R01MH086629

NARSAD Independent Investigator Award

Title: Incomplete marking of cerebral cortical interneuron expression patterns in Drd1 receptor BAC transgenic mice

Authors: *Y. JIN[1], K. M. MONEY[3,4], V. G. FUENTES[5], L. R. ANDERSON[1], G. D. STANWOOD[1,2,5];
[1]Dept. of Biomed. Sci., [2]Ctr. for Brain Repair, Florida State Univ. Col. of Med., Tallahassee, FL; [3]Neurosci. Grad. Program, Vanderbilt Univ., Nashville, FL; [4]Vanderbilt Med. Scientist Training Program, [5]Dept. of Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: The use of bacteria artificial chromosome transgenic reporter mice allow for the identification of cell populations not easily labeled otherwise, including low abundance G-protein coupled receptors. In the case of dopamine D1 (Drd1) receptors, Drd1-tdTomato and Drd1-eGFP transgenic mice have greatly enabled electrophysiological and neuroanatomical studies and appear to correctly and unambiguously report D1 receptor expression in most brain regions and cell types. However, in cortical GABAergic interneurons, we report that these D1 receptor reporter lines under-report Drd1 expression in GAD-67-, parvalbumin- and calbindin-expressing GABAergic interneurons in adult mice. Using double-label immunohistochemistry in Drd1-tdTomato (line 6) mice, only 18% of GAD-67+ interneurons also expressed the Drd1-driven transgene, which is far below that previously reported in colocalization studies. The case was even more dramatic for some GABAergic neuron subpopulations, with < 1% of parvalbumin+ cortical interneurons expressing the transgene. We used RNAScope to sensitively measure endogenous Drd1 transcript and found similar levels of Drd1/parvalbumin co-expression to previous reports in rodents (~50%). Transgene expression by excitatory pyramidal neurons closely matched predicted transcript patterns, suggesting that the issue in these lines is specific to interneurons. Thus while the reporter lines continue to be powerful tools to study the localization and connectivity of some D1 receptor-expressing neurons, one cannot assume that the expression patterns are totally congruent with endogenous D1 receptor expression patterns.

Disclosures: Y. Jin: None. K.M. Money: None. V.G. Fuentes: None. L.R. Anderson: None. G.D. Stanwood: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.08/G45

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR

NOSM/NOSMFA Research Development Fund

Title: Epigenetic regulation of phenyethanolamine N-methyltransferase: implications for adrenaline biosynthesis

Authors: *S. KHURANA[1], S. THARMALINGAM[1], K. VENKATARAMAN[2], T. C. TAI[1,3,4,5]; [1]Med. Sci. Div., Northern Ontario Sch. of Med., Sudbury, ON, Canada; [2]Gerontology, Huntington Univ., Sudbury, ON, Canada; [3]Biol., [4]Chem. and Biochem., [5]Biomolecular Sci., Laurentian Univ., Sudbury, ON, Canada

Abstract: Adrenaline, a neurohormone and neurotransmitter, plays an extensive role in the physiological response to stress, and the sympathetic regulation of blood pressure. Phenylethanolamine N-methyltransferase (PNMT), the terminal enzyme in the catecholamine biosynthetic pathway, directly responsible for adrenaline synthesis, is elevated in hypertensive animals; genetic linkage studies have associated the PNMT gene to the development of hypertension. Epigenetic changes are heritable changes in gene expression that are a consequence of the modification of the DNA (methylation at CpG sites) or histones (e.g. by methylation, acetylation) that facilitate packaging the DNA into nucleosomes. Changes in the epigenome have been associated with incidences of cancer, metabolic disorders and cardiovascular pathologies. In this study, alteration in PNMT expression modifiable by epigenetic regulation was examined using the rat adrenal pheochromocytoma derived PC12 cells. *In vitro* methylation of a PNMT promoter driven luciferase construct, using CpG methylases, consequently lead to a radical decrease in promoter activation, even in presence of dexamethasone (Dex) or Forskolin (Fsk), otherwise potent activators of PNMT expression. Further, the influence of a DNA methylation inhibitor 5-aza-2'-deoxycytidine (5aza2DC), and histone deacetylase inhibitor valproic acid (VPA) was examined. Transcript analysis of endogenous PNMT, and PNMT promoter driven luciferase assays, both revealed that PNMT transcription is elevated in presence of these epigenetic modifiers, and this was synergistic with the activation by Dex or Fsk. The extent of CpG methylation at the promoter of PNMT using bisulphite-sequencing, and transcription factor binding sites that are sensitive to epigenetic modification using site directed mutagenesis, are currently being examined. The data suggests that PNMT regulation is sensitive to epigenetic modification which can have repercussions for adrenaline biosynthesis, and consequently on its role as a neurotransmitter.

Disclosures: S. Khurana: None. S. Tharmalingam: None. K. Venkataraman: None. T.C. Tai: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.09/G46

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Memory, a key component of dementias: serotonergic markers

Authors: *A. MENESES;
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Abstract: Dysfunctional memory seems to be a key component of diverse dementias and other neuropsychiatric disorders unfortunately no effective treatment exists for it; probably due to the absence of neural markers accompanying it. Evidence of neural markers and memory continues accumulating, and progress is allowing a better framing of earlier and new evidence. Several neurotransmission systems have implicated in memory, including serotonin. 5-hydroxytryptamine (5-HT) or serotonin has multiple pharmacological tools, well characterized downstream signaling in mammals' species and well-established 5-HT neural markers offering new insights about memory functions and dysfunctions. Serotonin in mammal species has multiple neural markers, including receptors (5-HT1-7), transporter (SERT) and volume transmission; which are present in brain areas involved in memory. For instance, growing number of researchers report that memory, amnesia and forgetting modify neural markers; this influence is bidirectional. Evidence is showing insights and therapeutic targets and diverse approaches support the translatability of using neural markers and cerebral functions and dysfunctions, including memory formation, amnesia. For instance, 5-HT2C, 5-HT4 and 5-HT6 receptors are involved in tau protein hyperphosphorylation in Alzheimer´s disease. In addition, at least, 5-HT1A, 5-HT4, 5-HT6 and 5-HT7 receptors as well as SERT seem to be useful neural markers and therapeutic targets. Hence, available evidence supports the notion that several mechanisms cooperate to achieve synaptic plasticity or memory, including changes in the number of neurotransmitter receptors and transporters.

Disclosures: A. Meneses: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.10/G47

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIDA R01-DA16736 (MPG)

Department of Veterans Affairs grant RX000458 (DMK)

Fund for Anesthesiology Research (MPG, FG).

Title: Proton magnetic resonance spectroscopy assessment of the neurochemical profile in mice genetically depleted of brain serotonin

Authors: *F. GHODDOUSSI[1], D. I. BRIGGS[2,4], D. M. KUHN[2,4], M. P. GALLOWAY[3]; [1]Anesthesiol. & Magnetic Resonance Core, [2]Psychiatry & Behavioral Neurosciences, [3]Psychiatry & Behavioral Neurosciences and Anesthesiol., Wayne State University, Sch. of Med., Detroit, MI; [4]Res. & Develop. Service, John Dingell VA Med. Ctr., Detroit, MI

Abstract: **Introduction**: Serotonin (5HT) neurons originating in the dorsal raphe innervate nearly all areas of the brain. As a neurotransmitter 5HT is involved in mediating neuronal development as well as behavioral and physiological processes including, sleep, aggression, aging , control of food intake and body weight. In addition, the dysfunction in the 5HT neuronal system has been linked to neuropsychiatric conditions such as depression, anxiety, obsessive compulsive disorder and suicide. Serotonin selective reuptake inhibitors (SSRIs) are the most common treatment for depression and their therapeutic effect is generally attributed to their ability to increase the synaptic levels of 5HT. Tryptophan hydroxylase-2 (TPH2), expressed selectively in 5HT neurons, is the initial and rate limiting enzyme in the biosynthesis of 5HT. Genetic ablation of the TPH2 gene provides a hyposerotonergic animal model to examine the influence of 5HT innervation on development and behavior. $TPH2^{-/-}$ mice show intense compulsivity and impulsivity, social communication deficits, exaggerated aggression and decreased levels of anxiety like behavior. 5HT neurons in these mice, although lacking 5HT, retain their characteristic electrophysiological properties as well as relatively normal brain development and elaboration of the 5HT neuronal system. This suggests that behavioral phenotypes of the $TPH2^{-/-}$ may be mediated via the absence of a modulatory effect of 5HT on other neurotransmitter systems such as Glutamate (GLU) and GABA. **Methods:** $TPH2^{-/-}$ mice were generated by deleting exon1 of the Tph2 gene and were on a mixed C57BL/6-Sv129 background. High resolution magic angle spinning (HR-MAS) proton magnetic resonance spectroscopy (^{1}H-MRS) *ex vivo* (translation potential to clinical MRS) was used to assess unbiased neurochemical profiles in different brain regions of 8 week-old WT (n=8) and KO

(n=12) mice. **Results:** MRS analysis showed significant decrease in the GLU/CRE and NAA/CRE ratios in nucleus accumbens (NAc) and significant increase in GLN/CRE & GLN/GLU in anterior cingulate cortex (ACC) in the KO mice in comparison to the WT. **Discussion:** The 5HT innervation of the NAc as well as principle cortico-striatal GLU neurons is substantial. Decreased indices of GLU in the NAc may reflect a developmental insult related to 5HT deficits in the critical period and may be associated with the unique behavioral phenotypes of the $TPH^{-/-}$mouse. Previous studies have established a link between increased glutamate activity in the NAc and depressive behavior while $TPH2^{-/-}$ does not show depression like behavioral phenotypes, therefore maybe the decrease in glutamate levels is a compensatory effect.

Disclosures: F. Ghoddoussi: None. D.I. Briggs: None. D.M. Kuhn: None. M.P. Galloway: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.11/G48

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant MH1011778

Title: Neurochemical profile of dorsal raphe projections to the rat medial prefrontal cortex

Authors: *E. W. PROUTY[1], B. D. WATERHOUSE[2];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Cell Biol., Rowan Univ. Sch. of Med., Stratford, NJ

Abstract: Serotonin (5-HT)-containing neurons in the dorsal raphe (DR) nucleus project throughout the mammalian forebrain and are implicated in a host of physiological processes and neuropsychiatric disorders. These neurons have been characterized in terms of their neurochemistry and anatomical organization, but a clear sense of whether these attributes align with specific brain functions or specific forebrain terminal fields is lacking. DR 5-HT neurons can co-express additional neuroactive substances, increasing the potential for individualized regulation of target circuits. The goal of this study was to link the neurochemical profile of DR neurons to a specific functional role by characterizing cells in terms of their efferent connectivity; in this case, cells projecting to the the medial prefrontal cortex (mPFC), a region responsible for higher-order cognition and regulation of emotion. After injecting the retrograde tracer FluoroGold (FG) into the mPFC of Sprague-Dawley rats, brainstem sections containing

the DR from these animals were collected and subjected to immunohistochemical staining for serotonin, glutamate, GABA, or nitric oxide using antibodies targeting tryptophan hydroxylase 2 (TPH2), vesicular glutamate transporter 3 (VGLUT3), glutamate decarboxylase 1 (GAD67), and neuronal nitric oxide synthase (nNOS), respectively. We found that 98% of the 5-HT neurons projecting to the mPFC co-express VGLUT3, 60% co-express nNOS, and 0.1% co-express GAD67. For comparison, we also examined the co-expression patterns of DR 5-HT neurons projecting the lateral geniculate nucleus of the thalamus (a subcortical relay site for visual information). There were no meaningful differences in VGLUT3 or GAD67 co-expression between DR 5-HT neurons projecting to mPFC vs LGN; however, a significantly greater proportion of mPFC-projecting cells co-expressed nNOS (60% vs 22%, $\chi^2 = 178.1$, $p < 0.001$). The co-expression of nNOS is of interest because of its putative role in stress. Overall, these results establish a co-transmitter profile of DR projections to mPFC and raise further questions about how these transmitter combinations, particularly 5-HT and nNOS, impact cortical circuits underlying cognitive function and mood. Ongoing studies will determine if the electrophysiological profile of mPFC-projecting DR neurons differs from that of neurons that project to other DR targets.

Disclosures: E.W. Prouty: None. B.D. Waterhouse: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.12/G49

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Imbalance of serotonin homeostasis during adulthood affects serotonergic neuronal circuitry

Authors: *M. PRATELLI[1], B. PELOSI[1,2], S. MIGLIARINI[1], M. PASQUALETTI[1,3];
[1]Univ. of Pisa, Pisa, Italy; [2]Univ. catholique de Louvain, Inst. of Neurosci., Brussels, Belgium; [3]Ctr. for Neurosci. and Cognitive Systems, Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: Serotonin (5-hydroxytryptamnine, 5-HT) is a monoaminergic neurotransmitter orchestrating a broad array of cognitive and behavioral processes in the adult brain. The early expression of its receptors during development and the requirement of maternal and placental sources of serotonin to the foetus have led to the hypothesis that 5-HT could act as growth regulator in the fine-tuning of specific morphogenetic events during neurodevelopment. Outcomes from genetic mouse models in which brain 5-HT homeostasis has been perturbed by targeting genes necessary for serotonin reuptake, metabolism or synthesis such as *SERT, MAO-A*

and *Tph2*, respectively, support this hypothesis. However, evidence of a role for 5-HT in adulthood as a growth regulator or its requirement for maintenance of the proper neuronal circuitry, which is known to be susceptible to 5-HT imbalance during early postnatal stages, is still missing. To bridge this gap we used the *Tph2* conditional knock-out (cKO) allele that allows an efficient abrogation of 5-HT synthesis in the adult brain, in combination with the *Tph2::GFP* allele, in which GFP reporter expression highlights 5-HT neuron fibers and somata. Beside previously reported data showing that the lack of brain serotonin in *Tph2* knock-out (KO) mice deeply affects serotonergic circuitry development with a brain region-specific effect, the abrogation of 5-HT synthesis during adulthood produces alterations of serotonergic innervation in rostral brain targets matching those observed in mice with a life-long depletion of brain serotonin. Remarkably, we reported that restoring brain 5-HT signaling in both *Tph2* KO and cKO mice by chronic administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) results in a significant reduction in the extent of serotonergic fiber innervation defects, thus demonstrating an unexpectedly high degree of plasticity of the adult serotonergic system in response to changes of 5-HT homeostasis. Moreover, 3D computer-based analysis of serotonergic axon terminal morphology showed that imbalances in brain 5-HT content exert their growth regulatory activity on 5-HT axon terminals by promoting sprouting. Altogether these data demonstrate that a correct 5-HT homeostasis is life-long required to maintain the proper serotonergic innervation of specific rostral brain regions.

Disclosures: M. Pratelli: None. B. Pelosi: None. S. Migliarini: None. M. Pasqualetti: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.13/G50

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Canada Research Chair (CRC)

Early Researcher Award (ERA)

Canada Foundation for Innovation (CFI)

Title: p11 corticostriatal neurons have distinctive 5-HT responses sensitive to chronic social isolation stress and to antidepressant treatment

Authors: *D. SARGIN[1], K. PERIT[1], E. F. SCHMIDT[2], R. C. UTHAIAH[3], N. HEINTZ[2,4], P. GREENGARD[3], E. K. LAMBE[1];
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Abstract: Major depression affects more than 15% of North Americans in their lifetime. The most commonly prescribed antidepressant medications are selective serotonin reuptake inhibitors (SSRIs) that target the serotonin (5-HT) system. SSRI therapeutic action requires a prolonged period of treatment and is not always effective. To improve therapies for depression, it is essential to fine-tune the mechanisms of antidepressant action. Recently, layer 5a corticostriatal neurons expressing p11 (S100a10) protein were found to be specifically affected in depression and distinctively responsive to antidepressant treatment. Initially identified as an interacting partner for a group of 5-HT receptors, p11 expression is decreased in depression and increased by SSRIs. Yet, the critical mechanisms underlying the antidepressant neurophysiology of p11 remain to be elucidated. To characterize the distinctive neurophysiological properties of p11 expressing neurons, we performed whole cell electrophysiology on acute brain slices of motor cortex from mice with eGFP-labeled p11 expressing neurons. Here, we find p11 neurons have excitatory responses to 5-HT. Subjecting mice to chronic social isolation stress reduces 5-HT excitatory responses in p11 neurons but chronic treatment with the SSRI fluoxetine restores them. We probe the receptors and signaling mechanisms underlying the unique 5-HT responses of p11 neurons. Identification of these underlying regulatory mechanisms will be important for developing novel treatment strategies for depressive disorders.

Disclosures: D. Sargin: None. K. Perit: None. E.F. Schmidt: None. R.C. Uthaiah: None. N. Heintz: None. P. Greengard: None. E.K. Lambe: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.14/H1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSERC

Canada Research Chair

Early Researcher Award

CIHR DRA

Title: Serotonin and cortical disinhibition: Novel co-operation between 5-HT_{1A} and 5-HT_{2A} receptors in prefrontal cortex

Authors: *M. K. TIAN, E. K. LAMBE;
Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: In medial prefrontal cortex, layer 6 pyramidal neurons play a critical role in attention and express receptors for serotonin (5-HT), a neuromodulator known to shape and bias attention. In primary sensory cortex, layer 6 neurons exert strong cortical gain control through their excitatory projections to interneurons. This phenomenon has not been investigated in prefrontal cortex. Furthermore, little is known about the direct electrophysiological consequences of 5-HT on layer 6 neurons themselves and on their regulation of local cortical activity. Here, we performed whole-cell recordings and pharmacological manipulations in acute brain slices from transgenic mice expressing either eGFP or a fusion protein of eGFP-channelrhodopsin in prefrontal layer 6 pyramidal neurons. Optogenetic excitation was used to test the effects of 5-HT on the inter-laminar excitatory circuits between layer 6 pyramidal neurons and layer 5 interneurons, which act as critical mediators of cortical inhibition. Prefrontal layer 6 pyramidal neurons are strongly inhibited by 5-HT through activation of 5-HT_{1A} and, surprisingly, 5-HT_{2A} receptors. This direct serotonergic suppression of neuronal excitability in layer 6 is complex, with the inhibition mediated by 5-HT_{2A} receptors strongest near threshold. Optogenetic investigation of the connections between layer 6 pyramidal neurons and both fast- and regular-spiking interneurons in layer 5 reveal a strong suppression on this circuit by 5-HT through both 5-HT1A and 5-HT2A receptors. These findings reveal a novel modulatory role of 5-HT on cortical excitability through strong inhibitory effects on layer 6 and its feedforward projections. Disturbances of normal serotonin signalling in deep cortex would thus have unexpectedly broad consequences for prefrontal cortex activation and signal-to-noise ratios in circuits important to attention.

Disclosures: M.K. Tian: None. E.K. Lambe: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.15/H2

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF-1154096: AugSTEM Grant

Augsburg College Biology Department

Title: The role of serotonergic signaling in Daphnia magna swimming

Authors: O. BURT, *M. L. BECKMAN;
Biol., Augsburg Col., Minneapolis, MN

Abstract: *Daphnia magna* are freshwater microcrustaceans that have been used in toxicology research for decades. One common test involves exposing *Daphnia* to a drug or chemical and assessing its movement. Daphnids possess a rich motor program, yet not much is known about the neurochemical control of movement. We sought to use *D. magna* as a model organism to determine the role of serotonin in movement. *Daphnia* were treated with agonist and antagonist drugs targeting 5-HT 1A and 5-HT 7 receptors. Animals were filmed for one minute from above to make 2D movies at various time-points following treatment with 10 μM drug solutions. CTRAX, an open-source software, was used to track *Daphnia* swimming. Statistical analysis showed the effects of two of these drugs on 5-HT 1A receptors significantly affected movement. Xaliproden hydrochloride, a 5-HT 1A receptor agonist showed inhibition by decreasing the total swimming distance; whereas, NAD 299 which is a 5-HT 1A antagonist, increased total swimming distance. Further, application of NAD 299, the antagonist, followed by Xaliproden HCl, the agonist, resulted in control levels of *Daphnia* swimming. These findings indicate that a serotonergic signaling pathway involving 5-HT1A receptors is involved in the neural control of *Daphnia* swimming. Additionally, this research supports that *Daphnia magna* is a viable model organism in studying the role of serotonergic signaling in animal movement.

Disclosures: O. Burt: None. M.L. Beckman: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.16/H3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant F30 MH099704

NIH Grant P50 MH096972

NIH Grant RO1 MH062723

Title: Adult brain serotonin deficiency causes hyperactivity, circadian disruption, and elimination of siestas

Authors: *M. S. WHITNEY[1], A. M. SHEMERY[2], A. M. YAW[2], L. J. DONOVAN[1], J. GLASS[2], E. S. DENERIS[1];
[1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Biomed. Sci., Kent State Univ., Kent, OH

Abstract: Serotonin (5-HT) is a critical neuromodulator implicated in many psychiatric disorders. Yet, after decades of study, its role in behavior remains poorly understood, in part, because of a lack of methods to specifically deplete 5-HT from the adult brain. Here, we have developed a genetic approach that achieves near-complete elimination of 5-HT from the adult brain ascending 5-HT system by stereotaxic injection of an adeno-associated virus expressing Cre recombinase (AAV-Cre) into the midbrain of mice carrying a loxP-conditional *tryptophan hydroxylase 2 (Tph2)* allele. We investigated the behavioral effects of a specific loss of brain 5-HT and discovered a novel compound phenotype. Surprisingly, 5-HT deficiency in the adult brain did not affect anxiety-like behavior in open-field, elevated plus maze, or light-dark box testing. However, it resulted in a robust hyperactivity phenotype in novel and home cage environments. In a 30-minute open-field test, 5-HT-depleted (*Tph2-CKO*) mice showed increased rates of activity as compared to control (*Tph2-CON*) mice during the entire test. In two independent cohorts, *Tph2-CKO* mice also exhibited increased activity in the light and dark periods in home cage monitoring studies, as compared to *Tph2-CON* mice. Additionally, *Tph2-CKO* mice displayed increased average and peak rates of activity explicitly during bouts of activity, definitively demonstrating the presence of absolute hyperactivity. Moreover, specific loss of adult brain 5-HT led to an altered pattern of circadian behavior characterized by an advance in the onset and a delay in the offset of daily activity, thus revealing a specific requirement for adult ascending brain 5-HT in the control of daily activity patterns. Notably, after normalizing for hyperactivity, we found that the normal prolonged break in nocturnal activity (siesta), a period of REM and NREM sleep, was absent in all animals in which 5-HT deficiency was verified. Thus, our findings have identified adult ascending 5-HT as a necessary neurotransmitter for siestas, implicating adult ascending 5-HT in sleep-wake homeostasis. Together, our results may have implications for the role of 5-HT in a variety of mental health disorders with hyperactivity and circadian/sleep disruption, including ADHD, Seasonal Affective Disorder, depression, bipolar disorder, and sleep disorders. Furthermore, our findings highlight the importance of our brain-specific 5-HT depletion approach in understanding 5-HT's role in controlling daily activity levels and patterns.

Disclosures: M.S. Whitney: None. A.M. Shemery: None. A.M. Yaw: None. L.J. Donovan: None. J. Glass: None. E.S. Deneris: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.17/H4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Chair in Neuroscience UAM - Fundación Tatiana Pérez de Guzmán el Bueno

Title: Noradrenaline innervation in the primate thalamus: first order sensory nuclei versus higher order nuclei

Authors: I. PÉREZ-SANTOS, *C. CAVADA;
Univ. Autonoma Madrid, Fac Medicina, Madrid, Spain

Abstract: Noradrenaline (NA) is thought to improve signal-to-noise ratios in sensory systems. This could be particularly relevant in the thalamus, where sensory inputs are transmitted and modulated within the first order sensory relay nuclei. NA also plays a critical role in higher brain functions, including learning and memory. These functions are more related to higher order thalamic nuclei, such as the pulvinar, mediodorsal and intralaminar nuclei; which receive little direct sensory input, and instead participate in extensive cortico-thalamo-cortical pathways.
To shed light on the role of NA in those two types of thalamic nuclei, we have mapped their NA innervation in the macaque monkey using immunohistochemistry against dopamine-beta-hydroxylase (DBH), the NA synthesizing enzyme. Maps of DBH innervation were generated using Neurolucida® software filters to transform real pictures into black and white images. The borders of thalamic nuclei were traced using sections processed for acetylcholinesterase adjacent to the immunoreacted ones.
The first order sensory thalamic nuclei receive a moderate NA innervation. In particular, the less innervated nucleus throughout the thalamus is the lateral geniculate nucleus, the first order visual relay nucleus.
The higher order nuclei receive a denser NA innervation than the first order sensory nuclei. Within the mediodorsal nucleus the most innervated region is its medial sector, which is particularly connected to the orbitofrontal cortex. In contrast, the pulvinar and lateral posterior nuclei receive a moderate NA innervation, similar to that present in first order sensory nuclei. In the parafascicular-centromedian complex the most innervated region is the caudal part of the parafascicular nucleus, which projects to limbic and associative striatum, and is connected with the prefrontal cortex, the frontal eye fields and the supplementary motor cortex. The centromedian nucleus, which projects to sensorimotor striatum and is connected with the precentral motor, premotor and somatosensory cortices, receives a moderate NA innervation.
These results suggest a prominent role for thalamic NA in cortico-thalamo-cortical and cortico-

thalamo-striatal circuits related to cognition and limbic processing, rather than in sensory transmission through the thalamus.

Disclosures: I. Pérez-Santos: None. C. Cavada: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.18/H5

Topic: F.04. Stress and the Brain

Support: NIH R01MH104373

Title: Norepinephrine activates synchronized inhibitory synaptic inputs to BLA principal neurons

Authors: *X. FU, J. TASKER;
Cell and Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: Emotional arousal facilitates memory formation and norepinephrine released in the basolateral amygdala (BLA) during arousal is thought to play an important role in this facilitatory effect. Diverse GABAergic interneurons in the BLA have been shown to be essential for gating the activities of the principle neurons through feed forward inhibition or disinhibition during fear conditioning (Wolff, 2014). Norepinephrine (NE) thus may regulate the activity of the local interneurons to control the concerted neural outputs from BLA. Using whole-cell patch clamp recordings in amygdala slices, we found that norepinephrine application induces a significant increase in the frequency and amplitude of inhibitory postsynaptic currents (IPSCs) in BLA principal neurons. Also, we consistently observed 3 different types of IPSC bursting activity induced by NE. The effects of NE on IPSCs were blocked by pre-application of the a1 adrenergic receptor antagonist prazosin and of the sodium channel blocker TTX, indicating that NE stimulates different subtypes of presynaptic interneurons in the BLA by acting on a1 adrenoceptors. Dual recordings revealed synchronization of the IPSC bursts between pairs of BLA principal neurons. These data suggest that NE can synchronize BLA network activity via a1 receptor-mediated activation of upstream inhibitory interneurons and feedforward synaptic inhibition. This work was supported by NIH R01MH104373.

Disclosures: X. Fu: None. J. Tasker: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.19/H6

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: UCSB Academic Senate Faculty Research Grant

UCSB URCA grants

Title: The brain serotonin matrix: platelet and microglial elements

Authors: K. A. RIPARETTI, M. E. VICARI, E. J. SOPIRA, S. L. STARR, *S. JANUSONIS; Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: In vertebrate brains, neural computations take place in a dense serotonin matrix, produced by axons (fibers) originating in the rostral raphe nuclei. In the adult brain, these fibers have meandering trajectories and carry bead-like swellings (varicosities), the primary sites of serotonin (5-HT) release and reuptake. Serotonergic signaling has been extensively studied in normal and diseased brains, but little is known about the fine structure of the serotonin matrix. This includes its stochastic properties and its interactions with other ubiquitous brain elements, such as microvasculature and microglia. The serotonin matrix is sensitive to traumatic injury and drugs of abuse (e.g., MDMA), and it is also altered in some mental disorders (e.g., autism). Analysis 1: Serotonergic varicosities in the brain parenchyma and blood platelets in brain microcapillaries share a number of physical and biochemical properties. In particular, both express the same serotonin transporter (SERT) and take up 5-HT from their environment. Recent neuroimmunological research suggests that platelets may be able to exit microvasculature and interact with neural and glial cells. To investigate the potential contribution of platelets to 5-HT signaling in the healthy brain, we selectively depleted platelets in mice and assessed the expression of serotonin receptors and SERT in the neocortex. The mRNA levels of the 5-HT1A, 5-HT2A and 5-HT4 receptors were measured with quantitative reverse-transcription PCR (qRT-PCR), and the protein levels of SERT were measured with Western blotting. Our preliminary data suggest changes in the expression of some of the serotonin receptors. We are currently using the observed effect sizes to guide the analysis of a larger sample. If confirmed, these findings would have clinical implications: for example, selective serotonin reuptake inhibitors (SSRIs) directly affect platelet 5-HT uptake. Analysis 2: Serotonergic fibers may interact with “resting” microglia, resident immune cells in the brain. A number of recent studies have shown that microglia play a major role in neural plasticity. We used double-label immunohistochemistry (5-HT/Iba1) and automated 3D-analyses to investigate physical interactions between serotonergic fibers and microglial cells in the mouse neocortex. We found close appositions between

microglial processes and serotonergic varicosities, which suggests that microglia may control 5-HT signaling. We are investigating the statistical properties of these contacts.

Disclosures: K.A. Riparetti: None. M.E. Vicari: None. E.J. Sopira: None. S.L. Starr: None. S. Janusonis: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.20/H7

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: MH002386

Title: The catecholamine receptors ADBR1 and ADBR2 exhibit differential coupling to the cyclic AMP sensor NCS-Rapgef2

Authors: *L. E. EIDEN[1], A. C. EMERY[1], C. WESTOVER[1], W. XU[1], M. V. EIDEN[2]; [1]Sec Molec Neurosci, [2]NIH, NIMH-IRP, Bethesda, MD

Abstract: The G-protein-coupled receptors (GPCRs) βAR1 and βAR2 for the catecholamines norepinephrine and epinephrine couple to $G_s\alpha$ and thus signal through the second messenger cyclic AMP, and its downstream effectors PKA and the cyclic AMP-activated guanine nucleotide exchange (GEF) proteins Rapgef3 and 4 (Epac1 and Epac2). βAR2 signaling also occurs via engagement of β-arrestin leading to activation of ERK. We have recently characterized a third cAMP sensor, a GEF related to Epac1/2 and to the previously characterized non-cAMP-activated GEF PDZ-GEF1, which we have named NCS (neuronal cyclic AMP sensor)-Rapgef2 (protein product of the Rapgef2 gene). This sensor mediates activation of ERK leading to neuritogenesis in the PC12 and NS-1 neuroendocrine cell lines (Emery et al., Sci. Signaling 6(281), ra51, 2013; Emery et al., J. Biol. Chem. 289: 10125, 2014). We have created NS-1 cell lines stably expressing hβAR1 and hβAR2, and examined signaling to each of the three cAMP sensors present in these cells following treatment with isoproterenol. In hβAR1-expressing cells, isoproterenol treatment caused Epac2/p38-dependent growth arrest; PKA-dependent CREB phosphorylation; and NCS-Rapgef2/ERK-dependent neuritogenesis. In hβAR2-expressing cells, isoproterenol initiated Epac2/p38-dependent growth arrest and PKA-dependent CREB phosphorylation, but not NCS-Rapgef2/ERK-dependent neuritogenesis. We created cognate hβAR1- and hβAR2-expressing NS-1 cell lines that stably co-expressed a luminescent cAMP biosensor (Emery et al., Peptides, 79: 39, 2016) to compare the profiles of cAMP elevation induced by isoproterenol upon activation of either adrenoceptor. Maximum

cyclic AMP activation by isoproterenol occurred within about 10 min and decreased thereafter to less than half-maximal in hβAR2-expressing cells, while cyclic AMP elevation was persistent at maximal levels for at least 40 in in hβAR1-expressing cells. We conclude that there is an inverse relationship between adrenergic receptor desensitization, and engagement of NCS-Rapgef2 of sufficient duration to support sustained activation of ERK, coupled to neuritogenesis, in NS-1 cells. NCS-Rapgef2-independent activation of ERK by hβAR2, as reported by ourselves (Emery et al. Sci. Signaling 6(281), ra51, 2013) and others (Lefkowitz and Shenoy, Science 308: 512, 2005), is likely to occur in a cellular compartment that, unlike NCS-Rapgef2-dependent ERK activation, is not coupled to downstream immediate early genes such as Egr1/Zif268 required for neuritogenesis in PC12/NS-1 cells (Ravni et al., Mol. Pharmacol. 73: 1688, 2008).

Disclosures: L.E. Eiden: None. A.C. Emery: None. C. Westover: None. W. Xu: None. M.V. Eiden: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.21/H8

Topic: F.04. Stress and the Brain

Support: Commonwealth Health Research Board of Virginia Award 34-10 to JKS, JHP, KFS, JJW

Title: Locomotor activity in vesicular monoamine transporter 1 knockout mice

Authors: *J. K. STEWART[1], K. A. WEBSTER[2], J. J. WINDLE[3,4], K. FISCHER-STENGER[4], Y. GENG[4], E. C. GONYE[4], J. H. PORTER[2];
[1]Biol., [2]Psychology, [3]Human and Mol. Genet., VA Commonwealth Univ., Richmond, VA; [4]Biol., Univ. of Richmond, Richmond, VA

Abstract: Vesicular monoamine transporter 1 (VMAT1) in humans and rodents is the primary vesicular transporter in adrenal medulla, but the VMAT1 gene also is expressed in specific areas of brain. Association of the VMAT1 gene SLC18A1 with psychiatric disorders motivated investigation of behavioral differences in VMAT1-/- and VMAT1+/+ (littermate control) mice in this study. Previously, we reported delayed acquisition of learning in an autoshaping task and reduced prepulse inhibition in VMAT1-/- mice at age 4-5 months compared to VMAT1+/+ mice of the same age. Previous immunohistochemical data also confirmed VMAT1 deficiency in the adrenals of VMAT1-/- mice as compared to VMAT1+/+ mice. We now report reduced locomotor activity in VMAT1 knockout mice, but not in wildtype control mice, when the

animals were exposed to mild food deprivation (food reduced to maintain 85% body weight) at 4-5 months of age. Under free-feeding conditions there were no significant differences in wild type and knockout mice in horizontal ambulation or thimotaxia (time spent near the perimeter of the cage), and the knockout mice actually displayed displayed significantly more vertical rearing than wildtype mice. Food restriction significantly reduced horizontal locomotion, thigmotaxia, and vertical rearing in knockout mice but had no significant effects on any of these locomotor behaviors in wildtype mice. We hypothesize that deficiencient storage and release of adrenal epinephrine in VMAT1-/- mice limited metabolic responses of these mice to food deprivation, which impacted locomotor activity. Nevertheless, we cannot rule out central neural effects of VMAT1 deficiency that may affect locomotion responses to the mild stress of food deprivation.

Disclosures: J.K. Stewart: None. K.A. Webster: None. J.J. Windle: None. K. Fischer-Stenger: None. Y. Geng: None. E.C. Gonye: None. J.H. Porter: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.22/H9

Topic: B.05. Transporters

Support: NIH Grant R01DA02121

Title: Mechanisms of vesicular monoamine transporter-2 degradation

Authors: *E. K. STACHOWSKI[1], D. O. SAMBO[2], H. KHOSHBOUEI[2], G. E. TORRES[3]; [1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Neurosci., [3]Dept. of Pharmacol. and Therapeut., Univ. of Florida, Florida, FL

Abstract: The vesicular monoamine transporter-2 (VMAT2) is essential for monoaminergic homeostasis, packaging monoamines into vesicles for exocytosis. VMAT2 also has a neuroprotective role, given that free monoamines in the cytosol have the potential to auto-oxidize and form dangerous free radicals that can ultimately contribute to oxidative stress. There are several pieces of evidence that low VMAT2 expression increases vulnerability to toxic insults, while VMAT2 overexpression is protective. Despite the importance of VMAT2, little is known about mechanisms involving VMAT2 degradation. The balance of protein synthesis and degradation is vital to maintain normal cellular function and dysregulation of degradation systems can be detrimental to neurons, as evidenced in disorders such as Parkinson's disease. There are two primary organelles responsible for degradation in mammalian cells: the lysosome and the 26S proteasome. Although synaptic vesicle proteins are thought to be degraded by the

lysosome via the endo-lysosomal pathway, little direct evidence exists for this. Furthermore, it remains unclear if these proteins can be differentially regulated or degraded at the vesicle. Utilizing an *in vitro* PC12 cell model stably expressing VMAT2-GFP, we examined whether VMAT2 is degraded primarily through the UPS (ubiquitin-proteasome system) or by the lysosome. Inhibition of the proteasome, but not the lysosome results in accumulation of VMAT2, as assessed by immunoblot and immunocytochemistry. Furthermore, inhibiting the lysosome has no impact on VMAT2 half-life, strongly indicating lysosome activity does not mediate VMAT2 degradation. Our data also suggests this UPS-related degradation of mature VMAT2 is a process independent of endoplasmic-reticulum-associated degradation (ERAD; a quality control mechanism likely involved with degradation of immature VMAT2). Our results strongly indicate that VMAT2 is not degraded via the endo-lysosomal pathway as traditionally believed, but is likely degraded by the UPS system.

Disclosures: E.K. Stachowski: None. D.O. Sambo: None. H. Khoshbouei: None. G.E. Torres: None.

Poster

218. Monoamine Signaling

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 218.23/H10

Topic: B.05. Transporters

Support: University of Saint Joseph, Teaching and Learning Center Research Grant

University of Saint Joseph, Graduate Research Grant

Title: Fluoxetine induced behavioral alterations in *Drosophila melanogaster*

Authors: N. ROSA, O. GROVER, M. ASTHA, *P. G. MANDELA;
Dept. of Pharmaceut. Sci., Univ. of St. Joseph, Hartford, CT

Abstract: The overall goal of the project is to develop a reliable and valid model of *Drosophila melanogaster* (fruit fly) for screening selective serotonin reuptake inhibitors (SSRIs). Depression is a wide spread and debilitating illness often treated by targeting the monoaminergic neurotransmitter transporters. Inhibition of serotonin transporter (SERT) by selective serotonin reuptake inhibitors (SSRIs) is the leading strategy employed to treat depression and related neuropsychiatric illness. *Due to the restrictions imposed on the current animal models of depression, Drosophila melanogaster* could be a potential alternative animal model for SSRI screening. Flies have a sophisticated nervous system, containing 40 serotonergic neurons that

facilitate many complex behaviors.
Flies synthesize biogenic amines, pre-synaptic transporters, receptors and signaling pathways that mediate serotonin effects. *dSERT* gene encodes a protein with a topological organization that is analogous to other members of the SLC6 family of transporters including human serotonin transporter (hSERT). Although dSERT facilitates rapid reuptake of 5HT, it differs in pharmacology and substrate specificity in comparison to its mammalian orthologs. However it is not clearly understood how these difference in pharmacological differences would translate into behavioral changes. We embarked on a project to identify behavioral changes that results from selective serotonin reuptake inhibitor (SSRI) treatment. We plan to use these behavioral alterations as a repertoire to screen new SSRI's. We have used imipramine, fluoxetine and bupropion treatments for our behavioral studies. Our results indicate that imipramine was least effective and fluoxetine was the most effective drug in altering behavior in Drosophila Melanogaster.

Disclosures: N. Rosa: None. O. Grover: None. M. Astha: None. P.G. Mandela: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.01/H11

Topic: B.02. Ligand-Gated Ion Channels

Support: BBSRC grant BB/L001977/1

NIH grant R01MH060252

Title: The development of subtype-selective negative allosteric modulators of the NMDA receptor for the study of synaptic plasticity and associated diseases.

Authors: *E. BURNELL[1], K. SAPKOTA[2], M. IRVINE[1], R. THATCHER[1], G. CULLEY[1], A. VOLIANSKIS[3], Z. BORTOLOTTO[1], G. L. COLLINGRIDGE[1,4,5], D. T. MONAGHAN[2], D. E. JANE[1];
[1]Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [2]Dept. of Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Barts and The London Sch. of Med. and Dent., Queen Mary Univ. of London, London, United Kingdom; [4]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [5]Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: *N*-Methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that consist mainly of tetrameric combinations of two subunit families, GluN1 and GluN2A-D. NMDARs are key mediators of synaptic plasticity, while over-activation of NMDARs is implicated in several neurological disorders. Attempts to develop competitive NMDAR antagonists and high affinity channel blockers to treat these disorders have been largely unsuccessful due to side effects associated with complete block of NMDAR function. Current research is driven by the need to develop selective NMDAR antagonists to (1) elucidate the function of the different receptor subtypes in synaptic plasticity, and (2) provide viable therapeutics for neurological diseases. Allosteric receptor sites typically have less amino acid conservation, and thus offer good targets for development of GluN2 subunit selective ligands. Additionally, negative allosteric modulators can function as partial antagonists, which offer better clinical safety compared to full antagonists. Recently work has investigated the GluN2A-D subunits as potential targets for the development of novel subunit selective negative allosteric modulators. A previous study by our group (Costa et al. 2012, Neuropharmacology 62:1730-36) highlighted that compounds based on 3-hydroxy-7-phenyl-2-naphthoic acid (UBP617) had partial antagonist effects on GluN2A and GluN2B but fully blocked GluN2C and GluN2D. Building on this work, we now present the results of our latest structure-activity relationship studies. By varying the substituents on the aromatic moiety at the 7-position of the 2-naphthoic acid ring we have developed a series of novel compounds that induce sub-maximal inhibition of the NMDAR and show a preference for inhibition of GluN2D. Such compounds may have an advantage over previous generations of antagonists, as they are partial antagonists and therefore may block the pathological response without interfering in normal CNS function. In summary, we have developed a series of novel, subunit-selective NMDAR negative allosteric modulators with partial antagonist activity. These compounds are likely to be valuable for studies of NMDAR function and may be valuable leads for the treatment of NMDAR hyperfunction-associated disorders, such as chronic pain.

Disclosures: E. Burnell: None. K. Sapkota: None. M. Irvine: None. R. Thatcher: None. G. Culley: None. A. Volianskis: None. Z. Bortolotto: None. G.L. Collingridge: None. D.T. Monaghan: None. D.E. Jane: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.02/H12

Topic: B.02. Ligand-Gated Ion Channels

Title: Activation of light sensitive adenylyl cyclase changes the time course of long-term potentiation at the Schaffer collateral - CA1 synapse in mouse hippocampal slices

Authors: *A. S. AVILA[1], J. GEORGIOU[1], K. OKAMOTO[2,1], G. L. COLLINGRIDGE[3,1]; [1]Mount Sinai Hosp., Sinai Hlth. Syst., Toronto, ON, Canada; [2]Mol. Genet., [3]Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: Long-term potentiation (LTP) is a synaptic property regarded as one of the underlying mechanisms of learning and memory. In the hippocampus, and specifically at the Schaffer collateral - CA1 synapse, LTP can be induced by multiple rounds of theta burst stimulation (TBS). Importantly, the interval between these rounds of TBS determines the molecular pathway that is recruited during synaptic plasticity. Specifically, multiple, spaced TBS episodes favour the activation of a protein kinase A (PKA)-dependent LTP. In the present study, we have begun to study the spatial-temporal consequences of cAMP generation in CA1 synaptic plasticity.
We generated and analysed three light-sensitive mouse lines that each expressed Photoactivatable Adenylyl Cyclase (PAC) within the hippocampal subregions in a unique pattern and level. In our initial electrophysiological experiments, we combined TBS with an overlapping blue-light photo-activation of PAC, and found that PAC-Line1 showed an enhanced potentiation of CA1 synaptic responses that persisted for up to an hour after TBS.
The results of this study show that photo-activation of light sensitive adenylyl cyclase during TBS-induced LTP induction changes the extent and time course of LTP. The data are in agreement with the idea that cAMP could act as a primer for the establishment of PKA-dependent LTP. In future experiments we will manipulate the timing and spatial localization of PAC photoactivation to analyse the detailed spatial-temporal requirements and molecular effects downstream of cAMP.

Disclosures: A.S. Avila: None. J. Georgiou: None. K. Okamoto: None. G.L. Collingridge: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.03/H13

Topic: B.02. Ligand-Gated Ion Channels

Support: GACR 14-09220P

Title: Distinct structural determinants within the GluN2C subunit regulate the surface delivery of NMDA receptors

Authors: *M. KANIAKOVA, K. LICHNEROVA, K. SKRENKOVA, L. VYKLICKY, M. HORAK;
Inst. of Physiol. CAS, Praha, Czech Republic

Abstract: The *N*-methyl-D-aspartate receptors (NMDARs) are ion channels that play an essential role in glutamatergic neurotransmission. The functional NMDARs are heterotetramers composed of GluN1, GluN2A-D and/or GluN3A-B subunits. All NMDAR subunits share the same structural features including four membrane-spanning segments (M1-M4), an extracellular N-terminus and an extracellular loop between M3 and M4, and an intracellular C-terminus. It is expected that the numbers and types of the NMDARs present at neuronal cell surface are regulated at multiple levels including their synthesis, subunit assembly, endoplasmic reticulum (ER) processing, intracellular trafficking and degradation. Although distinct regions within the GluN1 and GluN2 subunits regulate ER processing and the trafficking of functional NMDARs, subunit-dependent differences among the GluN2 subunit types which contribute to the early NMDAR processing have not yet been studied in detail. Here, we investigated the mechanisms that underlie the trafficking of GluN1/GluN2C receptors. By combining confocal microscopy and electrophysiology in heterologous cells and cultured cerebellar granule cells, we found that the surface expression of GluN1/GluN2C receptors is reduced compared to GluN1/GluN2A and GluN1/GluN2B receptors. Furthermore, using mutated GluN2C subunits, we identified three distinct regions in the GluN2C subunit (within the N-terminus, M3 domain, and C-terminus) that regulate the surface expression of GluN2C-containing NMDARs. We conclude that the GluN2C subunit controls the early processing of functional NMDARs by a unique combination of regulatory mechanisms.

Disclosures: M. Kaniakova: None. K. Lichnerova: None. K. Skrenkova: None. L. Vyklicky: None. M. Horak: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.04/H14

Topic: B.02. Ligand-Gated Ion Channels

Support: Grant Agency of the Czech Republic (14-02219S)

Title: The characterization of N-glycans on cerebellar and recombinant NMDA receptor subunits

Authors: *M. HORAK, K. LICHNEROVA, M. KANIAKOVA, K. SKRENKOVA;
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Abstract: *N*-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors which form heterotetrameric complexes composed mostly of GluN1 and GluN2 subunits. We examined the *N*-glycan composition of cerebellar and recombinant NMDARs using deglycosylating enzymes, lectin-based biochemistry, and electrophysiology. Our results revealed that cerebellar NMDARs associate with 23 different lectins that have unique specificities for glycan structures. Using electrophysiology, we found that three specific lectins altered the functional properties of NMDARs expressed in either cultured cerebellar granule cells or HEK293 cells. Finally, using biochemistry we found that most of the putative *N*-glycosylation sites in GluN1 and GluN2B subunits are occupied by glycans. Together, these data shed light on the glycan composition of NMDARs, revealing potential targets for the development of novel therapeutic approaches.

Disclosures: M. Horak: None. K. Lichnerova: None. M. Kaniakova: None. K. Skrenkova: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.05/H15

Topic: B.02. Ligand-Gated Ion Channels

Support: GACR 14-02219S

GAUK 1520-243-227060

Title: The role of N-glycosylation in trafficking of NMDA receptors

Authors: *K. SKRENKOVA[1,2], K. LICHNEROVA[1], M. KANIAKOVA[1], S. P. PARK[3], Y.-X. WANG[4], R. S. PETRALIA[4], Y. H. SUH[3], M. HORAK[1];
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Abstract: *N*-methyl-D-aspartate receptors (NMDARs) play critical roles in excitatory neurotransmission, synaptic plasticity, and excitotoxicity. Surface expression of NMDA receptors is regulated at multiple levels, which include processing in the endoplasmic reticulum (ER), intracellular trafficking and processing in the Golgi apparatus, internalization, recycling, and degradation. Previous studies have shown that posttranslational modifications as

phosphorylation and palmitoylation regulates NMDA receptor trafficing. However, the role of *N*-glycosylation, one of the most common posttranslational modifications, in regulating NMDAR processing has not been studied in detail. We examined whether the *N*-glycosylation sites present in GluN1, GluN2A, GluN2B and GluN3A receptors are necessary for surface delivery of the receptors. Using a combination of microscopy, biochemistry, and electrophysiology in heterologous cell lines and primary hippocampal neurons, we found that there are two critical *N*-glycosylation sites within the GluN1 subunit (Asn-203 and Asn-368) which are required for releasing of NMDARs from ER. No single *N*-glycosylation site within the GluN2A, GluN2B and GluN3A subunits appear to be essential for the surface delivery of NMDARs. Furthermore, we found that removing *N*-glycans from native NMDAR saltered the receptor affinity for glutamate. Our results suggest a novel mechanism by which neurons ensure that postsynaptic membranes contain sufficient numbers of functional NMDARs.

Disclosures: K. Skrenkova: None. K. Lichnerova: None. M. Kaniakova: None. S.P. Park: None. Y. Wang: None. R.S. Petralia: None. Y.H. Suh: None. M. Horak: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.06/H16

Topic: B.02. Ligand-Gated Ion Channels

Support: National Institute of Health P20GM103546

small research grant from Mnemosyne Pharmaceuticals

Title: Structural basis for negative allosteric modulation of GluN2A-containing NMDA receptors

Authors: *F. YI[1], T.-C. MOU[2,3], R. A. VOLKMANN[4], F. S. MENNITI[5], S. R. SPRANG[2,3], K. B. HANSEN[1,2];
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Abstract: NMDA receptors mediate slow excitatory synaptic transmission and regulate synaptic plasticity in the central nervous system, but their dysregulation is also implicated in numerous psychiatric and neurological disorders. Subunit- selective modulators that only target NMDA receptor subtypes in specific brain regions or cell types could have unique therapeutic potentials.

We evaluated three negative allosteric modulators (NAMs), TCN-201, MPX-004 and MPX-007, all of which selectively inhibit GluN2A-containing NMDA receptors. The negative allosteric modulation is sensitive to the glycine concentration, and mutant GluN1 subunits, in which an engineered disulfide bond locks the ligand binding domain (LBD) in the closed agonist-bound conformation, abolish NAM inhibition. This experiment suggests that NAMs inhibition of GluN2A-containing NMDA receptors stabilizes the *apo*-state of the GluN1 LBD, which is incapable of triggering channel gating. Crystal structures of GluN1/GluN2A LBDs in complex with NAMs show the modulatory binding site in the interface between GluN1 and GluN2A subunits. We also uncover intra- and inter-subunit structural changes induced by NAM binding that correlate with structural and pharmacological differences between the NAMs. This work provides structural and mechanistic insight to allosteric NMDA receptor inhibition, thereby facilitating the development of novel classes NMDA receptor modulators as treatments in neurological diseases.

Disclosures: **F. Yi:** None. **T. Mou:** None. **R.A. Volkmann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Luc Therapeutics, Inc. **F.S. Menniti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Luc Therapeutics, Inc.. **S.R. Sprang:** None. **K.B. Hansen:** 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; Luc Therapeutics, Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Luc Therapeutics, Inc.. F. Consulting Fees (e.g., advisory boards); Luc Therapeutics, Inc..

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.07/H17

Topic: B.02. Ligand-Gated Ion Channels

Title: X11 and X11L regulate the non-synaptic NMDA receptor localization

Authors: *R. MOTODATE, Y. SAITO, T. SUZUKI;
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Abstract: X11/Mint1 (Munc18-1 interacting protein 1), X11-like (X11L/Mint2), and X11-like 2 (X11L2/Mint3) are adaptor proteins whose are highly conserved evolutionarily. The X11 family proteins comprise a PTB (Phosphotyrosine binding) and two PDZ (PSD95/discs large/ZO-1)

domains in their carboxyl terminal half along with a poorly conserved N-terminal region [1]. X11 is predominantly and X11L is specifically expressed in brain neurons, while X11L2 expresses ubiquitously in many tissues. Previous reports indicate that X11s regulate localization of NMDA receptor and AMPA receptor *in vitro* or in *C. elegans* [2-4], suggesting that X11 and X11L play an important role in the postsynaptic function, but little is known for their exact roles in the postsynapse *in vivo*. Here, we analyzed X11/X11L double-deficient mice (X11/X11L DKO) to evaluate the role of X11 and X11L in postsynapse. Postsynaptic density (PSD) fraction and non-PSD fraction of mouse cortex and hippocampus regions were prepared with sucrose gradient centrifugation. The amount of NMDA receptor subunits were not changed in PSD, but non-synaptic NMDA receptor subunits were altered in X11/X11L DKO mouse. To evaluate of NMDA receptor function, I measured the activity of CREB in nuclear fraction from mouse cortex and hippocampus. In X11/X11L DKO, the CREB activity was changed. These data suggest that X11 and X11L regulate the non-synaptic NMDA receptor function through the regulation of their localization.

<References>

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Disclosures: R. Motodate: None. Y. Saito: None. T. Suzuki: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.08/H18

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS intramural Research program (K.W.R.)

US NIMH (R.A.N.)

Title: $STEP_{61}$ differentially affects NMDARs and AMPARs

Authors: *S. WON[1], S. INCONTRO[2], R. NICOLL[2], K. ROCHE[1];
[1]NINDS, NIH, Bethesda, MD; [2]Dept. of Cell. and Mol. Pharmacol., Univ. of California, San Francisco, CA

Abstract: NMDARs and AMPARs are expressed throughout the nervous system and responsible for a variety of processes in mammalian brain including neuronal development, excitatory neurotransmission, learning and memory, and synaptic plasticity. NMDARs are heterotetramers formed between GluN1 and GluN2 (A-D) subunits. Calcium influx through NMDARs is thought to be critical in synaptic plasticity, a cellular mechanism for learning and memory. AMPARs are composed of four subunits (GluA1-4), which combine to form tetramers, mostly heterotetrameric. In addition AMPARs conduct the majority of fast synaptic transmission throughout the CNS and their modulation is the critical mechanism that underlies much of the plasticity at excitatory transmission. The precise regulation of NMDAR and AMPAR expression, trafficking, and localization is critical for proper neuronal function. Phosphorylation regulates surface and synaptic expression of NMDARs and AMPARs. In terms of phosphorylation of NMDARs and AMPARs, serine and threonine phosphorylation of NMDARs and AMPARs has been well studied, whereas the potential role of tyrosine phosphorylation of NMDARs and AMPARs has not received as much attention. It has been reported that tyrosine phosphorylation by Src family kinases regulates NMDAR and AMPAR trafficking via endocytosis. STEP (STriatal-Enriched protein tyrosine Phosphatase) is a brain-specific non-receptor tyrosine phosphatase. There have been previous studies that STEP binds to NMDARs and regulates their surface expression via dephosphorylation of Y1472 within endocytic motif of GluN2B. Using STEP knockdown, we now have elucidated a novel mechanism of $STEP_{61}$ regulation of GluN2B-containing NMDARs including regulation of extrasynaptic NMDARs. Indeed we find that STEP and PSD-95 play opposing roles with PSD-95 stabilizing NMDARs and destabilizing STEP, whereas STEP dephosphorylates GluN2B and reduces GluN2B-containing NMDARs via endocytosis. In the case of AMPARs, little is known about potential mechanisms of STEP regulating surface or synaptic expression of AMPARs. We find that $STEP_{61}$ binds to AMPARs in rat cultured cortical neurons and mouse hippocampus synaptosomes. In contrast to previous reports, we have examined AMPAR subunit-specific binding to $STEP_{61}$ showing differential regulation of GluA2 vs. GluA1. We have also investigated AMPAR vs. NMDAR subcellular expression in STEP KO mice showing differential regulation. Taken together, these results reveal a novel mechanism by which $STEP_{61}$ differentially regulates NMDARs and AMPARs.

Disclosures: S. Won: None. S. Incontro: None. R. Nicoll: None. K. Roche: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.09/H19

Topic: B.02. Ligand-Gated Ion Channels

Support: BMRC09/1/21/19/617

Title: Ontogenic profile and synaptic distribution of GluN3 proteins in rat brain and hippocampal neurons

Authors: *C.-M. LOW[1], S.-L. WEE[2], C. TAN[3], Y.-P. CHEONG[3], S. KHANNA[4];
[2]Pharmacol., [1]Yong Loo Lin Sch. of Med., Singapore, Singapore; [3]Pharmacol., [4]Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: N-Methyl-D-aspartate receptors are localized to synaptic and extrasynaptic sites of dendritic spines and shafts. Here, the ontogenic profiles of GluN3A and GluN3B subunits in the rat brain were determined. A developmental switch from GluN3A to GluN3B proteins was detected within the first two postnatal weeks of crude synaptosomes prepared from forebrain and midbrain. Further fractionation of crude synaptosomes revealed the preferential localization of GluN3B to synaptic regions from P7 onwards. Immunolabeling and biochemical fractionation of rat P7 cultured hippocampal neurons showed that GluN3B was predominantly at synaptic sites. Unlike GluN2A and GluN2B, both GluN3 subunits were mostly associated with peripheral components of the postsynaptic density (PSD) rather than its core. When considering the non-PSD fraction, the overall extrasynaptic/synaptic spatial profile of GluN3B differed from GluN3A. Heterologous expression of GluN3B with GluN1 in HEK293FT cells could not be co-immunoprecipitated with PSD-95 unless co-expressed with a PSD-95-interacting GluN2 subunit, suggesting that anchoring of GluN3B at synaptic sites may require co-assembly with another scaffold-interacting NMDAR subunit.

Disclosures: C. Low: None. S. Wee: None. C. Tan: None. Y. Cheong: None. S. Khanna: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.10/H20

Topic: B.02. Ligand-Gated Ion Channels

Support: R01-HL-116916

Title: The nuclear localization of NMDA receptor in human pulmonary artery smooth muscle cells

Authors: Y. DONG, *D. R. LYNCH;
Pediatrics and Neurol., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: NMDA receptor is ligand-gated ion channel which plays important roles in synaptic plasticity, memory formation, neural development, and many neurological disorders. NMDA receptor is also present in peripheral non-neuronal tissues including lung where NMDA receptor activation is implicated in airway contraction, lung injury and pulmonary arterial contractility. We previously showed that NMDA receptor is expressed in human pulmonary artery smooth muscle cells both in vivo and in vitro. Here we present the evidence that in addition to the plasma membrane localization, NMDA receptor (NR1/NR2A) is also localized in the nucleus of human pulmonary artery smooth muscle cells. Immunocytochemistry using antibodies against either N-terminus or C-terminus of NR2A subunit identifies the nuclear localization of NR2A in cultured human pulmonary artery smooth muscle cells but not in human embryonic kidney 293 cells transfected with NR1 and NR2A subunit. In order to confirm this result, we performed subcellular fractionation and found that both full length NR1 and NR2A subunit appeared in the nuclear fraction of human pulmonary artery smooth muscle cells. In contrast, neither NR1 nor NR2A subunit was detected in the nuclear fraction of rat cortical neurons. These results indicate that the subcellular localization of NMDA receptor is cell type specific. As functional NMDA receptor consists of both NR1 and NR2 subunit, the presence of full length NR1 and NR2A subunit in the nucleus of human pulmonary artery smooth muscle cells suggests that NMDA receptor in the nucleus of human pulmonary artery smooth muscle cells is potentially functional. The biological significance of NMDA receptor in the nucleus of human pulmonary artery smooth muscle cells remains to be investigated.

Disclosures: Y. Dong: None. D.R. Lynch: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.11/H21

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH- 5T32DA7234-24

DA07304

Title: Adaptation of NMDARs during exposure to HIV-1 Tat requires GluN2A-subunit signalling

Authors: *M. GREEN[1], S. THAYER[2];
[1]Neurosci., [2]pharmacology, Univ. of Minnesota, Minneapolis, MN

Abstract: HIV-associated neurocognitive disorders (HAND) affect about half of HIV-infected patients. Infected non-neuronal cells in the brain release neurotoxic factors such as transactivator of transcription (Tat) which potentiate neuronal NMDA receptor (NMDAR) function. Changes in NMDAR function regulate synaptic changes observed following exposure to HIV proteins such as Tat, which may underlie cognitive impairment in HAND patients. Here we used patch clamp recordings to measure NMDAR-mediated currents to investigate the mechanism of changes in NMDAR function. Exposure to Tat for 4 or 16 h potentiated NMDA-evoked whole cell current and increased the NMDAR:AMPAR ratio of evoked EPSCs. These changes adapted back to baseline amplitudes after 24 h exposure. Inhibition of GluN2A-containing NMDARs with the GluN2A-preferring antagonist, NVP-AAM 007, prevented this adaptation from occurring. Inhibition of GluN2B-containing NMDARs with the GluN2B-preferring antagonist, ifenprodil, did not prevent adaptation of NMDARs. Furthermore, inhibition of the E3 ubiquitin ligase, Mdm2, pharmacologically with nutlin-3, or genetically by expression of p14-ARF, also prevented adaptation of NMDARs. Inhibition of Mdm2 may prevent proteasomal degradation of PSD-95, a scaffolding protein that binds NMDARs and modulates their function. Future studies will investigate changes in NMDAR level and location by using live cell imaging to track expression of NMDAR subunits during exposure to Tat. Adaptation to the continued presence of Tat may be a neuroprotective response that also contributes to the synaptic loss that is a hallmark of HAND.

Disclosures: M. Green: None. S. thayer: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.12/H22

Topic: B.02. Ligand-Gated Ion Channels

Title: An in-vivo target engagement assay for NMDA receptor positive allosteric modulation

Authors: *M. WEBER[1], J. ELSTROTT[2], Y. CHEN[5], T.-M. WANG[1], Z. JIANG[1], B. LIEDERER[3], G. DESHMUKH[3], C. CHAN[3], B. SELLERS[4], M. VOLGRAF[4], J. SCHWARZ[4], D. HACKOS[1], R. WEIMER[2], M. SHENG[1], K. SCEARCE-LEVIE[6], J. HANSON[1];
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Abstract: Positive allosteric modulators (PAMs) of NMDA receptors may have therapeutic potential for neuropsychiatric & degenerative disorders. One critical step towards advancing

these compounds to the clinic is to demonstrate target engagement in-vivo. Since NMDA receptor antagonists like MK-801 or SDZ220,581 are known to increase locomotor activity, we reasoned that locomotor measures could also serve to demonstrate in-vivo target engagement for NMDA receptor PAMs. Using drug-drug interaction and pharmacogenetic approaches, we show that GNE-0723, a selective, potent and brain-available PAM that acts on GluN2A subunit-containing NMDA receptors, altered locomotor and rearing activity in a manner indicative of on-target activity. Assays of locomotor activity can serve as simple, yet reliable in-vivo indicators of target engagement for GluN2A NMDA receptor PAMs, and help accelerate early in-vivo profiling of putative novel therapeutics.

Disclosures: **M. Weber:** A. Employment/Salary (full or part-time): Genentech. **J. Elstrott:** A. Employment/Salary (full or part-time): Genentech. **Y. Chen:** Other; Employee of Genetech when experiments were conducted. **T. Wang:** A. Employment/Salary (full or part-time): Genentech. **Z. Jiang:** A. Employment/Salary (full or part-time): Genentech. **B. Liederer:** A. Employment/Salary (full or part-time): Genentech. **G. Deshmukh:** A. Employment/Salary (full or part-time): Genentech. **C. Chan:** A. Employment/Salary (full or part-time): Genentech. **B. Sellers:** A. Employment/Salary (full or part-time): Genentech. **M. Volgraf:** A. Employment/Salary (full or part-time): Genentech. **J. Schwarz:** A. Employment/Salary (full or part-time): Genentech. **D. Hackos:** A. Employment/Salary (full or part-time): Genentech. **R. Weimer:** A. Employment/Salary (full or part-time): Genentech. **M. Sheng:** A. Employment/Salary (full or part-time): Genentech. **K. Scearce-Levie:** Other; Employee of Genentech when experiments were conducted. **J. Hanson:** A. Employment/Salary (full or part-time): Genentech.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.13/H23

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant MH045817

NIH Grant MH105056

Title: Inhibition of NMDA receptors by the uncharged form of memantine

Authors: ***N. G. GLASGOW**, J. W. JOHNSON;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: NMDA receptors (NMDARs) are a class of ionotropic glutamate receptors with unique biophysical properties including high Ca^{2+} permeability and highly voltage-dependent Mg^{2+} block. Ca^{2+} influx through NMDARs is critical for many types of synaptic plasticity and learning and memory. In contrast, aberrant activation of NMDARs is implicated in many central nervous system disorders. The clinical benefits of the NMDAR open channel blockers memantine and ketamine have spurred hope that NMDARs can serve as useful therapeutic targets. Although NMDAR inhibition by memantine and ketamine share similar features, their clinical effects vary substantially. Further understanding of how each drug inhibits NMDARs may yield insight into their clinical utility. One difference between the drugs' inhibition of NMDARs is that memantine, but not ketamine, inhibits at two sites on the receptor. Both drugs bind at a site deep within the channel pore that overlaps with the Mg^{2+} binding site (the deep site). Only memantine binds at a second site thought to be near the channel gate (the second site). Due to difficulties isolating inhibition at the deep and second sites, it is unclear what role binding of memantine, but not ketamine, to the second site may play in the drugs' differential inhibitory actions. Despite these difficulties, binding at the second site has been correlated with several characteristics of memantine inhibition, including: (a) the ability to bind in the absence of agonist; (b) paradoxically slow unbinding kinetics combined with a high IC_{50}; and (c) minimal voltage dependence. We tested the hypothesis that the uncharged form of memantine binds to the second site on NMDARs, which offers a parsimonious explanation for these characteristics because uncharged memantine: (a) is relatively hydrophobic, and so may be able to access the channel in the absence of agonist; (b) may bind with a very low IC_{50} (consistent with slow unbinding kinetics) that appears much higher at physiological pH where only ~0.1% of memantine is uncharged; and (c) should be insensitive to membrane voltage. To test our hypothesis we compared memantine inhibition of NMDARs at pH 7.2 with inhibition by the same total memantine concentration at pH 9.0, a pH at which uncharged memantine concentration is ~60-fold greater. We found that inhibition at the second site is greatly enhanced at pH 9.0. We also found that channel block by Mg^{2+} occluded memantine inhibition at the second site, suggesting a complex interaction between memantine and Mg^{2+} binding. Modification of pH provides a useful tool to investigate the distinct contributions of the uncharged and charged forms of memantine to NMDAR inhibition.

Disclosures: N.G. Glasgow: None. J.W. Johnson: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.14/H24

Topic: B.02. Ligand-Gated Ion Channels

Title: A novel NMDAR positive allosteric modulator that potentiates currents via interaction with the GluN1 transmembrane domain

Authors: *T.-M. WANG[1], D. H. HACKOS[1], B. M. BROWN[1], B. D. SELLERS[2], P. J. LUPARDUS[3], H. J. A. WALLWEBER[3], E. WONG[1], M. VOLGRAF[2], J. B. SCHWARZ[2], J. E. HANSON[1];
[1]Neurosci., [2]Discovery Chem., [3]Structural Biol., Genentech, South San Francisco, CA

Abstract: Ionotropic glutamate receptors (iGluRs) mediate fast excitatory neurotransmission and are key drug targets in the nervous system. While diverse pharmacological tools have yielded insight into iGluR extracellular domain function, less is known about function of the transmembrane domain (TMD). We have discovered a novel NMDAR positive allosteric modulator, GNE-9278, that stabilizes a high open probability state once receptors are activated. An alanine scan identified residues in the TMD that are critical to GNE-9278 potentiation. Remarkably, swapping 3 critical residues in the GluN1 pre-M1 region from NMDARs to AMPARs was sufficient to confer GNE-9278 sensitivity to AMPARs. Mutation of a single GluN1 M3 residue could convert GNE-9278 effects from positive to negative allosteric modulation. Consistent with a unique mode of TMD action, NMDARs become insensitive to extracellular domain pharmacology during GNE-9278 modulation. These results reveal a new modulatory site in NMDARs and suggest the broader potential of iGluR modulation via the TMD.

Disclosures: **T. Wang:** A. Employment/Salary (full or part-time): Genentech. **D.H. Hackos:** A. Employment/Salary (full or part-time): Genentech. **B.M. Brown:** A. Employment/Salary (full or part-time): Genentech. **B.D. Sellers:** A. Employment/Salary (full or part-time): Genentech. **P.J. Lupardus:** A. Employment/Salary (full or part-time): Genentech. **H.J.A. Wallweber:** A. Employment/Salary (full or part-time): Genentech. **E. Wong:** A. Employment/Salary (full or part-time): Genentech. **M. Volgraf:** A. Employment/Salary (full or part-time): Genentech. **J.B. Schwarz:** A. Employment/Salary (full or part-time): Genentech. **J.E. Hanson:** A. Employment/Salary (full or part-time): Genentech.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.15/H25

Topic: B.02. Ligand-Gated Ion Channels

Title: The new hallucinogen, ephenidine, is an n-methyl-d-aspartate receptor antagonist.

Authors: *H. KANG[1], P. PARK[1], Z. A. BORTOLOTTO[1], G. L. COLLINGRIDGE[1,2,3], J. WALLACH[4], S. BRANDT[5], D. LODGE[1];
[1]Med. and Dent., Univ. of Bristol, Bristol, United Kingdom; [2]Univ. of Toronto, Toronto, ON, Canada; [3]Lunnenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada; [4]Univ. of the Sci., Philadelphia, PA; [5]Liverpool John Moores Unversity, Liverpool, United Kingdom

Abstract: Since misuse of arylcyclohexylamines, such as ketamine, 'Special K', has become illegal in many countries, research chemicals have been developed to replace the banned substances. Recently the diaryl ethylamine, ephenidine, has become popular among those seeking a dissociative agent. We have investigated this compound as a potential N-methyl-D-aspartate (NMDA) antagonist in electrophysiological and receptor binding assays. Using extracellular recording from rat hippocampal slices, 4 hour of exposure to 1 and 10 μM ephenidine caused 23 ± 3 % and 81 ± 3 % reductions respectively of the NMDA- receptor mediated field epsp but was without effect on that mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In whole cell patch clamp experiments using a magnesium-free artificial cerebro-spinal fluid (aCSF), 10 μM ephenidine caused a greater reduction of inward current relative to outward current resulting in a rectification factor of 2.3 at -40/+40 mV, which compares with the ratio of 1.0 at the same holding potentials in control aCSF. When compared with effects of ketamine, D-2-amino-5-phosphonopentanoate (D-AP5) and magnesium ions on the same electrophysiological parameters, ephenidine's profile was very similar to that of ketamine. In receptor binding studies of rat brain membranes, ephenidine had nanomolar potency versus ^{3}H-MK-801 but less affinity at other neural receptors. Our data show that ephenidine is a selective, potent and voltage-dependent NMDA receptor antagonist, which is likely to explain its dissociative hallucinogenic effects in man and to account for its popularity among the population of party-goers looking for a replacement for ketamine. It may, therefore, suffer from many of the adverse effects associated with ketamine.

Disclosures: H. Kang: None. P. Park: None. Z.A. Bortolotto: None. G.L. Collingridge: None. J. Wallach: None. S. Brandt: None. D. Lodge: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.16/H26

Topic: B.02. Ligand-Gated Ion Channels

Support: University of Genoa

Title: Functional interaction between presynaptic receptors on dopaminergic and noradrenergic nerve terminals of the rat central nervous system

Authors: *M. PADOLECCHIA, G. OLIVERO, J. CHEN, A. PITTALUGA, M. MARCHI, M. GRILLI;
Dept. of Pharm., Univ. of Genoa, Genova, Italy

Abstract: It is well known that cross-talk between receptors represent an important mechanism of neuromodulation and plasticity. Although, these interactions have been mostly localized post-synaptically, receptors cross-talk which involves common intracellular pathways have been reported to occur also at the presynaptic level (for a review see Marchi et al 2015). Neuronal nicotinic acetylcholine receptors (nAChRs) in the CNS are located mostly presynaptically and have been implicated in facilitating release of neurotransmitter. It has been shown that dopaminergic and noradrenergic axon terminals in the nucleus accumbens and hippocampus possess nAChRs mediating enhancement of dopamine (DA) and noradrenaline (NA) release respectively. We investigated whether nAChRs and N-methyl-D-aspartic acid (NMDA) receptors interact on the same nerve endings using rat (male Sprague–Dawley, 200–250 g) synaptosomes pre-labelled with [^{3}H]DA or [^{3}H]NA. The *in vitro* short-term pre-exposure of synaptosomes (10 min) to different concentrations of acetylcholine (from 0.01 μM to 10 μM) caused a significant reduction (maximal effect: -54 % at 10 μM) of the 100 μM NMDA-evoked [^{3}H]DA overflow in the rat nucleus accumbens. This inhibitory effect was completely abolished when nerve endings were pretreated with acetylcholine plus dihydro-b-erythroidine indicating a mechanism that involved the activation of β_2* nAChR subtypes. Conversely, the pre-exposure to acetylcholine in presence of atropine (0.1 μM) was ineffective; these results completely excluded a role of muscarinic receptors.
Moreover, the pre-treatment of synaptosomes with nicotine (from 0.01 μM to 30 μM) caused a significant reduction of the 100 μM NMDA-evoked [^{3}H]NA overflow in the rat hippocampus. The pre-exposure of synaptosomes to nicotine or acetylcholine also caused a marked reduction of the nicotine-induced [^{3}H]DA or [^{3}H]NA overflow (-85% and -91.2 % respectively) suggesting that these nAChRs could undergo an agonist-induced receptor desensitization. Washout in vitro experiment clearly demonstrated that the nicotinic modulation of NMDA receptors function was time dependent and completely restored after 16 min.
Our results show that the function of presynaptic NMDA receptors can be dynamically regulated in different neurons trough a brief incubation with endogenous and exogenous nicotinic agonists.
Marchi et al., (2015) *Front. Pharmacol., doi: 10.3389/fphar.2015.00089*

Disclosures: M. Padolecchia: None. G. Olivero: None. J. Chen: None. A. Pittaluga: None. M. Marchi: None. M. Grilli: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.17/I1

Topic: B.02. Ligand-Gated Ion Channels

Support: P20GM103546

Title: Structural and pharmacological evaluation of a class of GluN2A-selective competitive NMDAR antagonists with novel binding mode

Authors: *G. E. LIND[1], A. PINTO[2], L. TAMBORINI[2], P. CONTI[2], K. B. HANSEN[1];
[1]Univ. of Montana, Missoula, MT; [2]Univ. of Milan, Milan, Italy

Abstract: NMDA receptors (NMDARs) are important targets for glutamate, the primary excitatory neurotransmitter in the mammalian central nervous system. As such, NMDARs are also implicated in many neurological and psychiatric disorders. These heterotetrameric receptors are ligand-gated ion channels formed by glycine-binding GluN1 and GluN3 subunits and glutamate-binding GluN2 subunits. There are four GluN2 subunits (A-D) that endow distinct biophysical properties and physiological roles to the different NMDAR subtypes. Subunit-selective antagonists that distinguish NMDARs based on GluN2-subunit composition are useful pharmacological tools. Recent advances have produced multiple classes of NMDAR modulators with selectivity for the different GluN2 subunits. However, subunit-selective competitive antagonists that can be used as pharmacological tools to study NMDAR subunit composition in synaptic transmission are lacking. NVP-AAM077 is a competitive antagonist at the glutamate binding site of NMDARs and pharmacological evaluation showed that NVP-AAM077 binding affinity is ~5-fold higher for GluN2A- over GluN2B-containing NMDARs. We have developed a series of competitive glutamate-site antagonists with nanomolar binding affinities and using Schild analysis, we show that these compounds possess up to 15-fold greater binding affinity for N2A-containing receptors over N2B-containing receptors. This represents a major improvement over current competitive antagonists with preference for GluN2A. By solving the crystal structure for the GluN1/GluN2A ligand binding domain heterodimer in complex with four of these compounds, we have revealed a novel binding mechanism in the glutamate binding pocket. The crystal structures, in combination with electrophysiological recordings, have been used to evaluate the mechanisms of antagonist binding and subunit selectivity for the compounds. These studies expand our understanding of NMDAR pharmacology and provide a foundation for future ligand design of novel pharmacological tools or compounds with therapeutic potential.

Disclosures: G.E. Lind: None. A. Pinto: None. L. Tamborini: None. P. Conti: None. K.B. Hansen: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.18/I2

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01 MH099378-01

Louisiana Board of Regents Fellowship

Hoffman-La Roche

Title: Glun2b-containing nmdars and depression-like behavior: synaptic, cellular, and circuit mechanisms

Authors: *O. H. MILLER[1], B. J. HALL[1,2];
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Abstract: A single, low dose of the NMDA-receptor (NMDAR) antagonist ketamine results in rapid antidepressant effects in Major Depressive Disorder patients and antidepressant-like behaviors in animal models of depression. Despite its short half-life, ketamine's antidepressant effects sustain up to a week, suggesting that this brief window of NMDAR antagonism initiates changes leading to long-lasting alterations in brain function. Research from our lab and others demonstrates that this single dose of ketamine initiates mTOR-dependent protein synthesis of excitatory synapse-associated proteins, and elevated synaptic inputs onto mPFC pyramidal neurons measured 24 hours later. While the molecular mechanisms underlying this process have been well studied, the field lacks an extensive understanding of the brain regions involved, how ketamine alters connectivity between distributed nuclei, and how alterations in neuronal electrophysiological properties underlie these circuit alterations.
We have previously demonstrated that genetic removal of GluN2B-containing NMDARs from forebrain pyramidal neurons mimics and occludes the antidepressant and synaptogenic effects of ketamine in vivo. Here we demonstrate that post-developmental, viral deletion of GluN2B from mPFC pyramidal neurons is sufficient to recapitulate these antidepressant behavioral effects in the absence of hyperlocomotion ((OFT distance traveled; Control: 901.22 +/- 84.51cm, 2BΔ: 804.08 +/- 16.23cm; ns.) (TST immobility time; Control: 186.40 +/- 11.69s, 2BΔ: 143.21 +/- 11.83s; $p<0.05$.) (FST immobility time; Control: 108.38 +/- 10.17s, 2bΔ: 66.90 +/- 17.12s; $p<0.05$)) and induce alterations both in the inputs received by (sEPSC Amplitude; Control: 12.12 +/- 0.65pA, 2bΔ: 18.38 +/-1.85pA; $p<0.01$) and the outputs distributed from these neurons (AP Frequency; Control: 2.38 +/- 0.86Hz, 2BΔ: 6.44 +/- 0.63Hz; $p<0.01$).
The Medial Dorsal Thalamus is an essential integrator of central nodes of limbic information

including the Dorsal Raphe, Amygdala, and Habenula, and projects to mPFC. Using in vivo stereotaxic injections of viral vectors followed by in vitro optogenetic techniques, we sought to determine if the strength of these inputs are altered by NMDAR antagonism or removal of GluN2B. Our findings potentially provide novel cellular and circuit-level targets for optimizing future preclinical studies and clinical therapies based on the profound antidepressant effects of ketamine.

Disclosures: **O.H. Miller:** A. Employment/Salary (full or part-time): Hoffman-La Roche. **B.J. Hall:** None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.19/I3

Topic: B.02. Ligand-Gated Ion Channels

Support: R01 MH081935

F31 MH109267

Title: Presynaptic NMDA receptors contribute to short-term plasticity at mossy fiber-CA3 synapses

Authors: ***P. J. LITUMA**[1], H. KWON[1], R. LUJAN[2], P. E. CASTILLO[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Univ. of Castilla-La Mancha Inst. de Investigacion en Discapacidades Neurologicas (IDINE), Ciudad Real, Spain

Abstract: Neurotransmitter release is a highly regulated process that exquisitely controls the strength of neuronal communication. Presynaptic Ca^{2+} rise is a key component of this process not only for triggering synchronous, action-potential driven transmitter release, but also for facilitating neurotransmitter release during repetitive presynaptic activity. Hippocampal mossy fiber synapses, which carry the main excitatory input to the hippocampus proper, express uniquely robust activity-dependent facilitation. The molecular mechanisms underlying this form of short-term plasticity remain poorly understood, although glutamate autoreceptors may participate. Presynaptic NMDA receptors (preNMDARs) at the mossy fiber bouton have been reported more than three decades ago (Siegel et. al, *PNAS*, 1994) but their role remains unexplored. Here we test the hypothesis that preNMDARs likely due to their high Ca^{2+} permeability contribute to mossy fiber robust short-term facilitation. Using immunoelectron microscopy we identified the obligatory NMDAR subunit GluN1 in rat mossy fiber boutons. To

assess the role of preNMDARs we used electrophysiology, selective pharmacology, and two-photon laser scanning microscopy in acute rat hippocampal slices. Bath application of MK-801 (50 μM) reduced both low-frequency (0.1 to 1.0 Hz steps) and burst-induced (5 stimuli, 25 Hz) facilitation when postsynaptic NMDARs were blocked by loading CA3 pyramidal cells with MK-801 (2 mM). Calcium imaging of mossy fiber giant boutons (200 μM, Fluo5F intracellularly loaded into dentate granule cells) revealed a dAPV-sensitive Ca^{2+} component during brief bursts of activity, suggesting preNMDARs participate in presynaptic Ca^{2+} rise. Together, our findings reveal that during repetitive activity preNMDARs facilitate glutamate release from mossy fiber boutons. Thus, preNMDARs may contribute significantly to dentate gyrus-CA3 information transfer.

Disclosures: P.J. Lituma: None. H. Kwon: None. R. Lujan: None. P.E. Castillo: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.20/I4

Topic: B.02. Ligand-Gated Ion Channels

Support: AHAEIA9100012

R01N5052669

Title: Local calmodulin coupling to NMDA receptors mediates their Ca^{2+}-dependent inactivation

Authors: *G. IACOBUCCI, G. POPESCU;
Biochem., State Univ. of New York At Buffalo, Buffalo, NY

Abstract: In the central nervous system, NMDA receptors produce large and highly regulated Ca^{2+} fluxes, which are critical for synaptic development, plasticity, and apoptosis. Among the mechanisms regulating NMDA receptor currents, Ca^{2+}-dependent inactivation (CDI) is a rapid, reversible, and specific process by which intracellular Ca^{2+} reduces channel activity in a calmodulin (CaM)-dependent manner. Impaired NMDA receptor CDI may contribute to epilepsy pathogenesis and the neurologic symptoms of patients with calmodulinopathies, but the process is poorly understood.
Here, we used single-channel and macroscopic current recordings from GluN1-2a/GluN2A receptors expressed in HEK293, under controlled Ca^{2+}-buffering conditions, to identify the CaM molecules responsible for NMDA receptor CDI in response to local Ca^{2+} signals. These results, combined with mathematical models of local Ca^{2+} diffusion indicated that the CaM molecules

mediating NMDA receptor CDI most likely reside within 10 nm from the NMDA receptor pore. This spatial arrangement allows for fast and highly specific auto-inhibition in response to Ca^{2+} influx from the parent channel.
Next, we used a live-cell functional assay using overexpression of engineered CaM molecules to probe the sequence of events mediating NMDA receptor CDI. Our results provide the first evidence in live cells that NMDA receptor-driven Ca^{2+} nanodomains trigger CDI by specifically activating apoCaM molecules that are preassociated to the C0 cassette of the GluN1 subunit, consistent with CaM's proximal location to the pore.
Together, these results begin to dissect the sequence of events underlying NMDA receptor CDI to provide a more rigorous understanding of molecular mechanism that tune NMDA receptor-mediated Ca^{2+} fluxes and the downstream signaling cascades they control.

Disclosures: G. Iacobucci: None. G. Popescu: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.21/I5

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R15 AREA Award 1R15AG045820-01A1

GMU 4-VA Innovation Award

Title: Activity-dependent postsynaptic signaling in hippocampal neurons is altered by transgenic expression of chimeric NMDA receptor GluN2 subunits

Authors: *S. HUSSAIN[1], J. P. FOTANG[2], M. P. GREER[2], J. M. OILVER[2], M. DARAB[2], G. P. MAI[2], T. C. DUMAS[2];
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Abstract: N-methyl-D aspartate receptors (NMDARs) at excitatory synapses in the hippocampus are central players in the synaptic plasticity required for learning and memory. Two predominant signaling properties of NMDARs that have been independently linked to hippocampal plasticity are calcium conductance into the postsynaptic spine and direct intracellular protein interactions. These properties vary with the composition of the NMDAR such that, compared to NMDARs with GluN2A subunits, NMDARs with GluN2B subunits conduct calcium for a longer period after activation and display greater affinity for the obligatory synaptic plasticity protein, CaMKII. Thus, it is not possible to determine the separate influences

of these NMDAR properties by switching the entire GluN2 subunit. To overcome this obstacle, GluN2 chimeras have been created and engineered into transgenic mice. While changes synaptic plasticity and behavior have been documented in these animals, direct measurement of intracellular signaling has not been performed. We generated two transgenic mouse lines, one having the amino (A)-terminus and transmembrane domains (TMDs) of GluN2A fused to the carboxy (C)-terminus of GluN2B (termed ABc) and, vice versa, the other line having the A-terminus and TMDs of GluN2B fused to the C-terminus of GluN2A (termed BAc). These chimeric GluN2 subunits were expressed in transgenic mice using the tet-off expression system with tetracycline transactivator protein (tTA) expression under transcriptional control of the CaMKII minimal promoter. tTA expression was seen in many forebrain regions, but predominantly in hippocampal pyramidal cells. To induce synaptic plasticity in the hippocampus, animals were briefly exposed to a Y-maze in a novel testing environment. We quantified intracellular signaling through immunohistochemistry and analysis of expression level and colocalization of plasticity-related proteins. Pairs of antibodies were applied (anti-pCaMKII and anti-CaMKII or anti-PSD95 and anti-calmodulin). Currently, we observe an increase in the PSD95 signal and an increase in the PSD95 to calmodulin ratio in maze-exposed animals compared to naïve controls. Also, while the number of samples is low, we see no effect of maze or genotype on pCaMKII or CaMKII. These experiments will reveal which functional property of NMDARs is altered to the greatest extent in mice expressing chimeric GluN2 subunits. Findings from this study will begin to clarify how NMDARs regulate synaptic plasticity and learning and memory.

Disclosures: S. Hussain: None. J.P. Fotang: None. M.P. Greer: None. J.M. Oilver: None. M. Darab: None. G.P. Mai: None. T.C. Dumas: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.22/I6

Topic: B.02. Ligand-Gated Ion Channels

Support: the Eunice Kennedy Shriver National Institute Of Child Health & Human Development (NICHD) of the National Institutes of Health (NIH) R01HD082373

the National Institute of Neurological Disorders and Stroke (NINDS) of the NIH R01NS036654 and R01NS043277

the Xiangya-Emory Medical Schools Visiting Student Program

Emory+Children's Pediatric Center Seed Grant Program

the National Center for Advancing Translational Sciences of the National Institutes of Health UL1TR000454

Title: Evaluation of NMDA receptor human mutations suggests a role for pre-M1 helix in gating

Authors: *M. J. MCDANIEL[1], K. K. OGDEN[1], W. CHEN[1], S. A. SWANGER[1], C. HU[1], A. TANKOVIC[1], H. KUSUMOTO[1], G. J. KOSOBUCKI[2], A. J. SCHULIEN[2], Z. SU[1], J. PECHA[1], S. BHATTACHARYA[1], E. AIZENMAN[2], S. F. TRAYNELIS[1], H. YUAN[1];
[1]Pharmacol., Emory Univ., Atlanta, GA; [2]Neurobio., Univ. of Pittsburgh Sch. of Med. and Pittsburgh Inst. for Neurodegenerative Dis., Pittsburgh, PA

Abstract: NMDA receptors are tetrameric complexes of GluN1 and GluN2 subunits. Recently, a number of de novo mutations have been identified in the GRIN gene family encoding NMDA receptor subunits. Several missense mutations have been identified within the pre-M1 two turn helix that lies parallel to the plane of the membrane, is in van der Waals contact with the M3 gate, and is immediately upstream of the first transmembrane domain. We studied GluN1(D552E), GluN1(P557R), GluN2A(A548T), GluN2A(P552R), and GluN2B(P553L), which are de novo mutations found in children with developmental delay and/or epilepsy. Interestingly, no polymorphisms have been identified among the healthy population in the pre-M1 helix, suggesting that missense mutations in this region may contribute to neurological problems. Here we explore the effects of mutations on NMDA receptor expression and surface localization and response time course. Consistent with data implicating this short pre-M1 helix in channel gating, these mutations had functional consequence, with GluN1(D552E), GluN1(P557R), and GluN2A(A548T) showing reduced current responses, GluN2B(P553L) showing complete loss of function, and GluN1(P557R) and GluN1(D552E) showing reduced surface expression when co-expressed with GluN2A and GluN2B, respectively. In addition, there was significantly reduced overall expression of GluN1 (P557R) when co-expressed with GluN2B, but no change in expression levels of GluN2A(A548T). GluN2A(P552R) prolonged the response to brief synaptic-like stimulation and markedly increased charge transfer. Transfection of cultured neurons with GluN2A(P552R) triggered dendritic swelling, consistent with enhanced response of synaptic NMDARs containing GluN2A(P552R). Results from this study suggest that the pre-M1 helix is critical for receptor gating, such that mutations in this region are sufficient to disrupt receptor functioning and, ultimately, neuronal health.

Disclosures: M.J. McDaniel: None. K.K. Ogden: None. W. Chen: None. S.A. Swanger: None. C. Hu: None. A. Tankovic: None. H. Kusumoto: None. G.J. Kosobucki: None. A.J. Schulien: None. Z. Su: None. J. Pecha: None. S. Bhattacharya: None. E. Aizenman: None. S.F. Traynelis: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Janssen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeurOp. F. Consulting Fees (e.g., advisory boards); NeurOp, Pfizer, Janssen. H. Yuan: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.23/I7

Topic: B.02. Ligand-Gated Ion Channels

Title: PSD-95 blocks amyloid-beta induced depression by preventing NMDA receptor metabotropic actions

Authors: *K. B. DORE[1], Z. CARRICO[2], R. MALINOW[2];
[1]Neurosciences, [2]UCSD, La Jolla, CA

Abstract: Previous studies show that amyloid-beta (Aβ) depresses excitatory synapses. The underlying mechanisms are not well understood. We observed that PSD-95 overexpression prevented the depressive effects of amyloid-beta on synaptic transmission. This effect was also observed in slices made from GluR1 KO animals suggesting that PSD-95 effects were not due to synaptic incorporation of GluR1 receptors leading to potentiation. Recent evidence suggests that Aβ-induced synaptic depression requires ligand binding to NMDA receptors (NMDARs) but not ion-flux. Here we use FRET-FLIM (Forster resonance energy transfer, fluorescence lifetime imaging) to show that Aβ can modify the conformational configuration of the NMDAR c-terminal domain without ion-flux. Spines of neurons expressing GluN1 subunits tagged with GFP and mCherry were imaged using FLIM. Spines from neurons also expressing a construct that increases Aβ production displayed reduced FRET. The reduced FRET between GluN1 subunits is similar to what was observed upon ion-flux independent chemical long-term depression (cLTD), which supports a common mechanism for Aβ induced depression and cLTD dependent on metabotropic actions of NMDARs. Interestingly, we observed that PSD-95 overexpression froze the NMDAR cytoplasmic domain in a non-depressed conformation, even in the presence of Aβ, possibly through increased PSD-95/NMDAR interactions. These results indicate that PSD-95 prevents the effects of amyloid-beta by interfering with NMDAR metabotropic function.

Disclosures: K.B. Dore: None. Z. Carrico: None. R. Malinow: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.24/I8

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant AA006399

NIH Training Grant AA007471

Title: A long non-coding RNA induced in alcoholics reduces NMDA receptor expression without modifying GABA-A nor glycine receptor expression

Authors: *C. BORGHESE, K. J. LAWRENCE, E. OSTERNDORFF-KAHANEK, S. P. FARRIS, R. A. HARRIS;
Waggoner Ctr. for Alcohol and Addiction Res., Univ. of Texas At Austin, Austin, TX

Abstract: Long non-coding RNAs (lncRNAs) are defined as RNA molecules of more than 200 bases in length with no protein-coding capacity. They have been shown to interact with DNA, RNA and proteins in myriad ways, and their multiple roles are barely beginning to be characterized.
Chronic alcohol exposure led to differential expression of a human lncRNA (LINC01559) and its mouse counterpart (4930425L21Rik) in nucleus accumbens. Weighted Gene Co-Expression Network Analysis (WGCNA) showed that LINC01559 was negatively correlated to a module of genes that included a significant number of genes involved in CNS (glutamatergic) plasticity, which could indicate a regulatory role for LINC01559 in glutamatergic transmission. This lncRNA is located near the gene encoding the GluN2B NMDA receptor subunit in both human and mouse chromosomes, further supporting a link with glutamatergic transmission. We co-injected human LINC01559 (LNC) with cRNA encoding NMDA, $GABA_A$ or glycine receptors in *Xenopus laevis* oocytes, and tested the receptor function using two-electrode voltage clamp. Co-injection of LNC with NMDA receptors (either GluN1+GluN2B or GluN1+GluN2A) resulted in receptors that showed decreased maximal currents but concentration-response curves similar to controls. This suggests that LNC interacted with either the cRNA encoding these subunits or with the protein, ultimately interfering with their expression on the oocyte surface. Co-injection of LNC with $GABA_A$ $\alpha1\beta2\gamma2$ or glycine $\alpha1$ receptors resulted in concentration-response curves and maximal currents similar to controls. Thus, there is a specificity of action of LNC on NMDA receptors.
The precise mechanism by which LNC interacts with NMDA receptors is yet to be determined. However, this is, to our knowledge, the first reported interaction of a lncRNA with a specific receptor RNA or protein. Supported by grants from the National Institutes of Health: AA006399 (RAH) and Training Grant AA007471 (SPF).

Disclosures: C. Borghese: None. K.J. Lawrence: None. E. Osterndorff-Kahanek: None. S.P. Farris: None. R.A. Harris: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.25/I9

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS088479

Title: Missense mutations reveal a key role for the M4 eukaryotic specific transmembrane segment in NMDA receptor gating

Authors: J. AMIN[1], K. CHAN[2], Q. GAN[2], *L. P. WOLLMUTH[3,4];
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Abstract: NMDA receptors (NMDAR) are glutamate-gated ion channels that have a wide distribution in the nervous system. Dysfunction of NMDARs is implicated in numerous chronic (Parkinson's, Alzheimer's), acute (stroke, epilepsy) and psychiatric (schizophrenia) brain disorders. Recently, a variety of *de novo* missense mutations in NMDAR subunits have been identified that are associated with neurodevelopment disorders. NMDARs are obligate heterotetramers typically composed of GluN1 and GluN2 subunits. Each subunit contributes three transmembrane segments, M1, M3, & M4, and an M2 pore loop to forming the iGluR ion channel. The central core of the ion channel, which is homologous to an inverted K^+ channels, is formed by M1-M3. In AMPA receptors, the M4 segment is involved in receptor assembly whereas in NMDARs it appears to have a more prominent role in facilitating channel opening in the presence of agonist. Highlighting this feature, we find that *de novo* missense mutations in the M4 segment the GluN1 and GluN2A subunits strongly attenuate NMDAR pore opening or gating in the presence of agonist. Notable in the M4 segment is a highly conserved glycine, that is present in >80% of all eukaryotic iGluRs. Missense mutations at this position are found in the GluN1 and GluN2B subunits, and these mutations dramatically attenuate pore opening. Even substitution of alanine at this conserved G impedes the ability of the receptor to open. These results have strong implications for how such disorders are caused at the ion channel level and highlight an important role for the M4 segment in normal NMDAR gating.

Disclosures: J. Amin: None. K. Chan: None. Q. Gan: None. L.P. Wollmuth: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.26/DP02 (Dynamic Poster)

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01GM062868-14

Title: Molecular dynamics simulations reveal the importance of glycans on N-methyl-D-aspartate (NMDA) receptors

Authors: *A. SINITSKIY[1], N. STANLEY[1,2], V. PANDE[1];
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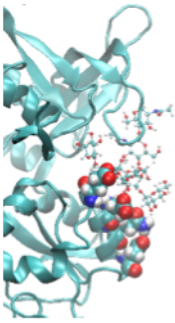
Abstract: N-methyl-D-aspartate (NMDA) receptors (NMDARs) are transmembrane proteins expressed in cells in the nervous system. Malfunctioning of NMDA receptors correlates with various neurological disorders, including schizophrenia, epilepsy, intellectual disability and autism, but specific molecular mechanisms for these correlations remain largely unknown. NMDARs *in vivo* are heavily glycosylated. At least 9 glycans are attached to each GluN1, at least 4 glycans to each GluN2A, and at least 7 glycans to each GluN2B subunit. Surprisingly, glycans attached to NMDARs have not received due attention in previous studies of the structure and function of NMDARs. We hypothesize that an inappropriate glycosylation of NMDARs may contribute to the development of some neurological disorders, such as schizophrenia. We use computational approach, specifically, all-atom molecular dynamics simulations analyzed with machine learning based on Markov state models, which allows us to address important questions about NMDA receptors that current experimental methods alone do not answer. In particular, we predict that glycosylation at specific sites on NMDA receptors, including Asp440 residue in the GluN1 subunit, changes the stability of various conformations of NMDARs, and by doing so, glycans on NMDARs effectively play an agonist-like role. This conclusion could change the state of affairs in the field of ion channel research, where glycans attached to ion channels have not received any significant attention so far. Partially, this gap in knowledge can be explained by tremendous technical difficulties of selectively controlling and measuring glycosylation patterns in ion channels. Overall, computational modeling provides a unique source of information about NMDA receptors, complementary to the information available from modern experimental techniques.



Disclosures: A. Sinitskiy: None. N. Stanley: None. V. Pande: None.

Poster

219. NMDA Receptors I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 219.27/I10

Topic: B.02. Ligand-Gated Ion Channels

Support: Lundbeck Foundation R118- A11608

Brødrene Hartmann Foundation R74-A21755

Lundbeck Foundation / Research Initiative on Brain Barriers and Drug Delivery

Title: Transient neuronal depolarization induces rapid, NMDA receptor-dependent, endogenously reversible and recurrent fission of the neuronal endoplasmic reticulum in physiology and disease *In vivo*.

Authors: *K. KUCHARZ[1], M. LAURITZEN[1,2];
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Abstract: BACKGROUND: The neuronal endoplasmic reticulum (ER) is the major intracellular Ca2+ store that modulates synaptic transmission and plasticity. The ER maintains structural continuity through all neuronal compartments, allowing long-distance intracellular diffusion of Ca2+ and protein trafficking, and integrates spatially separated synaptic events with gene expression. The transition to discontinuous state of neuronal ER is regarded as a hallmark of cell death. Here, in contrast, we provide the first longitudinal description of ER dynamics *in vivo* and show that the brain has an innate ability to reversibly and recurrently alter neuronal ER continuity in response to synaptic input in physiology and disease with important implications for neuronal function. **METHODS:** We used *in vivo* 2-photon microscopy in transgenic mice expressing soluble EGFP targeted to neuronal ER lumen. The ER was assessed in the

somatosensory cortex during whisker pad stimulation and in pathology during cortical spreading depolarizations (CSD) that accompany stroke, brain trauma and migraine. The ER continuity was monitored using developed real-time profile plot variance analysis and with fluorescence recovery after photobleaching. Intracellular Ca2+ was monitored with the red-shifted Ca2+ indicator and simultaneously with imaging we performed *in vivo* electrophysiological recordings of direct current and local field potentials (LFPs; electrocorticogram, ECoG). **RESULTS:** The neuronal ER in its basal state was continuous, as assessed up to 450 μm below the brain surface, with clear spine-ER morphology. However, during whisker pad stimulation the ER in spines exhibited rapid (<15 s) loss of connectivity with dendritic ER. The process was reversible (<1-2 min) and depended on NMDA receptor activation. In pathology, with neuronal depolarization during CSD, the ER underwent rapid (~10 s) and widespread fission in dendrites into numerous, optically isolated structures. With the repolarization, the ER gradually regained its pre-CSD morphology (~1 min; ER fusion). Interestingly, a fraction of ER remained isolated and the increase and decline in the amount of isolated ER over time after depolarization mirrored the degree of loss and regain of neuronal activity (LFPs/ECoG). The ER fission-fusion could occur multiple times within the same neuron, correlated spatio-temporally with the rise in intracellular Ca2+, and was compatible with cell survival. **SUMMARY:** Rapid ER fission-fusion in the brain is a previously unknown *in vivo* phenomenon that may affect neuronal Ca2+ signaling, homeostasis, synaptic activity and thus many vital aspects of brain function in physiology and disease.

Disclosures: K. Kucharz: None. M. Lauritzen: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.01/I11

Topic: B.04. Ion Channels

Support: Canadian Institutes of Health Research

Alberta Innovates-Health Solutions (AIHS)

QEII Studentships

Postdoctoral Fellowships from University Calgary Eyes High

Alberta Heritage Foundation for Research

Title: Cav3.1-mediated calcium entry triggers CaM / αCaMKII-dependent CREB activation and potentiation of the parallel fiber EPSP in Purkinje cells

Authors: *H. ASMARA, I. MICU, A. P. RIZWAN, G. SAHU, B. A. SIMMS, F.-X. ZHANG, P. K. STYS, G. W. ZAMPONI, R. W. TURNER;
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Abstract: At the circuit level memory formation is signified as a change in synaptic efficacy as a result of previous activity. A long term potentiation (LTP) of synaptic efficacy depends on calcium influx into the cell that triggers a signaling cascade and initiates gene transcription in the nucleus. Calmodulin (CaM) is an important signalling molecule that regulates a vast array of second messenger cascades that can also lead to gene transcription. Low voltage-activated calcium channels of the Cav3 family have the important role of mediating low threshold calcium influx, but were not believed to interact with CaM. We find a constitutive association between CaM and the Cav3.1 channel at rest that is lost through an activity- and Cav3.1 calcium-dependent CaM dissociation. Moreover, Cav3 calcium influx activates αCaMKII in the cytoplasm and phosphorylation of CREB in tsA-201 cells, cultured hippocampal cells and lobule 9 cerebellar Purkinje cells. Recent work has revealed a Cav3.1 channel-dependent LTP of cerebellar parallel fiber input to Purkinje cells. Optogenetic excitation of parvalbumin-ChR2 expressing Purkinje cells in vitro is sufficient to activate αCaMKII and evoke a Cav3.1-mediated LTP of a simulated postsynaptic EPSP in Purkinje cells. Our findings thus establish that T-type channel calcium influx invokes a novel dynamic interaction between CaM and Cav3.1 channels and triggers a signaling cascade directly relevant to postsynaptic LTP of the parallel fiber EPSP in Purkinje cells.

Disclosures: H. Asmara: None. I. Micu: None. A.P. Rizwan: None. G. Sahu: None. B.A. Simms: None. F. Zhang: None. P.K. Stys: None. G.W. Zamponi: None. R.W. Turner: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.02/I12

Topic: B.04. Ion Channels

Support: NIH Grant DC009433 to A.L.

NIH Grant NS084190 to A.L.

Carver Research Program of Excellence Award to A.L.

Austrian Science Fund P24079 to G.J.O

Austrian Science Fund F4406 to G.J.O

NIH Grant NS17660 to M.B.K

NIH Grant NS028710 to M.B.K

Title: Densin-180 regulates the cell-surface density of voltage-gated $ca_v1.2$ Ca^{2+} channels

Authors: *S. WANG[1], J. HAGEN[1], R. I. STANIKA[2], G. J. OBERMAIR[2], M. B. KENNEDY[3], R. J. COLBRAN[4], A. LEE[1];
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Abstract: Densin-180 (densin) is an excitatory synapse protein that interacts with and facilitates voltage-gated $Ca_v1.3$ (L-type) Ca^{2+} channels. Mice lacking densin (densin KO) exhibit impaired spatial memory and increased anxiety-related behaviors. Similar phenotypes are found in mice with forebrain-specific knockout of the major L-type channel in the brain, $Ca_v1.2$, but not in mice lacking $Ca_v1.3$. These findings suggest that densin could be a crucial regulator of $Ca_v1.2$ as well as $Ca_v1.3$. Therefore, we tested the hypothesis that densin functionally interacts with $Ca_v1.2$. When expressed in transfected HEK293T cells, densin coimmunoprecipitates with $Ca_v1.2$ channels and increases $Ca_v1.2$ current density in part by increasing the number of functional channels. These results are physiologically relevant since densin coimmunoprecipitates with $Ca_v1.2$ from mouse brain, and Ca_v1-mediated Ca^{2+} currents are smaller in cortical neurons from densin KO than from control mice. Moreover, overexpression of densin increases the clustering of $Ca_v1.2$ channels in dendrites of hippocampal neurons. Cell-surface levels of $Ca_v1.2$ protein are reduced in densin KO brain, which likely explains the reduction in $Ca_v1.2$-mediated phosphorylation of the transcription factor CREB in densin KO neurons. The effect of densin on cell-surface $Ca_v1.2$ channels is likely due to an enhancement of forward trafficking rather than a reduction in endocytosis of $Ca_v1.2$, based on analyses of channel dynamics in transfected HEK293T cells. We conclude that densin is a component of $Ca_v1.2$ complexes in the brain, which augments the number of cell-surface channels that are available for excitation-transcription coupling. Our results underscore the importance of $Ca_v1.2$ channel-protein interactions for neuronal Ca^{2+} signaling, and suggest that dysregulation of such interactions may lead to cognitive and affective phenotypes such as those in densin KO mice.

Disclosures: S. Wang: None. J. Hagen: None. R.I. Stanika: None. G.J. Obermair: None. M.B. Kennedy: None. R.J. Colbran: None. A. Lee: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.03/J1

Topic: B.04. Ion Channels

Support: CONACYT 152326

Title: Cdk5 phosphorylates Cav1.3 channels and regulates its activity.

Authors: ***S. LOYA**[1], R. GONZÁLEZ[2], A. SANDOVAL[3], M. RODRIGUEZ SANCHEZ[1], R. FELIX[3], D. ERLIJ[4], B. FLORÁN[1];
[1]Ctr. De Investigación Y Estudios Avanzados Del Inst. Politécnico Nacional, Ciudad De México, Mexico; [2]Hosp. Manuel Gea González, Mexico City, Mexico; [3]Biología Celular y Mol., CINVESTAV-IPN, Mexico City, Mexico; [4]State Univ. of New York, New York, NY

Abstract: Cyclin-dependent kinase 5 (cdk5) has been implicated in neurotransmission. Neurotransmission depends largely on calcium movement across ion permeable channels. Several studies suggest a significant role of cdk5 in direct regulation of various ion channels; in hippocampal synaptosomes, phosphorylation of P/Q type calcium channels prevents its interaction with SNAP25 and synaptotagmin 1, thereby inhibiting glutamate release from these terminals.
In this work, we study the possible regulation by phosphorylation of cdk5 to Cav1.3 calcium channels, which regulate significantly the GABA release of striato-nigral terminals. Also we studied the implication of this phosphorylation in the electrophysiological properties of Cav1.3. Through electrophysiology experiments in HEK293 cells transfected with Cav1.3 channel and its respective auxiliary subunits, we found that, by inhibiting cdk5 with olomoucine (50 μM), there is an increase of current density. This speaks of an inhibitory mechanism exerted on Cav1.3 and is also probably due to phosphorylation by cdk5 of a serine residue located at the carboxyl terminus of this calcium channel.
Our results suggest a novel mechanism of regulation of neurotransmission by a phosphorylation event, whose understanding may be useful for the development of therapeutic alternatives against neurodegenerative diseases such as Parkinson's disease.

Disclosures: S. Loya: None. R. González: None. A. Sandoval: None. M. Rodriguez Sanchez: None. R. Felix: None. D. Erlij: None. B. Florán: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.04/J2

Topic: B.04. Ion Channels

Support: FWF P24079

SFB F4415

Title: Presynaptic alpha2delta subunits of voltage-gated calcium channels modulate postsynaptic $GABA_A$-receptor abundance

Authors: *S. GEISLER, C. L. SCHÖPF, R. STANIKA, L. TRAXLER, G. J. OBERMAIR; Dept. of Physiol., Med. Univ. Innsbruck, Innsbruck, Austria

Abstract: Auxiliary $\alpha_2\delta$ subunits modulate membrane trafficking and current properties of voltage-gated calcium channels and they have been implicated in synapse formation. By employing a cellular $\alpha_2\delta$ triple knockout model in cultured hippocampal neurons, we could recently identify $\alpha_2\delta$ subunits as key regulators of glutamatergic synaptogenesis. In contrast, synapses of GABAergic neurons seemed unaffected by knockout of all three subunits and thus the role of $\alpha_2\delta$ subunits in GABAergic synapses remained unclear. Surprisingly, however, overexpression of $\alpha_2\delta$-2 induced a mismatched localization of postsynaptic $GABA_A$-receptors ($GABA_AR$) opposite glutamatergic terminals. This puzzling observation could be explained in two ways: firstly, since overexpression of $\alpha_2\delta$-2 increases presynaptic calcium channel abundance, postsynaptic $GABA_AR$ may be recruited to compensate for excessive excitatory synaptic activity. In this scenario we expect $GABA_AR$ abundance at inhibitory GABAergic synapses to remain unchanged. Secondly, presynaptic $\alpha_2\delta$-2 may actively participate in the recruitment and/or anchoring of postsynaptic $GABA_AR$. Here, $GABA_AR$ density should be increased both in glutamatergic and GABAergic synapses. In order to distinguish between these two hypotheses we established cultures of striatal neurons, which consist of approximately 95% inhibitory medium spiny neurons (MSN). In line with previous observations the proper maturation of dendritic arbors and spines of MSN required the co-culture with glutamatergic cortical neurons. To test whether $\alpha_2\delta$ subunits affect the composition of GABAergic synapses, we transfected MSN with individual $\alpha_2\delta$ isoforms together with soluble eGFP. Finally, differentiated MSN (DIV24) were immunolabeled for pre- and postsynaptic constituents of GABAergic synapses (vGAT/ $GABA_AR$ $\beta_{2/3}$ subunit). High-resolution fluorescence microscopy revealed a significant increase of both size and intensity of postsynaptic $GABA_AR$ clusters opposite presynaptic terminals of MSN overexpressing $\alpha_2\delta$-2. Conversely, $GABA_AR$ content in $\alpha_2\delta$-3 expressing synapses was strongly reduced when compared with $\alpha_2\delta$-1 transfected or control (eGFP) neurons. Therefore our findings suggest that presynaptic $\alpha_2\delta$ subunits regulate the

postsynaptic composition of GABAergic synapses in an isoform specific manner. Moreover, our results point towards an active involvement of $\alpha_2\delta$-2 in the recruitment and/or anchoring of postsynaptic $GABA_AR$.

Disclosures: S. Geisler: None. C.L. Schöpf: None. R. Stanika: None. L. Traxler: None. G.J. Obermair: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.05/J3

Topic: B.04. Ion Channels

Support: NIH R01 NS078792

NIH R01 MH097887

Title: Phosphorylation of S1928 controls regulation of L-type Cav1.2 channel activity by the β2-adrenergic receptor in neurons

Authors: *J. L. PRICE[1], T. PATRIARCHI[2], E. HAMMES[1], C.-Y. CHEN[2], F. HOFMANN[3], M. NAVEDO[2], J. HELL[2];
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[3]Pharmacol., Tech. Univ. of Munich, Munich, CA

Abstract: The L-type Ca2+ channel (LTCC) Cav1.2 is the predominant LTCC in the brain. It controls neuronal excitability, gene expression, and NMDA-independent forms of synaptic plasticity in the hippocampus such as mGluR-dependent LTD and LTP induced by a 3 min / 5 Hz stimulus train that coincides with beta adrenergic stimulation (prolonged theta tetanus LTP, PTT-LTP). We have identified a mechanism for specific desensitization of beta-adrenergic upregulation of Cav1.2 activity in neurons. Residue S1928 in the C-terminal tail of Cav1.2 is prominently phosphorylated by PKA upon beta adrenergic stimulation. We found that S1928 is part of the binding site for the beta2 adrenergic receptor and that its phosphorylation displaces the beta1 adrenergic receptor from Cav1.2 for transient desensitization of Cav1.2 regulation by beta2 adrenergic receptor stimulation.. Stimulation of the beta2 adrenergic receptor also induces AMPAR phosphorylation and increases their activity. However it does not result in dissociation of the beta2 adrenergic receptor from the AMPAR complex nor in a refractory period for re-stimulation AMPAR phosphorylation by a second pulse of beta2 adrenergic receptor stimulation. PTT-LTP requires the interaction between the beta2 adrenergic receptor and Cav1.2. Further

work with S1928A knock-in mice is being performed to determine functional significance of this particular site in Cav1.2 regulation.

Disclosures: J.L. Price: None. T. Patriarchi: None. E. Hammes: None. C. Chen: None. F. Hofmann: None. M. Navedo: None. J. Hell: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.06/J4

Topic: B.04. Ion Channels

Support: University of Virginia School of Medicine

Title: Glycosylation of Cav3.2 channels contributes to hyperalgesia in peripheral neuropathy of type 1 diabetes

Authors: *S. M. TODOROVIC[1], S. L. JOKSIMOVIC[1], J. G. EVANS[2], P. EGGAN[1], V. JEVTOVIC-TODOROVIC[1];
[1]Anesthesiol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [2]Univ. of Virginia, Charlottesville, VA

Abstract: Introduction: Recent studies have implicated plasticity of the Cav3.2 isoform of T-type Ca^{2+} channels in the development of painful peripheral diabetic neuropathy (PDN). Furthermore, inhibitors of glycosylation, such neuraminidase (NEU), fully reverse abnormalities of Cav3.2 channels in nociceptive sensory neurons *in vitro*, and reverse hyperalgesia in mice with Type 2 diabetes *in vivo* (Orestes et al., 2013). Here we investigated if glycosylation of Cav3.2 channels could lead to neuronal hyper-excitability, which could in turn contribute to the development of painful PDN in Type I diabetes.
Methods: We studied *in vitro* effects of NEU on biophysical properties of recombinant human Cav3.2 channels stably expressed in human embryonic kidney (HEK)-293 cells grown in hyperglycemic cell culture medium. For *in vivo* studies, we injected NEU intra-plantary (i.pl.) into peripheral receptive fields of sensory neurons and measured heat sensitivity and mechanical sensitivity of healthy mice and mice with streptazocin (STZ)-induced painful PDN.
Results: Whole-cell recordings of current-voltage relationships demonstrated that Cav3.2 current densities were profoundly decreased (about 2-fold) after NEU treatment. Furthermore, NEU diminished macroscopic current activation and inactivation kinetics, induced a depolarizing shift of around 7 mV in the steady-state activation and steady-state inactivation curves while having very little effect on current deactivation kinetics.

For *in vivo* study 3 cohorts of mice were injected with STZ and developed hyperglycemia ranging from 400-600 mg/dl: C57Bl/6 wild type (WT) mice, $Ca_V3.2$ knock-out (KO mice) and Swiss-Webster (SW) mice. Either 10 µl of 1.5 U/ml NEU (same concentration as used *in vitro* studies) or vehicle (saline) were injected i.pl. in the right hind paws of adult female diabetic mice 14 days post-STZ. We found that injections of NEU, but not vehicle, completely reversed thermal and mechanical hyperalgesia in diabetic WT mice. In contrast, NEU did not affect baseline thermal and mechanical sensitivity in diabetic Cav3.2 KO mice and SW mice, both of which are resistant to the development of painful PDN.
Conclusion: Our results demonstrate that glycosylation-induced alterations in Cav3.2 current kinetics and density can directly influence neuronal excitability and that NEU can be used to ameliorate painful symptoms in Type 1 diabetes. We expect that our studies will lead to a better understanding of the molecular mechanisms underlying painful PDN in an effort to facilitate the discovery of novel treatments for this intractable disease.

Disclosures: S.M. Todorovic: None. S.L. Joksimovic: None. J.G. Evans: None. P. Eggan: None. V. Jevtovic-Todorovic: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.07/J5

Topic: B.04. Ion Channels

Support: NINDS NS29709 (JLN)

Title: Early development of thalamic low-threshold T-type calcium current in the VPM of C57BL/6 mice

Authors: *Q. MIAO[1,2], J. L. NOEBELS[3];
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Abstract: Transient (T)-type calcium current plays an important role in regulating the excitability of thalamocortical (TC) neurons and endows the neural network with oscillatory properties that are critical for brain functions. Abnormal T current was demonstrated to be involved in mental diseases, such as childhood absence epilepsy (CAE) which is a common epilepsy syndrome starting between 4-8 years with peak age between 5-7 years. Several lines of evidence support an essential role of T-current in TC relay cells plays in generating CAE. However, it is unclear how T current normally matures in these cells during early development

and how this might affect the functional properties of TC cells. To better understand the normal development of this current, we performed whole-cell recordings of relay cells in the thalamic ventral posteromedial nucleus (VPM) of slices made from mice at postnatal day 19-20 (P19-20). We found that somatic measurement of T current density was highly dependent on whether tetraethylammonium (TEA, 10 mM) and 4-aminopyridine (4-AP, 0.5 mM), two potassium channel blockers, were present in the artificial cerebrospinal fluid (ACSF) (T current density in normal ACSF: 7.83 ± 0.94 pA/pF, n = 10; in ACSF with TEA and 4-AP: 22.21 ± 1.16 pA/pF, n = 12; $p = 9.3\times10^{-9}$). This nearly three-fold increase is consistent with the evidence that T-type calcium channels are densely distributed on thalamic relay cell dendrites, and that potassium channel blockade improves the space clamp of dendritic current in soma recordings. We next investigated the early development of T current from P13 to P21, a time coinciding with the onset of absence epilepsy in tottering mice and other monogenic mouse models of this seizure type. ACSF with TEA and 4-AP was used. Interestingly, we found a significant increase in T current density in the VPM during the early development of wildtype mice (P13-14, 12.75 ± 1.78 pA/pF, n = 13; P19-20, 22.2± 1.2 pA/pF, n = 12; $p = 0.00022$). Analysis of dendritic morphology of recorded neurons is ongoing. Together, these results demonstrate the importance of blocking membrane potassium channels to assess whole cell T currents, and reveal a significant change of thalamic T current in the wildtype mice during the third postnatal week.

Disclosures: Q. Miao: None. J.L. Noebels: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.08/J6

Topic: B.04. Ion Channels

Support: ERC Advanced Grant 268548

Austrian Science Fund P24909-B24

Title: Presynaptic R-type (Cav2.3) channels mediate glutamate release at habenulo-interpeduncular nucleus synapses

Authors: *D. VANDAEL, P. BHANDARI, R. SHIGEMOTO, P. JONAS;
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Abstract: Synapses between medial habenula (MH) and interpeduncular nucleus (IPN) neurons are thought to play crucial roles in sleep, stress, and nicotine addiction, presumably through

corelease of glutamate and acetylcholine (Ren et al., 2011, Neuron 69:445-452; Frahm et al., 2015, Neuron 69:445-452). To address the mechanisms of transmitter release at MH-IPN synapses, we combined electron microscopy with patch-clamp recording in acute slices from 18- to 23-day-old mice. We previously reported predominant presynaptic localization of R-type Ca^{2+} channels (Cav2.3) in these synapses (Parajuli et al., 2012, J. Neurosci. 32:13555-13567). Immunogold labeling revealed 24% of gold particles in the active zone, 10% in the perisynaptic region, and 66% in the extrasynaptic region. Extracellular electrical stimulation of MH axons in the fasciculus retroflexus evoked robust EPSCs in putative GABAergic IPN neurons (-415 ± 80 pA, n=14). Synaptic currents showed a mean latency of 6.8 ± 0.5 ms, a 20-80% rise time of 1.2 ± 0.1 ms, and a decay time constant of 7.8 ± 0.7 ms. During trains of stimuli, MH-IPN EPSCs showed mostly depression, with mean multiple-pulse ratios of EPSC10 / EPSC1 of 0.7 ± 0.2 and 0.84 ± 0.3 for 10 Hz and 50 Hz, respectively. Furthermore, trains of stimuli evoked marked asynchronous release during and after the train. SNX-482 (500 nM - 1 μM) almost completely blocked both synchronous (93%, n=6) and asynchronous release components (76 %, n=6), indicating that evoked release was primarily mediated by R-type Ca^{2+} channels. Although the slow component of transmission was previously attributed to nicotinic receptor mediated currents (Ren et al., 2011), both synchronous and asynchronous EPSCs were fully blocked by 20 μM of the AMPA-type glutamate receptor antagonist CNQX (n=6). Bath application of EGTA-AM (100 μM) blocked 75 ± 2.3 % (n=5) of synchronous release and 34 ± 7 % of asynchronous release, indicating loose coupling between R-type Ca^{2+} channels and release sensors. Thus, MH-IPN synapses show highly specialized transmission properties: Release primarily relies on R-type presynaptic Ca^{2+} channels, and asynchronous release mediates a slow component of synaptic signaling. Our results indicate that asynchronous release of glutamate rather than acetylcholine mediates slow components of synaptic signaling.

Disclosures: D. Vandael: None. P. Bhandari: None. R. Shigemoto: None. P. Jonas: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.09/J7

Topic: B.04. Ion Channels

Support: NIH Grant NS090644

MDA Grant 295271

Title: Modeling voltage sensor movement in Cav2.1 calcium channels

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Abstract: The P/Q-type voltage-gated calcium channel, Cav2.1, is a key player in synaptic transmission. Dysfunction of this channel has been implicated in a variety of neurological diseases, and it is the target for newly developed gating modifiers (GV-58) with therapeutic potential. Therefore, Cav2.1's mechanism of activation is clinically relevant. A well-supported model of activation for all voltage-gated ion channels is the sliding helix model. Briefly, the voltage-sensing S4 segments rotate outward within the depolarized membrane. In this model, the movement of the positively-charged S4 residues through the hydrophobic membrane interior is proposed to be catalyzed by a series of transient electrostatic interactions between these residues and the residues of neighboring segments. It has previously been impossible to determine whether Cav2.1 gates via the sliding helix mechanism, since no crystal structure is available for this channel. Fortunately, the recently-published cryo-electron microscopy structure of a closely related calcium channel, together with the wealth of structural information available on voltage gated sodium channels, make it feasible to predict the structure of Cav2.1 via homology modeling. Using multiple templates, we modeled the resting and active states of the voltage-sensing regions of each of Cav2.1's four heterologous domains. Our resting-state models reveal electrostatic interactions between arginines and lysines in the S4 segment, and conserved residues in neighboring segments. These include salt bridges as well as a cation-pi interaction between a conserved phenylalanine (in S2) and arginine (in S4). These interactions may stabilize the resting state. Computational modeling of voltage sensor domain (VSD) movement reveals a rotation of S4 toward the center of the VSD, coupled with a displacement toward the extracellular region of less than 10 Å. In the process, the cation-pi interaction is broken and the salt bridges are reconfigured. We identified ionic interacting pairs that may stabilize individual conformation states. To refine our model, we are currently using site-directed mutagenesis of expressed channels in HEK293 cells to evaluate the contribution of the predicted electrostatic interactions to the voltage-dependence of channel activation. We will constrain the model based on our results.

Disclosures: S. Aldrich: None. M.H. Cheng: None. S.D. Meriney: None. I. Bahar: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.10/J8

Topic: B.04. Ion Channels

Title: Role of R-type calcium channel (Cav2.3) in medial habenula to interpeduncular nucleus pathway

Authors: *P. BHANDARI[1,2], L. K. PARAJULI[2], K. TAKAO[2,3], T. MIYAKAWA[2,4], Y. KOBAYASHI[5], K. F. TANAKA[6], R. SHIGEMOTO[1,2];
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Abstract: Medial habenula (MHb) neurons project almost exclusively to the interpeduncular nucleus (IPN) (Herkenham and Nauta, 1979; Agetsuma et al., 2010). MHb to IPN pathway plays a crucial role in various behavioral domains such as fear responses (Agetsuma et al., 2010), inhibitory control and cognition-dependent executive functions (Kobayashi et al., 2013), nicotine addiction (Frahm et al., 2011) and so on. In situ hybridisation data shows that medial habenula expresses predominantly mRNA transcripts of R-type (Cav2.3) but not P/Q- or N-type calcium channels (Allen Brain Atlas). We previously reported that in sharp contrast to its predominant postsynaptic localization in other brain regions, Cav2.3 is localized predominantly in presynaptic terminals in IPN (Parajuli et al., 2012). To address the role of Cav2.3 in the MHb to IPN pathway, we combined genetic, behavioral and pre-embedding electron microscopy techniques. Pre-embedding immunolabeling revealed specific localization of gold particles for Cav2.3 in presynaptic terminals making glutamatergic synapses in IPN, with densities of 7.31 particles/μm in active zone, 6.94 particles/μm in perisynaptic zone and 3.18 particles/μm in extrasynaptic zone. Behavioral studies in Cav2.3 null knock out (KO) mice showed altered behavior compared with heterozygous control . In open field test, KO mice travelled longer total distance ($p<0.0001$) than control showing hyperactivity. In social interaction test, KO mice showed lesser duration and number of contacts ($p<0.05$) denoting lesser social interaction. There was no significant difference in elevated plus maze test. In fear conditioning test, KO mice showed increased freezing percentage ($p=0.0003$) during conditioning and pretone period with altered context ($p=0.0007$) whereas no change in context testing. To further examine the role of Cav2.3 in MHb to IPN pathway, we used Flexible Accelerated STOP Tetracycline Operator (tetO)-knockin (FAST) technique (Tanaka et al., 2010) to generate Cav2.3 FAST KO mice, which could be rescued by crossing with Cre-driver mouse lines. We selected Gpr151-Cre mouse (Kobayashi et al., 2013) for the rescue of Cav2.3 expression in the MHb to IPN pathway, because Gpr151-Cre is selectively expressed in MHb (Kobayashi et al., 2013). Crossing with Ai9 reporter mice, we found that the majority of Cav2.3 positive terminals in IPN expressed tdTomato in Gpr151-Cre mice, whereas only 12% and 25% of Cav2.3 positive terminals expressed tdTomato in Tac1-Cre and ChAT-IRES-Cre mice, respectively. Preliminary results showed that Gpr151 Cre rescued some of FAST Cav2.3 KO phenotypes, suggesting a role of presynaptic Cav2.3 in the MHb to IPN pathway.

Disclosures: P. Bhandari: None. L.K. Parajuli: None. K. Takao: None. T. Miyakawa: None. Y. Kobayashi: None. K.F. Tanaka: None. R. Shigemoto: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.11/J9

Topic: B.04. Ion Channels

Title: Cacna1a loss-of-function mutations impair synaptic release properties from frontal cortical fast-spiking interneurons and result in cognitive and behavioural impairments in humans and mice

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Abstract: Loss-of-function mutations in the *CACNA1A* gene, which encodes the α1 subunit of voltage-gated $Ca_V2.1$ channels, result in episodic ataxia (EA2) in humans. These conditions are rarely associated with overt cognitive deficits. We investigated 16 patients from 4 non-consanguinous families carrying different *CACNA1A* loss-of-function mutations and revealed that the majority of them had moderate to significant neurocognitive impairment that includes a spectrum of impulsivity, learning difficulties, intellectual deficiency and autism. Targeted deletion of *Cacna1a* causing an ablation of voltage-gated $Ca_V2.1$ Ca^{2+} channels selectively in forebrain GABAergic interneurons (INs) in mice leads to selective synaptic impairment of parvalbumin (PV) fast-spiking basket cells that is sufficient to induce generalised epilepsy. We therefore, propose that a selective impairment of perisomatic inhibition resulting from PV-INs synaptic dysfunction in neocortical orbitofrontal circuits might contribute to the cognitive deficits observed. To understand the pathological mechanisms underlying these cognitive deficits, we studied the effects of $Ca_V2.1$ channel ablation in PV neurons that are thought to be critical for cognition. We generated mutant mice carrying a targeted heterozygous *Cacna1a* deletion restricted to PV neuronal populations ($PV^{cre};Cacna1a^{c/+}$), which targets cortical PV-INs post-natally. Using optogenetic, we demonstrated that this selective mutation significantly impairs perisomatic inhibition of pyramidal cells in the orbitofrontal cortex (cOF). We assessed the behavioural and cognitive abilities of these mutant mice in the Open Field, the Elevated Plus Maze, the Morris Water Maze and an Attention Set-Shifting Task. These investigations revealed that the haploinsufficiency of *Cacna1a* in PV-INs leads to impulsivity and reduced cognitive flexibility in $PV^{cre};Cacna1a^{c/+}$ mutant mice. Surprisingly, contrary to what we had observed in mice with homozygous *Cacna1a* deletion in cortical pyramidal cells, mice carrying a heterozygous *Cacna1a* deletion in pyramidal cells ($Emx1^{Cre};Cacna1a^{c/+}$) had intact cortical excitability and had no behavioural impairment. Finally, local AAV-Cre injections in the cOF of $Cacna1a^{c/+}$ mice recapitulated the reversal-learning deficit and cognitive rigidity phenotypes. Our results demonstrate that the haploinsufficiency of *Cacna1a* selectively impairs the synaptic

release properties from fast-spiking PV+ basket cells and that it results in significant cognitive and behavioural impairment in humans and mice, in part attributable to disrupted perisomatic inhibition in orbitofrontal circuits.

Disclosures: A. Lupien-Meilleur: None. X. Jiang: None. I. Riebe: None. L. Damaj: None. C. Vanasse: None. L. Gagnon: None. J. Lacaille: None. E. Rossignol: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.12/J10

Topic: B.04. Ion Channels

Title: Regulation of $Ca_V1.3$ channels by the cGMP-PKG signaling pathway

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Abstract: cGMP is a second messenger widely used in the nervous system as well as in other tissues including the vascular smooth muscle and the inner ear. One of the major effectors for cGMP is the serine/threonine protein kinase, cGMP-dependent protein kinase (PKG). PKG has been shown to catalyze the phosphorylation of a variety of physiologically relevant proteins including ion channels. Previously, it has been shown that the cGMP-PKG signaling pathway inhibits the high voltage-gated Ca^{2+} current recorded in rat vestibular hair cells. This current mainly flow through $Ca_V1.3$ L-type calcium channels, and may be important for controlling vestibular hair cell sensory function. However, the role of the PKG phosphorylation in channel function is still poorly defined. In the present report we used a strategy that combines molecular biology with patch clamp recordings to investigate whether the cGMP-PKG signaling cascade regulates recombinant $Ca_V1.3$ channels heterologously expressed in HEK-293 cells. We first evaluated the properties of the whole-cell currents through $Ca_V1.3$ channels, and then tested the action of 8-bromo-cGMP (8-Br-cGMP), a cell-permeable analog of cGMP, on $Ca_V1.3$ channel activity. Our results show that 8-Br-cGMP significantly inhibited the macroscopic current, while KT-5823, a specific inhibitor of PGK, prevented the inhibition generated by 8-BrcGMP. Further, mutating the putative phosphorylation sites to unphosphorylatable residues showed that the relevant PKG site for $Ca_V1.3$ L-type channel regulation centers on a single residue, serine 860, located in the intracellular loop connecting the II and III repeats of the Cavα1 ion conducting

subunit of the channel. These findings suggest a mechanism for how the cGMP-PKG signaling pathway may regulate $Ca_V1.3$ channels.

Disclosures: A. Sandoval: None. P. Durán: None. M.A. Gandini: None. V. Castillo: None. A. Andrade: None. R. Felix: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.13/J11

Topic: B.04. Ion Channels

Support: FWF P27031

FWF P27392

FWF F4415

FWF W1101

Title: AChR pre-patterning in early neuromuscular junction development does not require L-type calcium currents

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Abstract: During development neuromuscular junctions (NMJ) organize within a narrow endplate band in the center of muscle fibers. Whereas nerve-derived factors are important for the formation and stabilization of NMJ, the initial formation of acetylcholine receptor (AChR) clusters in the central endplate band—a process called AChR pre-patterning—occurs prior to the arrival of the nerve by muscle-intrinsic mechanisms. A recent study implicated an essential role of L-type calcium currents (LTCCs) in AChR pre-patterning (Chen et al., 2011, Nat. Neurosci. 14: 570-7). According to this study, LTCCs are required for proper AChR pre-patterning independent of the role of $Ca_v1.1$ as voltage sensor in excitation-contraction (EC) coupling. Our laboratory discovered a developmental splice variant of $Ca_v1.1$, which lacks exon 29 ($Ca_v1.1e$), is expressed at the critical stage for AChR pre-patterning and, at variance with the adult $Ca_V1.1a$ splice variant, conducts sizeable calcium currents (Tuluc et al., 2009, Biophys. J. 96: 35-44).

Here we used a range of genetic calcium channel mouse models to study whether LTCCs through $Ca_v1.1e$ are essential for AChR pre-pattering and how the developmental switch of $Ca_v1.1$ splice variants might contribute to the aggregation of AChR clusters in the endplate band. Consistent with the published evidence total lack of LTCCs and calcium release from sarcoplasmic reticulum (SR) in dysgenic ($Ca_v1.1^{-/-}$) mice led to failed AChR pre-patterning and unrestricted outgrowth of the phrenic nerve in E14.5 diaphragm muscle. In contrast, lack of SR calcium release by itself in dyspedic mice (ryanodine receptor, $RyR^{-/-}$) resulted in normal AChR pre-patterning. If spatial or temporal differences in the expression of the well-conducting $Ca_v1.1e$ and the low-conducting $Ca_V1.1a$ splice variants contributed to the organization of NMJ in the endplate band, AChR pre-patterning should fail in a mouse model in which $Ca_v1.1$ exon 29 is not spliced out during development ($Ca_v1.1\Delta E29$; Sultana et al., 2016, Development, 143: 1547-1559). However this was not the case. Therefore we re-examined the basic hypothesis that LTCCs are required for AChR pre-patterning, using a knock-in mouse model exclusively expressing non-conducting $Ca_v1.1$ ($DHPR^{nc/nc}$; Dayal et al., 2014, Biophys. J., doi: 10.1016/j.bpj.2013.11.748). Unexpectedly, when LTCCs are lacking but EC coupling remained intact, pre-patterning and innervation of AChR clusters were normal. If indeed calcium is involved in the regulation of AChR pre-patterning, calcium signals from either source (LTCCs or SR calcium release) may be sufficient.

Disclosures: M.M. Kaplan: None. N. Sultana: None. A. Benedetti: None. A. Dayal: None. M. Grabner: None. N.F. Linde: None. S. Papadopoulos: None. G.J. Obermair: None. B.E. Flucher: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.14/J12

Topic: B.04. Ion Channels

Support: "973" of China: 2013CB835100

NSFC of China: 31470054

Title: Cannabinoid CB1 and CB2 receptors dichotomously modulate L- and T-type Ca^{2+} channels in rat retinal ganglion cells

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Abstract: Endocannabinoid signaling system is involved in regulating multiple neuronal functions in the central nervous system by activating G-protein coupled cannabinoid CB1 and CB2 receptors (CB1Rs and CB2Rs). Growing evidence has shown that CB1Rs and CB2Rs are extensively expressed in the vertebrate retinas. Here, we explored possible modulation of Ca^{2+} channels by activating CB1Rs/CB2Rs in acutely isolated rat retinal ganglion cells (RGCs) using patch-clamp techniques. The CB1R agonist WIN55212-2 voltage- and dose-dependently suppressed both peak and steady-state Ca^{2+} currents, with IC_{50} being 9.6 μM and 8.4 μM, respectively. Under our conditions, the high- and the low-voltage-activated Ca^{2+} currents were mainly mediated by L- and T-type Ca^{2+} channels respectively. Immunocytochemistry further confirmed that $Ca_V1.2$ subunit of L-type Ca^{2+} channels, and $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ subunits of T-type Ca^{2+} channels were expressed in RGCs. Either WIN55121-2 or ACEA, another CB1R agonist, significantly suppressed both L- and T-type Ca^{2+} currents, and accelerated inactivation of T-type one. The effect of WIN55121-2 on L-type Ca^{2+} channels was mediated by intracellular cAMP/protein kinase A (PKA), mitogen-activated protein kinase (MPKA)/extracellular signal-regulated kinase (ERK) and calcium/calmodulin-dependent protein kinase II (CaMKII) signaling pathways, while only CaMKII signaling pathway was involved in the effect of WIN on T-type Ca^{2+} channels. Furthermore, neither CB65 nor HU308, two specific CB2R agonists, had effect on L-type Ca^{2+} currents. In contrast, CB65/HU308 significantly suppressed T-type Ca^{2+} currents, which was mediated by intracellular cAMP/PKA and CaMKII signaling pathways. Our results suggest that activation of CB1Rs and CB2Rs dichotomously modulates L- and T-type Ca^{2+} channels in rat RGCs, which was mediated by the distinct intracellular signaling pathways respectively. These results imply that cannabinoids may inhibit Ca^{2+} channels of RGCs and subsequently modulate visual integrative processing.

Disclosures: Z. Wang: None. W. Qian: None. N. Yin: None. F. Gao: None. Y. Miao: None. Q. Li: None. X. Yang: None. X. Sun: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.15/J13

Topic: B.04. Ion Channels

Support: NIH Grant 5 F31 NS086226-03

Title: Proteolytic processing of the L-type calcium channel Cav1.2 alpha$_1$ subunit occurs mostly at its C-terminus

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Abstract: The L-type Ca^{2+} channel $Ca_v1.2$ accounts for 80% of L-type current in neurons and strongly impacts Ca^{2+}-mediated gene expression, neuronal excitability, and synaptic plasticity. Posttranslational modifications that regulate this channel, particularly the pore-forming alpha$_1$ subunit, continue to emerge despite decades of focus. We characterized the main size variants of brain $Ca_v1.2$ using five different antibodies to demonstrate that alpha$_1$ exists mostly as two size forms of ~ 250 and 200 kD in molecular weight. Whole brain tissue and acute slices from mice and rats were homogenized for immunoblot analysis with these antibodies. Our work demonstrates that the alpha$_1$ short form in rodent brain lacks about 350 residues of the distal C-terminus compared to the 250 kDa full length form. Analogous results were obtained after surface biotinylation of slices and pull down with streptavidin. Importantly, specificity of all five antibodies was validated by absence of signal in whole brain lysate from conditional Cav1.2 knock-out mice. Very weak immunoreactivity (<2%) indicated the presence of additional, smaller (<200 kD) forms, although in much lower abundance. Detection of two prominent alpha$_1$ size variants of 250 and 200 kD is consistent with earlier findings that calpain cleaves the long alpha$_1$ C-terminus pretty exactly in its middle to yield a shorter channel form.

Disclosures: O. Buonarati: None. P.B. Henderson: None. J.W. Hell: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.16/J14

Topic: B.04. Ion Channels

Support: Italian Institute of Technology

Title: Alternative splicing of P/Q-type Ca^{2+} channels controls neurotransmitter release and short-term synaptic plasticity

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Singapore, Singapore, Singapore; [4]Natl. Neurosci. Inst., Singapore, Singapore; [5]MRC Lab. for Mol. Cell Biology, UCL, London, United Kingdom; [6]RIKEN Brain Sci. Inst., Wako, Saitama, Japan

Abstract: Alternative splicing (AS) of pre-mRNAs, a process with the potential to expand proteome diversity, is especially prominent in the mammalian nervous system. For example, AS of voltage-gated Ca^{2+} channel (VGCC) α_1 subunits can potentially generate thousands of isoforms with differential properties and expression patterns, but the impact of this molecular diversity on brain function, particularly on synaptic transmission that crucially depends on VGCCs, is unknown.
We have investigated how synaptic transmission and plasticity in hippocampal pyramidal neurons is controlled by two major isoforms of P/Q-type VGCCs. Specifically, we have devised experiments to bi-directionally change their relative abundance in primary hippocampal cultures and in the hippocampus *in vivo*, and used genetically encoded Ca^{2+} indicators (GCaMPs), synapto-pHluorin, electrophysiological recordings and optogenetics to assess how AS of P/Q-type Ca^{2+} channels affects presynaptic Ca^{2+} transients, synaptic vesicle release, synaptic transmission and short-term synaptic plasticity.
We find that alternative splicing of $Ca_v2.1$ affects short-term synaptic plasticity in opposite directions as a consequence of a differential coupling to the neurotransmitter release machinery, with one isoform promoting synaptic depression and the other synaptic facilitation.
We propose that alternative splicing of P/Q-type Ca^{2+} channels is a key mechanism for controlling neurotransmitter release and short-term synaptic plasticity.

Disclosures: A. Thalhammer: None. A. Contestabile: None. T. Soong: None. Y. Goda: None. L.A. Cingolani: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.17/J15

Topic: B.04. Ion Channels

Support: NIH grant NS055251 (DL)

Title: The cellular mechanism that controls cell-specific e37a inclusion during alternative splicing of voltage-gated $Ca_V2.2$ channel pre mRNA

Authors: *E. J. LOPEZ SOTO, S. E. ALLEN, K. PATEL, D. M. DUBREUIL, S. DENOME, D. LIPSCOMBE;
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Abstract: Voltage-gated calcium $Ca_V2.2$ channels control transmitter release at nociceptor terminals in the dorsal horn of the spinal cord. Nociception is severely impaired in mice lacking $Ca_V2.2$ channels, and neuromodulators that inhibit $Ca_V2.2$ channel gating via G protein coupled receptors (GPCR) are analgesic. Cell-specific alternative splicing of $Ca_V2.2$ pre mRNA generates $Ca_V2.2$ isoforms that differ by their biophysical properties, trafficking, and modulation by GPCRs. Splicing factors regulate the pattern of exon inclusion or skipping in different types of cells and at different developmental time points. In nociceptors, the pattern of exon inclusion during alternative splicing of $Ca_V2.2$ pre mRNA is different compared to most other neurons. Specifically, e37a rather than e37b is included with higher frequency in $Ca_V2.2$ mRNAs in nociceptors. The resultant $Ca_V2.2$-e37a channels are more efficiently trafficked to the plasma membrane, and more sensitive to GPCR regulation compared to $Ca_V2.2$-e37b channels. This cell-specific splicing event also impacts the efficacy of morphine analgesia against noxious thermal stimulation in naïve animals *in vivo*. Here we elucidate the likely cell-specific mechanism that facilitates inclusion of e37a over e37b during alternative splicing of $Ca_V2.2$ pre mRNA. We analyzed genome-wide chromatin immunoprecipitation and sequencing data in the e37 region of *Cacna1b* and located a strong binding signal for the multi-zinc finger DNA binding protein CCCTC-binding factor (CTCF). CTCF, an insulator of DNA, is implicated in promoting the recognition of weak splice junctions in the CD45 gene. CTCF binding is inhibited by methylation. We find that CTCF binding promotes e37a recognition during splicing and that methylation of DNA inhibits CTCF action. We used electrophoretic mobility shift assays to show that recombinant CTCF binds a 139 bp sequence of *Cacna1b* gDNA containing e37a, 22 bp of the 5' intron, and 20 bp of the 3' intron, but not the equivalent e37b region of gDNA. The major determinant of CTCF binding resides within a 60 bp region of *Cacna1b* containing e37a. We used 10 μM of 5-Azacitidine to inhibit DNA methylation in neuronal-derived F11 cell lines that express $Ca_V2.2$-e37b but not $Ca_V2.2$-e37a mRNAs. Following demethylation we detected e37a-containing $Ca_V2.2$ mRNAs in F11 cells. We generated a 15.3 Kb *Cacna1b* minigene containing e36 through e38. When we transduced this minigene in tsA201 cells, we found that overexpression of CTCF promoted e37a inclusion. Our data support the hypothesis that CTCF binding to *Cacna1b* gDNA within e37a is required to promote e37a recognition and its inclusion in $Ca_V2.2$ mRNA during alternative splicing.

Disclosures: E.J. Lopez Soto: None. S.E. Allen: None. K. Patel: None. D.M. DuBreuil: None. S. Denome: None. D. Lipscombe: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.18/J16

Topic: B.04. Ion Channels

Support: UNAM-DGAPA-PAPIIT IN218016

UNAM-DGAPA-PAPIIT IV100116

CONACYT #512960

Programa de Apoyo a los Estudios de Posgrado (PAEP)

Title: PIP_2 in pancreatic β-cells regulates voltage-gated calcium channels by a voltage-independent pathway

Authors: L. DE LA CRUZ[1], E. I. PUENTE[1], A. REYES-VACA[1], I. ARENAS[1], J. GARDUÑO[1], J. BRAVO-MARTÍNEZ[1], *D. E. GARCIA-DIAZ[1,2];
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Abstract: Phosphatidylinositol-4,5-bisphosphate (PIP_2) is a membrane phosphoinositide that regulates multiple physiological processes. Recent evidence has suggested that the PIP_2 molecule is required for the activity of many transporters and ion channels, including voltage-gated Ca^{2+} (Ca_V) channels. Ion channel regulation by PIP_2 has been reported in neurons and in some expression systems, however it has not been tested in insulin-releasing cells. Insulin secretion is primarily due to calcium influx through Ca_V channels. The purpose of this study was to investigate whether Ca_V channels are regulated by PIP_2 hydrolysis in pancreatic β- and INS-1 cells through the activation of a muscarinic pathway by oxotremorine methiodide (Oxo-M). Ca_V channel currents were recorded by using the patch clamp technique in whole cell configuration. Results showed that Ca_V current amplitude was reduced by the activation of muscarinic receptor 1 (M_1R) in the absence of kinetic changes in either pancreatic β- or INS-1 cells. Oxo-M inhibition exhibited the hallmarks of voltage-independent (VI) regulation, which was prevented by diC8-PIP_2 dialysis in pancretic β-cells. PIP_2 localization into the plasma membrane was examined by transfecting INS-1 cells with the pleckstrin homology domain of phospholipase C-δ1 (PH-PLCδ1), which revealed a close time-course relationship between PIP_2 hydrolysis and Ca_V channel inhibition. Furthermore, the depletion of PIP_2 by a voltage-sensitive phosphatase reduced Ca_V currents in a similar way to that observed following M_1R activation. Isolated L-type current was inhibited by M_1R activation suggesting that this current is regulated by PIP_2 as well. We conclude that the activation of the M_1R pathway results in VI inhibition of Ca_V channels in

pancreatic β- and INS-1 cells via PIP_2 depletion. Activation of cytosolic second-messenger pathways is not required for this inhibition on Ca_V channel currents. These findings support the hypothesis that membrane phospholipids regulate ion channel activity by interacting directly with Ca_V channels in insulin-releasing cells.

Disclosures: L. de la Cruz: None. E.I. Puente: None. A. Reyes-Vaca: None. I. Arenas: None. J. Garduño: None. J. Bravo-Martínez: None. D.E. Garcia-Diaz: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.19/J17

Topic: B.04. Ion Channels

Support: NICHD Intramural

Title: Loss of regulated Cav2.3 expression in a mouse model of Fragile X Syndrome

Authors: *E. E. GRAY[1], Y. LIU[2], I. TRANG[2], D. A. HOFFMAN[2];
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Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability in humans and arises from the loss of the fragile X mental retardation protein (FMRP), an mRNA binding protein that regulates translation in neurons following activation of group I metabotropic glutamate receptors (GpI mGluRs). When bound to its target mRNAs under basal conditions FMRP acts as a translational repressor, whereas GpI mGluR activity leads to the unbinding of FMRP to allow protein translation. In the FMRP knock-out mouse, expression of target mRNAs is no longer regulated and this ultimately results in aberrant spine formation, enhanced neuronal plasticity, and hyperexcitability. Little is known about which particular mRNAs are abnormally expressed in FXS or how they might impact neuronal function. A recent study found that FMRP binds the mRNA of Cav2.3 (Darnell et al, 2011), a voltage-gated calcium channel capable of generating large calcium spikes and shaping action potentials. Given that neurons lacking FMRP have alterations in calcium spiking and neuronal excitability, we sought to explore the possibility that FMRP regulates the expression of Cav2.3 mRNA. We first examined the basal levels of mRNA isolated from WT and FMRP KO mouse brains using qRT-PCR and found that Cav2.3 mRNA levels were altered in FMRP KO neurons ($p<0.05$). We were curious if this change in mRNA abundance would be reflected in the amount of Cav2.3 expressed in the FMRP KO brain, thus we performed Western blotting and found that Cav2.3 protein levels were significantly enhanced in the FMRP KO ($p<0.01$ vs WT). This FMRP-

dependent change in Cav2.3 expression should impact neuronal physiology; therefore we isolated Cav2.3 currents using whole cell electrophysiology in cultured hippocampal neurons. As predicted from our Western blot data, FMRP KO neurons exhibited greatly enhanced Cav2.3 current amplitudes ($p<0.005$), although the kinetics of the currents remained unchanged. To test the possibility that FMRP-dependent Cav2.3 expression can be further regulated by GpI mGluRs, we treated hippocampal neurons cultured from WT and FMRP KO mice with the GpI mGluR agonist DHPG. Following stimulation, Cav2.3 protein levels were increased in WT neurons ($p<0.005$) but not in neurons lacking FMRP. Taken together, our data suggests that FMRP represses translation of Cav2.3 under basal conditions and that activation of GpI mGluRs disinhibits FMRP to allow for active translation of Cav2.3. The loss of regulated Cav2.3 expression seen in the FMRP KO may underlie the atypical calcium signaling and neuronal excitability seen in FXS, and may be a potential therapeutic target in this disease.

Disclosures: E.E. Gray: None. Y. Liu: None. I. Trang: None. D.A. Hoffman: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.20/J18

Topic: B.04. Ion Channels

Support: RO1NS084190

T32NS045549

DC009433

Title: Molecular determinants masking Ca^{2+}-dependent facilitation of $Ca_v2.2$ channels

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Abstract: Voltage-gated $Ca_v2.1$ (P/Q-type) Ca^{2+} channels undergo Ca^{2+}- dependent inactivation (CDI) and facilitation (CDF), respectively, which contribute to short-term synaptic plasticity. Both CDI and CDF are mediated by calmodulin (CaM) binding to sites in the C-terminal domain of the $Ca_v2.1$ α_1 subunit, including a consensus CaM-binding IQ-domain. Closely related $Ca_v2.2$ (N-type) channels display CDI but not CDF, despite conservation of the IQ-domain and additional sites (Pre-IQ, EF-hand) implicated in regulation of CDF of $Ca_v2.1$. Here, we test the hypothesis $Ca_v2.2$ does not undergo CDF because it lacks additional key elements in the C-

terminal domain that are required for CDF. We find that alternative splicing of exons in the proximal and distal C-terminal domain, which regulates CDF of $Ca_v2.1$, has no effect on CDF of $Ca_v2.2$. However, replacement of the entire C-terminal domain of $Ca_v2.2$ with that of $Ca_v2.1$ produces robust CDF of the chimeric channel. Further analyses reveal that transfer of the proximal C-terminal domain of $Ca_v2.1$, which includes the EF-hand, Pre-IQ- IQ domains, and a downstream CaM-binding domain (CBD), is sufficient to support CDF in chimeric $Ca_v2.2$ channels. Our results highlight the importance of the C-terminal domain in distinguishing Ca^{2+} feedback regulation of $Ca_v2.2$ and $Ca_v2.1$, and underscore how molecular distinctions may underlie the unique contributions of these channels in regulating neurotransmitter release.

Disclosures: J.R. Thomas: None. J. Hagen: None. A. Lee: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.21/K1

Topic: B.04. Ion Channels

Support: CNPq

CAPES

Funcap

Title: Essential oil of Alpinia zerumbet inhibits low and high voltage activated calcium channels in sensory neurons

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Abstract: Among natural products, essential oils and their constituents are promising sources of therapeutic agents with pharmacological efficacy and low toxicity. *Alpinia zerumbet* have widespread use in folk medicine. The essential oil of *Alpinia zerumbet* (EOAZ) is documented to have diverse pharmacological effects, including antinociceptive activity and inhibitory effects on nervous excitability. However, despite its use, little is known about its mechanism of action on excitable tissues, especially on the peripheral sensory nervous system. This study aims to investigate whether EOAz blocks the neuronal voltage-dependent Ca^{+2} current which, believed to be important for several pharmacological effects, is thought to be important for nociception mechanism of action. Dissociated small neurons of the dorsal root ganglion of Wistar rats were

used. The patch-clamp technique in the whole cell configuration was employed. EOAz (major constituents: 1-beta-pinene (23.25% of EO wheight), 1,8-cineole (21.75%), terpinen-4-ol (15.94%), gamma-terpinene (7.85%)) dose-dependently inhibited the High Voltage Activated (HVA) and Low Voltage Activated (LVA) Ca^{+2} currents. At the concentrations tested, OEAz (0.03 to 10 mg/ml) did not completely block LVA Ca^{+2} current; the maximal inhibition and IC_{50} of LVA were 67.88 ± 1.93 % and 1.6 ± 0.19 mg/ml (n = 6). Concerning the HVA Ca^{+2} current, it was completely blocked by OEAz (0.01 to 3 mg/ml) with 0.4 ± 0.3 mg/ml (n = 6) IC_{50}. The results show that the EOAz blocks the two types on voltage-dependent neuronal Ca^{+2} channels, being more efficient and powerful on HVA than LVA Ca^{+2} currents. The alterations in Ca^{+2} channels reported here might partially explain the antinociceptive effect of EOAz.

Disclosures: T. Santos-Nascimento: None. K.M. Veras: None. A.N. Coelho-de-souza: None. L. Moreira-júnior: None. J.H. Leal-cardoso: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.22/K2

Topic: B.04. Ion Channels

Support: MOST-Taiwan grant 102-2320-B-002-021-MY3

MOST-Taiwan grant 104-2320-B-010-011

Title: Protein degradation mechanism of $Ca_V2.1$ (P/Q-type) Ca^{2+} channel

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Abstract: The voltage-gated Ca^{2+} (Ca_V) channel is a multi-subunit membrane protein complex comprising the voltage-sensing, pore-forming α_1 subunit, as well as the auxiliary β and $\alpha_2\delta$ subunits. $Ca_V2.1$ (P/Q-type) is a distinct member of Ca_V α_1 subunit subfamilies that is expressed in central and peripheral neurons. The subcellular localization of $Ca_V2.1$ channels is characterized by their high densities at the active zones on the pre-synaptic membrane. In addition, $Ca_V2.1$ channels are also notably found in the dendrosomatic compartment of postsynaptic neurons, especially in Purkinje cells in the cerebellum. Therefore $Ca_V2.1$ channels play a critical role in regulating neurotransmitter release at presynaptic terminals, as well as setting postsynaptic membrane excitability. Mutations in the gene encoding the α_1 subunit of human $Ca_V2.1$ channels have been associated with the dominantly inherited cerebellar disease

episodic ataxia type 2 (EA2). Several lines of experimental evidence strongly suggest that EA2-associated mutations may result in impaired membrane trafficking and enhanced protein degradation of wild-type (WT) as well as mutant $Ca_V2.1$ α_1 subunits. The molecular nature and mechanistic role of the ubiquitin-proteasome pathway in $Ca_V2.1$ protein biosynthesis is currently unknown. In this study, we aim to ascertain the protein degradation mechanism of WT $Ca_V2.1$ and EA2 mutants by addressing how $Ca_V2.1$ channels are regulated by the ubiquitin-proteasome system. We identified a zinc finger protein that co-exists in the same protein complex and co-localizes with $Ca_V2.1$ channels in rat neurons. Upon heterologous expression in human embryonic 293T (HEK293T) cells, the zinc finger protein regulates the protein level, turn-over rate, and ubiquitination of human $Ca_V2.1$ channels. Disruption of the endogenous expression of the zinc finger protein in HEK293T cells significantly increases protein level of WT human $Ca_V2.1$ channels, as well as reversing the defective protein expression of EA2 mutants. Together our data suggest that the zinc finger protein may serve as a ubiquitin ligase that contributes to EA2-associated protein degradation of human $Ca_V2.1$ channels.

Disclosures: C. Tang: None. S. Fu: None. C. Jeng: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.23/K3

Topic: B.04. Ion Channels

Support: R01EY020850

R01DC009433

R01NS084190

T32NS007421

R25GM058939

Title: C-terminal splice variation reveals new insights into calmodulin regulation of $Ca_v1.4$ channels

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Abstract: In synaptic terminals of retinal photoreceptors, $Ca_v1.4$ (L-type) Ca^{2+} channels mediate Ca^{2+} influx that promotes neurotransmitter release. Mutations in $Ca_v1.4$ are associated with multiple vision disorders including congenital stationary night blindness (CSNB2). $Ca_v1.4$ does not undergo Ca^{2+}-dependent inactivation (CDI) – a negative feedback mechanism seen for other L-type channels (e.g., $Ca_v1.2$ and $Ca_v1.3$) mediated by calmodulin (CaM) binding to a consensus IQ domain in proximal C-terminal domain (CT) of the pore-forming subunit. The lack of CDI in $Ca_v1.4$ is due to a C-terminal automodulatory domain (CTM), located in the distal CT of $Ca_v1.4$. The CTM is thought to suppress CDI of $Ca_v1.4$ channels by competing with CaM binding to sites in the proximal CT. A CSNB2-causing mutation (K1591X) in $Ca_v1.4$ that deletes the CTM promotes CaM binding and CDI, but also causes channel activation at more negative potentials than full-length channels ($Ca_v1.4_{FL}$). Here, we demonstrate that similar properties are exhibited by a naturally occurring human $Ca_v1.4$ splice variant lacking exon 47 ($Ca_v1.4\Delta ex47$), and characterize an unexpected role for CaM in the regulation of this channel. By quantitative PCR, we found that $Ca_v1.4\Delta ex47$, which lacks the initial 43 amino acids of the CTM, is highly expressed in primate but not mouse retina. In electrophysiological recordings of transfected HEK293T cells, $Ca_v1.4\Delta ex47$ Ca^{2+} currents activate at more negative voltages and display stronger CDI than $Ca_v1.4_{FL}$, similar to K1591X. These effects were blunted by IQ domain mutations known to disrupt CaM binding to $Ca_v1.4$. Mutations that prevent Ca^{2+} binding to either N- or C-terminal lobes of CaM suppress CDI of $Ca_v1.4\Delta ex47$. However, mutations in the N-terminal but not the C-terminal lobe of CaM abolish the hyperpolarizing effect of exon 47 deletion on voltage-dependent activation of Ca^{2+} currents. We conclude that exon 47 contains key molecular determinants within the CTM for regulating CDI and activation, and that CaM plays distinct roles in these processes.

Disclosures: B. Williams: None. V. Kerov: None. F. Haeseleer: None. A. Lee: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.24/K4

Topic: B.04. Ion Channels

Support: GM-102525

Title: The role of T-type calcium channels in the development of incisional pain model in rats

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Abstract: Introduction: Recent studies have implicated T-type Ca^{2+} channels in regulation of neuronal excitability under physiological conditions, as well as in pathological states, such as epilepsy and chronic pain. However, their role in acute pain resulting from the tissue injury is yet to be established. Therefore, the aim of our study was to investigate if T-type Ca^{2+} channels (T-channels) are involved in development of acute post-operative pain.
Methods: Incisional pain model in Sprague-Dawley rats was developed by performing deep tissue incision of plantar surface of hind paw. To investigate changes in expression of T-channel subtypes in incised vs. sham animals, ipsilateral L4-L6 dorsal root ganglia (DRG) were harvested and tissue samples were analyzed using qRT-PCR. Changes in current densities and kinetic properties of these channels were measured by using a patch-clamp technique on dissociated DRG cells. To assess antihyperalgesic efficacy of selective T-channel antagonist (TTA-P2), hind paw responses were measured after thermal and mechanical stimulation of the plantar paw surface.
Results: Our qRT-PCR experiments revealed a significant increase of mRNA expression levels of all three T-channel subtypes ($Ca_V3.1$, 3.2 and 3.3) 24 h post-incision vs. sham group, with the highest (three-fold) increase of $Ca_V3.1$ isoform. Patch-clamp recordings from dissociated DRG cells showed that current densities for both activation and steady-state inactivation protocols were profoundly increased (about 2-fold) at 2 h post-surgery, as compared to the control (sham) group. The same trend was noticed for the 24 h post-surgery group. When applied intrathecally in healthy rats, TTA-P2 exerted antinociceptive effect to both thermal ($F(1,210)=7.46$; $p<0.001$) and mechanical stimuli ($F(1,198)=7.18$; $p<0.001$). In incised rats, a single preemptive intrathecal injection of TTA-P2 significantly reduced thermal hyperalgesia post-incision ($F(1,60)=11.59$; $p=0.005$). Mechanical hyperalgesia was also reduced ($F(1,36)=13.61$; $p=0.004$) when TTA-P2 was applied in three consecutive post-operative days.
Conclusion: We found increased T-current density in DRG cells from incised rats as well as increased expression of mRNA for all three T-type Ca^{2+} channel isoforms, with $Ca_V3.1$ isoform exhibiting the highest mRNA levels. Furthermore, when applied either before or after skin incision, a significant
analgesic effect of T-channel blocker was detected in *in vivo* study. In conclusion, our results suggest that T-type Ca^{2+} channels play an important role in the development of acute pain and could be considered as new drug target for alleviating postsurgical hyperalgesia.

Disclosures: S. Joksimovic: None. S.M. Joksimovic: None. H. Osuru: None. P. Eggan: None. V. Jevtovic-Todorovic: None. S.M. Todorovic: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.25/K5

Topic: B.04. Ion Channels

Support: DFG SFB 894

DFG SFB 1027

FWF P24079

SFB F4406

Title: $\alpha_2\delta2$ controls function and trans-synaptic coupling of $Ca_v1.3$ channels in mouse inner hair cells and is essential for normal hearing

Authors: *J. ENGEL[1], B. FELL[1], S. ECKRICH[1], K. BLUM[1], T. ECKRICH[1], D. HECKER[1], G. J. OBERMAIR[2], V. FLOCKERZI[1], B. SCHICK[1];
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Abstract: Auxiliary $\alpha_2\delta$ subunits can modulate the abundance and function of voltage-gated calcium channels. Here we show that of the four $\alpha_2\delta$ isoforms 1-4, $\alpha_2\delta2$ mRNA is expressed in neonatal and mature inner hair cells (IHCs). In a functional $\alpha_2\delta2$ null mouse, the ducky mouse (du), auditory brainstem response (ABR) click and frequency-dependent hearing thresholds were elevated whereas otoacoustic emissions were not impaired. Peak Ca^{2+} channel currents of mature du/du IHCs were reduced by 30% in the lower frequency range and by 40% in the range of best hearing, respectively. Moreover, gating properties such as voltage of half-maximum activation and voltage sensitivity were altered, indicating that $Ca_v1.3$ channels normally co-assemble with $\alpha_2\delta2$ at IHC presynapses. Depolarization-evoked exocytosis in du/du IHCs was reduced, reflecting their reduced Ca^{2+} currents, but Ca^{2+} efficiency of exocytosis was unaltered. $Ca_v1.3$ and $Ca_v\beta2$ protein expression was unchanged in du/du IHCs, forming clusters at synaptic ribbons. However, the close apposition of presynaptic $Ca_v1.3$ clusters with postsynaptic glutamate receptors 4 (GluA4) and PSD-95 clusters was significantly impaired in du/du mice. This implies that in addition to controlling both expression and gating properties of $Ca_v1.3$ channels, the largely extracellularly localized $\alpha_2\delta2$ subunit plays a so far unknown role in mediating trans-synaptic alignment of presynaptic Ca^{2+} channels and postsynaptic glutamate receptors.

Disclosures: J. Engel: None. B. Fell: None. S. Eckrich: None. K. Blum: None. T. Eckrich: None. D. Hecker: None. G.J. Obermair: None. V. Flockerzi: None. B. Schick: None.

Poster

220. Voltage-Gated Ca2+ Channels

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 220.26/K6

Topic: B.04. Ion Channels

Support: Telethon Foundation (grant # GGP15110)

Austrian Science Fund (FWF P27809)

Title: Disruption of the Cav1.3 C-terminal auto-modulatory domain attenuates Cav 1.3/BK channel coupling in mouse chromaffin cells

Authors: *L. GUARINA[1], D. H. F. VANDAEL[2], A. MARCANTONI[1], J. STRIESSNIG[3], E. CARBONE[1];
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Abstract: Cav 1.3 L-type Ca^{2+} channels play key roles in controlling excitability and pacemaking of central neurons and neuroendocrine cells (*Vandael et al., 2010*). In particular, incomplete Cav1.3 channel inactivation as it occurs in human congenital multiorgan syndrome and "de novo" mutations (*Azizan et al., 2013; Scholl et al., 2013; Pinggera et al, 2015*), lead to marked L-type Ca^{2+} current increases and originate autism and intellectual impairment. To study the role of Cav1.3 channel inactivation in the regulation of cell excitability, we used the Cav1.3DCRD$^{HA/HA}$ knockin (KI) mutant mouse that was generated by replacing part of the "distal C-terminal regulatory domain" (DCRD) in exon 49 of the CACNA1D gene by homologous recombination with an HA-epitope (*Scharinger et al., 2015*). The newly available KI mice possess Cav1.3 channel isoforms with faster and more complete inactivation than wild-type mice. Here, we further investigated the effects of Cav1.3DCRD$^{HA/HA}$ mutation in mouse chromaffin cells (MCCs), that were shown to cause hyperpolarization of resting potential, reduced number of spontaneously firing cells and higher steady-state adapting frequency during sustained stimulation. Our new findings suggest that a major effect on cell excitability could derive from the attenuated coupling of Cav1.3 to BK channels in Cav1.3DCRD$^{HA/HA}$ KI MCCs. BK channels are highly expressed in MCCs and control action potential shape, pacemaking and firing modes (Marcantoni et al., 2010; Vandael et al., 2015). We found that BK channels of KI MCCs preserve their voltage-dependence but lose most of their Ca^{2+}-dependence. BK currents of KI mice change very little when activated by different Ca^{2+} preloading steps either following increasing step depolarizations (-40 to +40 mV) or increasing the duration of preloading steps (10 to 110 ms at +10mV). In few cells, BK currents were even absent. A reduced activation of BK channels was also evident by observing the reduced size of the after-hyperpolarization potential of single APs generated by brief depolarizing pulses (30-120 pA; 5 ms). Our data

suggest that Cav1.3 channels are tightly coupled to BK channels in MCCs and that alteration of the C-terminal modulatory domain can induce profound changes to the Cav1.3/BK channel activation and thus to AP shape, pacemaking and spontaneous firing.

Disclosures: L. Guarina: None. D.H.F. Vandael: None. A. Marcantoni: None. J. Striessnig: None. E. Carbone: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.01/K7

Topic: B.07. Synaptic Transmission

Support: Wellcome Trust 094385/Z/10/Z

BBSRC BB/M02556X/1

Title: Improved probes for investigating glutamate neurotransmission

Authors: N. HELASSA[1], C. DURST[2], U. ARIF[1], C. SCHULZE[2], T. OERTNER[2], *K. TÖRÖK[1];
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Abstract: Genetically-encoded fluorescent glutamate indicator iGlu-'sniffer' (iGluSnFR) has enabled detection of neurotransmitter glutamate release in tissue slice and *in vivo* experiments, however its slow rise and decay rates limit the temporal resolution of glutamate release in response to high frequency action potential firing. To improve the kinetics of iGluSnFR, selected amino acid residues coordinating glutamate at the binding site were mutated, the resulting variant proteins were expressed in *E. coli*, purified and characterised with regard to fluorescence dynamic range, affinity, glutamate association and dissociation kinetics and temperature dependence, using steady-state fluorescence and stopped-flow kinetics. Novel variants with the most improved kinetics were $iGlu_f$ (iGlu fast) and $iGlu_u$ (iGlu ultrafast). F_{max}/F_{min} values of 4 to 5 in solution were comparable to that of iGluSnFR for both $iGlu_f$ and $iGlu_u$. K_d values for glutamate were 140 μM and 600 μM, with 1.5 ms and 0.83 ms decay half-times, respectively, at 34 ^{o}C. In hippocampal slices, CA3 neurons were electroporated with an iGluSnFR construct under the control of synapsin promoter and action potentials were triggered by patch-clamp either by paired pulse ISI with a 48 ms interval or by firing 10 action potentials at 100 Hz. Post-synaptic terminals on the dendritic arbour in CA1 pyramidal neuron were imaged by two-photon microscopy. Glutamate release was detected at pre-synaptic terminals (boutons) in CA1 stratum

radiatum imaged by two-photon microscopy at 34^{o}C using spiral line scans at 500 Hz. Compared to iGluSnFR (tau_{off} of 15 ms), the $iGlu_f$ and $iGlu_u$ variants showed improved time resolution for the detection of glutamate release at the synapse with fluorescence decay times tau_{off} of 5 and 2 ms, making both novel probes suitable for application in high frequency stimulation paradigms. Models are presented for the kinetic mechanism of iGlu variants and for the synaptic handling of glutamate.

Disclosures: N. Helassa: None. C. Durst: None. U. Arif: None. C. Schulze: None. T. Oertner: None. K. Török: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.02/K8

Topic: B.07. Synaptic Transmission

Support: JSPS KAKENHI Grant Number 16J00633

Title: An integrated analysis for the dynamical responses of pre- and postsynaptic neurons through synaptic encoding in the *C. elegans* salt-chemotaxis circuit

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Abstract: Neuronal circuits are an assembly of synaptic connectivity among neurons. Analysis of both the patterns of synaptic connections in a circuit and the roles of each synapse in network activity helps to understand characteristics of a neural encoding for behavioral outputs. Synaptic connectivity in the *C. elegans* nervous system has been identified, but it is not clear how each synapse is contributed for the spatio-temporal responses of each postsynaptic neuron and for behaviors. We have focused on the *C. elegans* salt-chemosensory circuit to understand the role of each synapse on a neural encoding and to describe a computational model on this neuronal circuit. This chemosensory circuit is composed of the salt-sensory neurons ASE, their postsynaptic neurons such as AIB and AIY, and further downstream neurons. To describe the patterns of each neuronal response in the circuit, as a first step, we measured calcium response of the presynaptic ASER sensory neuron triggered by salt concentration changes. The calcium indicator G-GECO was expressed specifically in the ASER neuron, and using this transgenic animal, we collected response patterns of ASER to various patterns of salt concentration change. The ASER had a nonlinear response with the concentration change of salt. Based on these

imaging data, we generated a novel mathematical model representing its activity *in silico*. For the second step, to develop an expanded computational model in a circuit level, we examined activities of the first-layer interneurons which are connecting with the ASER and tried to evaluate the role and meaning of each synaptic connection for their activities.To analyze synaptic communications between the ASER and AIB or AIY neuron, we also monitored calcium responses of these interneurons to salt concentration changes. Our imaging analyses using the wild-type animals or sensory-input defective mutants, indicated that the ASER activates AIB interneurons whereas inactivates AIY interneurons when the salt concentration is decreased. Furthermore, by using synaptic transmission mutants and their cell-specific rescuing animals, we measured the single-input responses in postsynaptic neurons AIB and AIY, and compared their responses in animals showing variable patterns of synaptic connections. These data are used to generate *in-silico* modeling to represent each postsynaptic activity depending on both the presynaptic activity and synaptic patterns. Our analyses will suggest a novel aspect of the relationship between spatial distribution of synapse connectivity and postsynaptic responses in a neuronal circuit.

Disclosures: M. Kuramochi: None. M. Doi: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.03/K9

Topic: B.07. Synaptic Transmission

Title: Differential effects of dopaminergic D2 receptor activation on synaptic responses in a subset of layer V mouse medial prefrontal pyramidal neurons.

Authors: *J. M. LEYRER[1], M. THOMAS[2];
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Abstract: In humans, prefrontal cortical areas are known to support executive functions. In mice, these functions are mediated by homologous regions in the medial prefrontal cortex (mPFC). While it is well established that executive processes are critically dependent on optimal levels of dopamine (DA) in the PFC, the cellular mechanisms of DA modulation are incompletely understood. Stable patterns of neuronal activity may be sensitive to frequency dependent changes in synaptic transmission. In this study, we characterized the effects of D2 receptor (D2R) activation on short-term excitatory postsynaptic potential (EPSP) dynamics evoked at varying frequencies in layer V pyramidal neurons in mouse mPFC. Further, we aimed

to determine the effects of D2 receptor activation on the two major subtypes of layer V pyramidal neurons that show distinct axonal projection patterns to either the brainstem (type I cells) or the commissural mPFC (type II cells). We isolated NMDA receptor (NMDAR) and non-NMDAR receptor mediated components of EPSP trains evoked by stimulating fibers within layer V or layer I (tufts). D2R activation significantly decreased NMDAR-mediated EPSPs in type I pyramidal neurons with tuft stimulation, yet had no effect on type II cells. Additionally, D2R activation had no effect on NMDAR-mediated EPSPs with layer V stimulation in type I or type II cells. Interestingly, D2R activation also had no effect on non-NMDAR mediated EPSPS with layer I or layer V stimulation in either cell type. These results suggest that D2 receptor activation may prevent persistent activity, partly through inhibiting coincidence detection, in a specific class of (type I) layer V pyramidal neurons. Additionally, D2R activation may play a role in inhibiting synaptic plasticity and stabilizing top-down signals during working memory tasks. These data provide further insight into mechanisms of dopamine's modulation of executive functions.

Disclosures: J.M. Leyrer: None. M. Thomas: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.04/K10

Topic: B.07. Synaptic Transmission

Title: A distance-dependent gradient in presynaptic release probability along tapering dendrites

Authors: *F. GRILLO, J. BURRONE;
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Abstract: Neurons receive a large number of heterogeneous synaptic inputs all along their vast dendritic trees. Although recent advances have shed light on the biased distribution of postsynaptic compartments and their role on neuronal integration, very little is known about the distribution of presynaptic boutons along dendrites. Here, we show that the structure and function of presynaptic boutons along the thin tapering basal dendrites of CA1 pyramidal neurons decreases with distance from the soma. Recordings of EPSCs measured electrophysiologically in response to trains of stimuli delivered at varying locations in the stratum oriens of acute slices showed that distal inputs facilitate more than proximal inputs, suggesting a distance-dependent change in release probability. In agreement with our functional data, we find that the size of the active zone (as well as the volume) of presynaptic terminals also decreased with distance to the soma, as measured by 3D reconstructions of dendrites obtained

from serial block-face scanning electron microscopy (3 view). In parallel, we find that postsynaptic spines and their postsynaptic densities closely followed the distribution of presynaptic boutons, decreasing in size with both distance and dendrite diameter. We propose that the changes in synaptic transmission dynamics (short-term plasticity) linked to the graded distribution of release probability fine-tunes different sections of dendrite to respond to specific input frequencies. Our findings describe a novel form of distance-dependent distribution of synaptic properties that allow neurons to efficiently sample across large frequency domains.

Disclosures: F. Grillo: None. J. Burrone: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.05/K11

Topic: B.07. Synaptic Transmission

Support: ANR

Marie Curie

Title: Spine neck morphology shapes postsynaptic potentials in hippocampal neurons

Authors: *J. TONNESEN[1,2], D. CATTAERT[1,3], V. NÄGERL[1,2];
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Abstract: It has been a long-standing fundamental question whether the dendritic spine neck influences excitatory postsynaptic potentials and their somatic integration. While the importance of the spine neck for biochemical compartmentalization is well established, its role in electrical signaling remains highly controversial. Two main obstacles have hindered progress. 1) The average spine neck diameter is around 150 nm, which is too small to be resolved by conventional light microscopy; 2) The inaccessibility of the spine head to direct electrophysiological recordings. We have developed a new approach to address this controversy and test the hypothesis that spine necks can provide a sufficient electrical resistance to affect the EPSPs measured at the soma. It is based on a combination of STED microscopy, two-photon glutamate uncaging and patch-clamp electrophysiology in organotypic hippocampal slice cultures. To discriminate between the effects of synaptic conductance and synaptic morphology on measured voltages, we obtained matched electrophysiological recordings of synaptic conductance (g_{syn})

and voltage (V) for the same spine by performing the 2-photon glutamate uncaging both in voltage-clamp and current-clamp. In addition, we acquired super-resolved STED microscopy images to correlate the electrophysiological data to spine morphology. This strategy allowed us to normalize the synaptic EPSP measured at the soma to the strength of the synapse in terms of conductance. The V/g_{syn} ratio (or 'synaptic gain') for a given spine was compared to spine geometry obtained from the corresponding STED image. We identify a correlation between neck geometry and synaptic gain. Specifically, the longer and thinner the neck is, the lower the synaptic gain. We further find that spines with high neck resistances show a more pronounced sub-linear response to repetitive stimulation than neighboring spines with lower neck resistances. Finally, we present numerical simulations to model and explore these relationships in more detail. Our results provide strong new evidence for an influential role of spine geometry in electrical signaling, indicating that high neck resistances boost the spine head voltage to the point of gradual saturation of the synaptic potential. Conversely, spines with low neck resistances (i.e. with shorter and wider necks) can convey larger synaptic currents during strong synaptic stimulation.

Disclosures: J. Tonnesen: None. D. Cattaert: None. V. Nägerl: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.06/K12

Topic: B.07. Synaptic Transmission

Support: National basic research program of China

Natural science foundation of China

Title: Subquantalized synaptic transmission at single active zone contained central synapses

Authors: ***M. KUNPENG**[1,2], J. DAI[1], Q. ZHU[1], P. SAH[3], J. SUN[1];
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Abstract: As the classic Katz's quantal theory proposed, the small all-or-none unit, termed miniature postsynaptic potential or current(mini), in respect to presynaptic vesicle fusion,represents the basic unit or quantum of synaptic signal. However, justification of this notion, particularly the nature of mini events, has never been completed. Emerging evidences pointed out that the uniquantal events, from minis to single vesicle capacitances, are various in

size.Based on analysis ofthe amplitude of minis, it was hypothesized that each Katz's quantum could be composed of smaller subunits. This subquantal hypothesis was rigorously tested and ended in suspense because all the experimental and theoretical studies were not sufficient to support or exclude the existence of the subquantal elements. The major arguments are: all the studies were based on histogram analyses and no confidence limits have been given to evaluate the significance of these subunits; increasing the sample size of recordings decreased, instead of increased, the prominence of subunit in histogram; the subunit sizes estimated from the different subsets of the same recording were different. We attribute these discrepancies to less quantitative analyses and that the minis wererecorded from multiple synapses,because the variation of the subunit amplitude and postsynaptic sensitivity from multiple synaptic recordings prevented the detection of small subunits.Here, we selectively observed the miniatures from single active zone(AZ) contained axo-soma synapses and took the advantage of well electrical access of the somatic whole cell(SWC) recording to quantitatively analyze single quantal events with much less variability of postsynaptic response. Our study revealed significant subquantalized synaptic transmission and strongly suggested that synaptic information could be processed at higher precision and capacity than the estimation by Katz's theory.

Disclosures: M. Kunpeng: None. J. Dai: None. Q. Zhu: None. P. Sah: None. J. Sun: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.07/K13

Topic: B.07. Synaptic Transmission

Support: MINECO DPI2015-65833-P, TIN-2012-30883

MINECO FIS2013-43201-P

ONRG grant N62909-14-1-N279

Title: Selectivity to temporal information arising from the interaction between dynamic synapses and intrinsic subthreshold oscillations

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Abstract: Subthreshold oscillations underlie key mechanisms of information discrimination in single neurons while dynamic synapses provide channel-specific input modulation. Previous studies have shown that intrinsic subthreshold oscillations constitute a biophysical means for the emergence of non-trivial single-cell input/output preferences. It has also been shown that synaptic dynamics, in the form of short-term depression and/or facilitation, can provide a channel-specific mechanism for the enhancement of the post-synaptic effects of temporally specific input sequences. The interplay between intrinsic oscillations and synaptic dynamics can lead to more selective and complex temporal input processing.
Here, we investigated theoretically and computationally how the combination of intrinsic subthreshold oscillations and short-term synaptic dynamics can act together in enabling the emergence of robust and channel-specific selectivity of temporal inputs. We calculated analytically the voltage trajectories and spike output of Generalized Integrate-and-Fire (GIF) model neurons in response to temporally distinct triplets, and analyzed the model output as intrinsic and synaptic parameters were varied.
Our results show that intrinsic and synaptic dynamics interact coactively for the emergence of specific and complex input-output transformations. In particular, precise non-trivial preferences emerge from synergic intrinsic and synaptic dynamics, while broader discrimination selectivity is observed for mismatched neuronal and synaptic dynamics. We discuss the conditions for robustness of the observed input/output relationships, including those under the action of noise, and also show their presence in more realistic biophysical models.
We conclude that the interaction of intrinsic subthreshold oscillations and synaptic dynamic properties can enable complex and channel-specific mechanisms for the emergence of temporal selectiveness in neuronal responses. We also discuss the impact of single-channel/single-neuron input discrimination in the context of information processing based on heterogeneous synaptic and neuronal elements.

Disclosures: P. Varona: None. F. Baroni: None. R. Latorre: None. J.J. Torres: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.08/K14

Topic: B.07. Synaptic Transmission

Support: Lundbeck Research USA, Inc.

Title: Impact of vortioxetine on synaptic integration in prefrontal-subcortical circuits: comparisons with escitalopram.

Authors: *S. CHAKROBORTY[1], E. DALE[2], A. PEHRSON[2], C. SÁNCHEZ MORILLO[2], A. R. WEST[1];
[1]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]Lundbeck Res. USA, Inc., Paramus, NJ

Abstract: Prefrontal-subcortical and related limbic circuits support executive functions (e.g., cognition, motivated behaviors, emotional processes) which often become dysfunctional in psychiatric and neurological disorders. Current therapies for these disorders are limited and often produce considerable side effects. Vortioxetine (VORT) is an atypical antidepressant which is currently used in the clinic to treat major depressive disorder. Mechanisms of action of VORT include serotonin (5-HT) transporter blockade, $5HT_{1A}$ receptor agonism, $5HT_{1B}$ receptor partial agonism, and $5HT_{1D}$, $5HT_3$, and $5HT_7$ receptor antagonism. VORT has been shown to facilitate 5-HT transmission in the medial prefrontal cortex (mPFC), however the impact of this compound on 5-HT signaling in related prefrontal-subcortical circuits is less clear. Thus, the current study examined the impact of systemic VORT administration (0.8 mg/kg, i.v.) on spontaneous and afferent driven spike activity of electrophysiologically identified neurons recorded in the anterior cingulate cortex (ACC), medial shell of the nucleus accumbens (msNAc), and lateral septal nucleus (LS) of urethane-anesthetized rats. Similar studies were performed using the selective 5HT reuptake inhibitor (SSRI) escitalopram (ECP; 1.6 mg/kg, i.v.) to enable comparisons between the multimodal actions of VORT and SSRI-mediated effects. VORT did not affect spontaneous or mPFC-evoked spike activity recorded in the ACC, but did induce a decrease in the number of spontaneously active cells recorded per electrode track. Furthermore, following VORT administration putative projection neurons recorded in the msNAc exhibited decreased responsiveness to mPFC stimulation delivered alone, or immediately following, train stimulation (30Hz, 500ms) of the hippocampal fimbria (HF). VORT also decreased spontaneous firing in the LS, an effect which was mimicked by ECP. ECP administration also reproduced the above effects of VORT on HF-evoked firing recorded in the msNAc. In contrast to VORT, ECP administration increased the number of spontaneously active cells recorded per track in the ACC and facilitated mPFC-evoked firing recorded in the LS. Taken together, the current studies indicate that VORT is likely to modulate afferent drive from the HF in the msNAc and spontaneous firing in the LS via 5-HT transporter blockade and increased 5HT transmission. Contrasting effects of VORT and ECP on population firing in the ACC and mPFC evoked firing in the msNAc and LS point to other complex 5HT receptor-dependent effects of VORT which may contribute to its unique impact on the function of prefrontal-subcortical circuits and motivated behavior.

Disclosures: S. Chakroborty: None. E. Dale: None. A. Pehrson: None. C. Sánchez Morillo: None. A.R. West: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.09/K15

Topic: B.07. Synaptic Transmission

Support: DFG SFB 1089

Title: Neurons in the medial entorhinal cortex integrate locomotion speed information from glutamatergic septo-entorhinal projections

Authors: *D. JUSTUS[1], D. DALÜGGE[1], S. BOTHE[1], F. FUHRMANN[1], C. HANNES[1], H. KANEKO[1], D. FRIEDRICHS[1], L. SOSULINA[1], I. SCHWARZ[2], D. A. ELLIOTT[1], S. SCHOCH[3], F. BRADKE[1], M. K. SCHWARZ[2], S. REMY[1];
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Abstract: Spatially tuned neurons in the medial entorhinal cortex (MEC), especially grid cells, require information about the instantaneous movement speed. However, the source of this information is yet unknown. Due to its involvement in velocity dependent hippocampal functioning the medial septum and diagonal Band of Broca (MSDB) is an ideal candidate for providing a speed signal to the MEC.
We used extracellular tetrode recordings and cell-type specific fiber-optometric measurements of GCaMP fluorescence to assess the activity of neurons in the MSDB and found a glutamatergic cell population that exhibits strongly speed modulated outputs.
Using mono-transsynaptic retrograde tracing we identified VGluT2$^+$ neurons in the MSDB with projections into MEC layer 2/3. We then characterized these projections and their targets in the MEC by combining in-vitro whole-cell recordings with optogenetic stimulations of ChR2 expressing septo-entorhinal axons.
We found that the septo-entorhinal pathway provides glutamatergic input to several MEC cell types, but most prominently to MEC pyramidal cells. Integrating the precise electrophysiological characterization in a computational model of MEC stellate cells, pyramidal cells and fast-spiking interneurons we demonstrated that this speed-signal excites all L2/3 MEC neurons in a speed dependent manner, but is integrated most effectively by L2/3 pyramidal cells. This is in full agreement with high membrane time constants and slow EPSP kinetics that we observed in pyramidal cells.
Thus, we identified the glutamatergic cell population in the MSDB as a source for the speed-information that is necessary for a spatial representation of the environment by neurons of the MEC.

Disclosures: D. Justus: None. D. Dalügge: None. S. Bothe: None. F. Fuhrmann: None. C. Hannes: None. H. Kaneko: None. D. Friedrichs: None. L. Sosulina: None. I. Schwarz: None. D.A. Elliott: None. S. Schoch: None. F. Bradke: None. M.K. Schwarz: None. S. Remy: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.10/K16

Topic: B.07. Synaptic Transmission

Support: American Heart Association Postdoctoral Fellowship 16POST27250302

NIH R01 NS089662-01

NIH R21 NS089439-01

NIH R01 MH099045-03

NIH F31 MH105043-01

Title: Disruption of coordinated pre- and postsynaptic maturation underlies the defects in hippocampal synapse stability and plasticity in Abl2/Arg-deficient mice.

Authors: *X. XIAO[1], A. LEVY[1], B. ROSENBERG[1], M. HIGLEY[2], A. KOLESKE[3]; [1]Mol. Biophysics and Biochem., [2]Yale Univ., New Haven, CT; [3]Yale Univ., NEW HAVEN, CT

Abstract: Immature glutamatergic synapses in cultured neurons contain high release probability (*P*r) presynaptic sites coupled to postsynaptic sites bearing GluN2B-containing N-methyl-D-aspartate receptors (NMDARs), which mature into low *P*r, GluN2B-deficient synapses. Whether this coordinated maturation of high *P*r, GluN2B+ synapses to low *P*r, GluN2B-deficient synapses actually occurs in vivo, and if so, what factors regulate it and what role it might play in long-term synapse function and plasticity are unknown. We report that loss of the integrin-regulated Abl2/Arg kinase in vivo yields a subpopulation of "immature" high *P*r, GluN2B+ hippocampal synapses that are maintained throughout late postnatal development and early adulthood. These high *P*r, GluN2B+ synapses are evident in *arg-/-* animals as early as postnatal day (P) 21, a time that precedes any observable defects in synapse or dendritic spine number or structure in *arg-/-* mice. Using focal glutamate uncaging at individual synapses, we find only a subpopulation of *arg-/-* spines exhibits increased GluN2B-mediated responses at P21. As *arg-/-* mice age, these synapses increase in proportion and their associated spines enlarge. These

changes coincide with an overall loss of spines and synapses in the Arg-deficient mice. We also demonstrate that although LTP and LTD are normal in P21 *arg-/-* slices, both forms of plasticity are significantly altered by P42. These data demonstrate that the integrin-regulated Arg kinase coordinates the maturation of pre- and post-synaptic compartments at a subset of hippocampal synapses in vivo and this coordination is critical for NMDAR-dependent long-term synaptic stability and plasticity.

Disclosures: X. Xiao: None. A. Levy: None. B. Rosenberg: None. M. Higley: None. A. Koleske: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.11/K17

Topic: B.07. Synaptic Transmission

Support: Netherlands Organization for Scientific Research (NWO)

Title: Anatomical and physiological aspects of the cerebellar impact on thalamic relay neurons

Authors: ***S. V. GORNATI**[1], C. B. SCHAEFER[2], C. I. DE ZEEUW[2,3], F. E. HOEBEEK[2]; [1]Neurosci., [2]Erasmus MC, Rotterdam, Netherlands; [3]Netherlands Inst. for Neuroscience, Royal Dutch Acad. for Arts and Sci., Amsterdam, Netherlands

Abstract: The cerebellum has an important role in the control and coordination of movements. The afferents of the cerebellum are the cerebellar nuclei (CN) which project to the spinal cord (via relays in the brainstem) and to upper motor neurons in the cortex via thalamus. Previous anatomical studies showed that the innervation is not confined to the motor thalamus (ventroanterior/lateral (VA/VL) complex), but it is widespread also in other nuclei such as VM, CL and CM which have specialized cortical connectivity. However, a detailed quantification of the anatomical and physiological characteristics of cerebello-thalamic connections with regional differentiation has not been reported. We recorded post-synaptic responses in thalamic cells located in VA/VL, VM, CL and CM in order to reveal if cerebellar output affects similarly the thalamic cells in the different nuclei. To test the strength of the cerebello-thalamic projection we used both electrophysiological and imaging techniques. We injected a virus encoding for Channelrhodopsin in the interposed/medial nuclei and we performed *in vitro* whole cell recordings in voltage- and current-clamp mode in thalamic relay neurons to evaluate the post-synaptic currents and whether or not cerebellar stimulation evoked action potential firing. Electrophysiological recordings revealed that between cells the maximally evoked post-synaptic

response varied substantially in a location-specific manner, i.e., showing greater amplitude in the VA/VL complex compared to the CM, CL and VM nuclei. Nevertheless, all the recordings exhibit paired-pulse depression upon stimulation frequencies higher than 10 Hz. Furthermore, washing in the AMPA blocker NBQX we could reduce EPSCs amplitude and abolish it completely with further addition of the NMDA blocker AP-5.To reconstruct the morphology of the recorded neurons we filled it with biocytin and stained the cerebellar terminals with vesicular glutamate transporter 2 (VGluT2) antibody. Using high-magnification confocal microscopy and deconvolution we analysed the structure and distribution of CN boutons finding that CN afferents synapse consistently onto proximal dendrites. Together these findings help us to better understand how effective CN neurons are in controlling thalamic firing within the different nuclei and thereby differentiate the cerebellar impact on cerebral cortices.

Disclosures: S.V. Gornati: None. C.B. Schaefer: None. C.I. De Zeeuw: None. F.E. Hoebeek: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.12/L1

Topic: B.07. Synaptic Transmission

Support: NIH Grant EY 15573

UCLA Oppenheimer Seed Grant

Plum Foundation

Veterans Administration Career Scientist Award

NSERC (Canada) Discovery Award 194640

Title: Pathways by which horizontal cell inhibitory amino acid transmitters inhibit cones

Authors: J. C. R. GROVE[1], K. FILER[1], A. A. HIRANO[1], N. C. BRECHA[1,2], *S. A. BARNES[3,1];
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Abstract: *Indirect feedback to photoreceptors:* Release and autoreception by horizontal cells of inhibitory amino acid transmitters (IAA), such as GABA, could mediate a pH shift in the

synaptic cleft (due to the HCO_3^- conductance of the horizontal cell IAA receptors) and this pH shift leads to modulation of photoreceptor Ca channels, which are widely accepted to be the target of inhibitory feedback. To test whether horizontal cells respond to their own GABA/IAA release, Cx57-tdTomato horizontal cells in slices of mouse retina were patch-clamped with solutions containing (mM): (internal) 110 $CsMeSO_4$, 2.8 NaCl, 4 EGTA, 5 TEA-Cl, 4 Mg-ATP, 0.3 Na_3-GDP, 20 HEPES, 10 phosphocreatine; and (external) 120 NaCl, 3 KCl, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10 glucose, 25 $NaHCO_3$. Horizontal cells were voltage clamped in steps from -50 to +40 mV and when currents obtained in 100 μM picrotoxin were subtracted from control currents, inward tail currents (~20 pA) at -60 mV, as well as inward current during steps to -30 mV and reversed outward currents at -10 mV and more positive steps, reaching 30 pA, were observed. To test the effects of removal of GABA/IAA inhibition, mouse, rat and guinea pig cones in slices were patch-clamped (same solutions as above) and responded to superfusion of picrotoxin with increased Ca channel currents, which analysis showed had activation curves whose Boltzmann function midpoints shifted by about -7 mV (-25 mV to -32 mV). HEPES (10 mM) eliminated the midpoint shifts in rat slices. *Direct feedback to photoreceptors:* A direct GABA/IAA-mediated pathway to photoreceptors has been established in the retinas of both mammalian and non-mammalian vertebrates but is refuted in some reports, possibly due to circadian regulation. Some of the experiments described above, carried out during subjective day, showed a two-fold effect during the application of picrotoxin to guinea pig cones. In addition to the modulation of the Ca channels, background conductance between potentials of -80 and -50 mV was reduced during picrotoxin application. These data support the hypothesis that there are two pathways by which GABA/IAA acts on two conductances in photoreceptors. GABA/IAA release in the outer plexiform layer may act 1) directly on photoreceptor GABA/IAA receptors and 2) indirectly on photoreceptor Ca channels, via a cleft pH change caused by HCO_3^- and Cl^- permeable GABA/IAA receptors on horizontal cells.

Disclosures: J.C.R. Grove: None. K. Filer: None. A.A. Hirano: None. N.C. Brecha: None. S.A. Barnes: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.13/L2

Topic: B.07. Synaptic Transmission

Support: Sunovion

Title: Identifying genetic markers associated with treatment response to lurasidone ; A genome-wide association study on two independent clinical trials.

Authors: *A. YOSHIKAWA[1], J. LI[2], H. Y. MELTZER[2];
[1]Psychiatry and Behavioral Sci., Northwestern University, Feinberg Sch. of Medici, Chicago, IL;
[2]Psychiatry and Behavioral Sci., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Introduction: Schizophrenia (SCZ) is a heterogeneous disorder with a diverse genetic contribution to its etiology and treatment response to antipsychotic drugs (APDs). About one third of patients fail to respond adequately to the standard treatment. There are currently no biomarkers which can predict the response to APDs in SCZ. “Trial and error” in selection of the optimal APD leads to delayed recovery, prolonged suffering, and low adherence of the patients, all of which may worsen the prognosis. Therefore, to identify the genetic markers associated with treatment response is of great interest to triage the patients and disclose the fundamental mechanisms of drug efficacy. Lurasidone is a more potent serotonin $(5\text{-HT})_{2A}$ than dopamine (DA) D_2 receptor blocker; a 5-HT_7 antagonist and 5-HT_{1A} partial agonist. **Material and Methods:** A genome-wide association study (GWAS) on two randomized clinical trials on Caucasian and African-American patients with SCZ was conducted by Affymetrix 6.0 SNP array to identify the genetic biomarkers associated with treatment response to lurasidone at last observation carried forward (LOCF). Genome-wide SNP imputation was performed by IMPUTE2/SHAPEIT. Treatment response was quantitatively evaluated by change in PANSS-Total (delta PANSS-T) from the baseline to LOCF. An unadjusted p value $< 1.0\times10^{-4}$ in the association test with delta PANSS-T was set as cutoff. **Results:** We identified common variants in genes related to synaptic function as the top predictors of response to lurasidone in Caucasians and African-American patients. The identified genomic loci mainly resided in synaptogenic adhesion genes and scaffolding proteins, both essential for synaptic function. *NRXN1* replicates previous findings for prediction of response to clozapine by us and others. Other synaptic genes predicting response included *NRG1/3*, *KALRN*, and neuron-specific splicing regulator, *RBFOX1*. Most of these genes and their associated pathways have been previously linked to SCZ. Decreased gene expression in post-mortem brain areas relevant to SCZ also supports our findings. Some of the top SNPs are potential cis-eQTLs. The expression of some of the top hits is significantly inversely correlated with HTR7 gene expression. **Discussion:** Our non-hypothesis driven GWAS study identified genetic biomarkers which predicted treatment response to lurasidone. As many of the identified genes have been previously reported as risk genes for SCZ, our results may indicate that lurasidone and other atypical APDs act through disease modifying genes. These findings suggest that synaptogenic adhesion and scaffolding proteins will be potential novel drug targets for SCZ.

Disclosures: A. Yoshikawa: None. J. Li: None. H.Y. Meltzer: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.14/L3

Topic: B.07. Synaptic Transmission

Support: Wellcome Trust

Brain Research Trust

Epilepsy Research UK

Title: Dendritic specific integration rules in hippocampal CA1 parvalbumin positive fast spiking interneurons

Authors: *J. CORNFORD, D. M. KULLMANN;
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Abstract: PV+ interneurons make up ~2% of interneurons in the CA1 region of the hippocampus. Despite their rarity, they are fundamental for a range of functions, from basic circuit processes such as the sharpening of principal-cell input coincidence detection, to more complex network operations such as place-cell field regulation (Hu *et al.*, 2014). This central importance of PV+ basket cells stems from their privileged position as mediators of both feedforward and feedback inhibition.
Recent work has shown that basket cells express synaptic receptors in an input specific distribution: local feedback connections onto oriens dendrites display a larger NMDA receptor component than feedforward inputs, which synapse predominately in the stratum radiatum (Le Roux *et al.*, 2013). This raises the possibility that PV+ basket cells handle synaptic input in a dendritic specific manner; synaptic connections from local pyramidal cells might be more able co-operate with each other via NMDA receptors (Branco & Häusser, 2011), whereas forward connections would be integrated in a linear or sub-linear manner.
In order to investigate the distinct expression of rectifying glutamate receptors and the resulting functional implications, we used a combination of compartmental modelling, multi-photon imaging, and immunohistochemistry. We report that dendrites located in the radiatum integrate synaptic inputs in a linear or sub-linear manner. In contrast, oriens dendrites display linear or supra-linear integration rules. We also present pharmacology and modelling experiments detailing the underlying biophysical mechanisms and the functional implications of these dendritic specific integration rules.
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Disclosures: J. Cornford: None. D.M. Kullmann: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.15/L4

Topic: B.07. Synaptic Transmission

Support: 1R21 NS090040

Title: Coordinated spiking in CA3 propagates to hilar mossy cells in juvenile mice but only rarely in adult mice

Authors: *T. HEDRICK, W. P. NOBIS, G. T. SWANSON;
Pharmacol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Temporal lobe epilepsy is a disorder of altered hippocampal network activity associated with aberrant excitability and widespread degeneration of hilar mossy cells (HMCs), suggesting that HMCs play an important role in hippocampal network activity. A clearer delineation of excitatory input to HMCs in physiological and pathological conditions will be essential for understanding how these cells contribute to circuit dynamics in the dentate gyrus. HMCs bridge distant hippocampal areas by relaying information from granule cells (GCs) and CA3 pyramidal neurons (CA3) to dentate gyrus; however, many aspects of GC-HMC and CA3-HMC synapses remain uncharacterized. To examine these HMC excitatory inputs, we are recording both spontaneous and evoked EPSCs in HMCs in acute slices from juvenile (~P20) and adult (~P65) mice, detailing excitatory synaptic physiology, the propagation of seizure-like events to HMCs, and how these properties evolve during development.
In vivo administration of certain drugs can induce chronic seizure states, but how HMCs participate in this altered network activity is unknown. We found that acute application of bicuculline and picrotoxin, pilocarpine, or 4-aminopyridine (4-AP) in acute slices induced coordinated firing in CA3 which propagated into the hilus resulting in compound EPSCs (cEPSCs) in HMCs (amplitude: 4255 ± 245 pA). cEPSC frequency was greatly diminished with

aging (juvenile: 2.19 ± 0.25 Hz; adult: 0.20 ± 0.16 Hz), suggesting that developmental changes in hippocampal circuitry diminish the strength of these coordinated bursts or their propagation to HMCs.
To examine if the decrease in cEPSCs with aging could be due to decreased connectivity of HMCs, we examined synaptic physiology at HMC synapses in juvenile and adult mice. We found that HMCs receive significant excitatory input from both CA3 and GCs. Evoked CA3-HMC EPSCs were of small amplitude and facilitated during paired stimulation (paired pulse ratio: 1.95 ± 0.15). In contrast, evoked GC-HMC EPSCs were large amplitude with modest facilitation (paired pulse ratio: 1.66 ± 0.09), which was surprising given that anatomically similar GC-CA3 synapses display much more robust facilitation during paired stimuli of mossy fibers. CA3-HMC and GC-HMC excitatory synaptic function was similar in juvenile and adult mice, suggesting that decreased HMC connectivity likely is unlikely to account for the reduction in cEPSCs with aging. These data show that HMCs receive comparatively weaker synaptic input from CA3 than from GCs, but that strong CA3 input drives the propagation of seizure-like events into the hilus in the presence of pro-epileptic agents.

Disclosures: T. Hedrick: None. W.P. Nobis: None. G.T. Swanson: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.16/L5

Topic: B.07. Synaptic Transmission

Title: Measuring the strength nicotinic synapses in rat sympathetic ganglia using stochastic nerve stimulation, voltage-clamp and dynamic-clamp

Authors: *J. P. HORN, P. H. M. KULLMANN;
Dept of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Synaptic integration in sympathetic ganglia depends upon the strength and number of converging nicotinic synapses and upon intrinsic postsynaptic excitability. Previous efforts to measure synaptic strength have relied on measurements of EPSP amplitude, while not accounting for damage introduced by microelectrode recording (Springer, Kullmann & Horn, J Physiol 2015) or trial-to-trial fluctuations in EPSP size. Here we describe experiments that utilized whole-cell patch electrode recording from the isolated intact superior cervical sympathetic ganglion at 36°C, together with stochastic activation of synapses using minimal presynaptic stimulation at an average frequency of 1 Hz. Synaptic strength was calculated by measuring the average EPSC amplitude at a given synapse under voltage-clamp and converting it

to a synaptic conductance (g_{syn}) using the driving force. Threshold-g_{syn} was then measured under dynamic clamp. We calibrated synaptic strength to excitability by dividing g_{syn} by thresh-g_{syn}. Release dynamics were assessed using 400-600 second trains of 1 Hz stimuli, delivered either at a constant rate or with random exponential timing that mimics physiological activity observed *in vivo*. The strengths of 17 synapses studied this way ranged from 5% to 3,044% thresh-g_{syn}. Interestingly, 5 of these synapses had strengths clustered close to 100% thresh-g_{syn}. The importance of this observation became evident when fluctuations of EPSC amplitude were measured. When regular and stochastic patterns of stimulation were compared, they each evoked similar average amplitudes. However, stochastic stimulation increased the coefficient of variation of EPSCs by up to 60% in 6 of 10 synapses. These results imply that many nicotinic EPSPs in sympathetic ganglia have the capacity to straddle threshold and they reveal for the first time that stochastic synaptic activity in this system produces a form of stochastic resonance that may enhance synaptic amplification (gain) in sympathetic ganglia. The results will be discussed in terms of our previous work on ganglionic gain theory and the implications for autonomic control of cardiovascular function.

Disclosures: J.P. Horn: None. P.H.M. Kullmann: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.17/L6

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH099054

Frank & Myra Weiser Scholar Award

Title: Interaction of AMPA and NMDA conductances in transducing synaptic drive into action potential output

Authors: C. LI, *A. T. GULLEDGE;
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Abstract: Attenuation of synaptic currents within dendrites limits the efficacy of distal excitatory inputs in driving action potential output at the axon. To reduce the impact of distance on synaptic efficacy, neurons employ a range of mechanisms to boost distal synaptic signals, including expression of active dendritic conductances and variations in synaptic structure. Key to excitatory synapses is the expression of ionotropic glutamate receptors, including AMPA and

NMDA receptors, that have distinct kinetics and voltage-dependencies. We used simulated barrages of excitatory input in simplified and morphologically realistic model neurons to explore the interaction of NMDA and AMPA conductances in synapse-spike coupling. Identical series of stochastic synaptic barrages were simulated along progressively more distal stretches of dendrite (100-μm-long, moving in 10 μm increments), while variability in the number and timing of resulting action potentials were measured for each dendritic location. When synapses contained only AMPA or NMDA conductances, the number of action potentials generated during any given trial was highly dependent on the dendritic location of synaptic input, albeit in opposite directions for the two conductance subtypes. In AMPA-only models (500 or 700 pS maximal synaptic conductances), efficacy of synaptic input in driving action potential output dropped precipitously with distance from the soma, while in NMDA-only models (200 pS or 700 pS), excitatory drive peaked when synaptic input occurred at distal locations. These results reflect overall attenuation of synaptic signals with distance from the soma, and enhanced voltage-dependent NMDA receptor activation in distal, high-impedance dendritic compartments. On the other hand, pairing AMPA and NMDA conductances (500 and 200 pS, respectively) reduced the location dependence of synapse-spike coupling relative to models containing either conductance alone. Variability in interspike interval also depended on the composition of conductances at the synapse. When synapses contained only AMPA conductances, spike timings were highly variable, while NMDA conductances, either alone or paired with AMPA, promoted regular firing due to prolonged NMDA decay kinetics. These preliminary results suggest that coexpression of NMDA and AMPA receptors at excitatory synapses may limit location-dependent differences in synapse-spike coupling, and will promote regular firing patterns in response to sustained excitatory drive.

Disclosures: C. Li: None. A.T. Gulledge: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.18/L7

Topic: B.07. Synaptic Transmission

Support: NIH/NINDS U01 NS090340

AHA 14POST20130031

Title: Characterization of a polysynaptic microcircuit involved in sensory vagal afferent activation of dorsomotor vagal neurons in mouse

Authors: *I. AIBA[1], J. L. NOEBELS[2];
[1]Dept. of Neurol., [2]Baylor Col. of Med., Houston, TX

Abstract: Abnormal parasympathetic activity is implicated in sudden unexpected death in epilepsy (SUDEP). Vagal sensory axons entering the brainstem are responsible for the majority of parasympathetic autonomic system input, and abnormal regulation of vagal motor efferents may contribute to the deleterious autonomic deregulation. The dorsomotor vagal (DMV) neurons contribute a majority of the unmyelinated C-fiber efferents, and one of the major regulatory inputs to DMV is the vagal sensory afferent nerve terminating within the dorsal medulla. The present study characterized the regulation of DMV neuron action potential generation by sensory vagal nerve activation in murine acute brainstem slices. Extracellular cell-attached recordings were made from visually identified DMV neurons. DMV neurons showed spontaneous action potentials (AP) with a frequency range between 1-3Hz. AP frequency was increased by bath application of glutamate and eliminated by GABA (0.5mM each). DMV neurons activated by afferent vagal nerve impulses were identified in response to bipolar stimulation of the vagal afferent nerve ending (tractus solitarius). Responsive neurons showed evoked action potentials with a variable probability (51.6±27.6%, n=52) and jitter (7.46±2.9ms). In a subset of recording, evoked EPSCs and IPSCs were subsequently recorded in the same neurons and stimulation by whole-cell recordings. Both evoked EPSCs and IPSCs showed an asynchronous waveform with a large variability in the amplitudes. A regression analysis revealed that the probability of evoked APs significantly correlated with the amplitude of evoked EPSC, but not the evoked IPSC. Inhibition of AMPA receptors with NBQX (5μM) completely abolished both evoked EPSCs and APs, while residual spontaneous EPSCs were still detected. In contrast to the expectation from regression analysis, inhibition of $GABA_A$ receptors (gabazine 10μM), but not glycine receptors (strychnine 1μM), significantly increased the probability of evoked action potential activity. It is likely that polysynaptic inhibitory circuits between sensory vagal and premotor neurons, rather than direct inhibitory monosynaptic current onto the DMV plays a predominant role in regulation of vagal premotor neurons. These results confirmed that glutamate and GABA are the principle excitatory and inhibitory neurotransmitters regulating the vagal afferent to excite DMV efferent neurons.

Disclosures: I. Aiba: None. J.L. Noebels: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.19/L8

Topic: B.07. Synaptic Transmission

Support: EPSRC EP/F500385/1

BBSRC BB/F529254/1

Wellcome Trust WT086602MF

Title: What are the synaptic input properties *In vivo*? A new method.

Authors: *M. C. VAN ROSSUM[1], M. JELITAI[2], I. DUGUID[2], P. PUGGIONI[3];
[2]Ctr. for Integrative Physiol., [3]Sch. of Informatics, [1]Univ. Edinburgh, Edinburgh, United Kingdom

Abstract: Knowledge of synaptic input is crucial to understand synaptic integration and ultimately neural function. However, in vivo the rates at which synaptic inputs arrive are high, that it is typically impossible to detect single events. We show here that it is nevertheless possible to extract the properties of the events, and particular to extract the event rate, the synaptic time-constants, and the properties of the event size distribution from in vivo voltage-clamp recordings. Applied to cerebellar interneurons our method reveals that the synaptic input rate increases from 600Hz during rest to 1000Hz during locomotion, while the amplitude and shape of the synaptic events are unaffected by this state change. This method thus complements existing methods to measure neural function in vivo.

Disclosures: M.C. Van Rossum: None. M. Jelitai: None. I. Duguid: None. P. Puggioni: None.

Poster

221. Synaptic Transmission: Synaptic Integration

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 221.20/L9

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH106906

Title: Voltage-imaging of electrical signaling in dendritic spines.

Authors: J.-Y. WENG, *D. ZECEVIC;
Dept Cell & Mol. Physiol, Yale Univ. Sch. Med., New Haven, CT

Abstract: Thousands of dendritic spines on individual neurons process information and mediate plasticity by generating electrical input signals using a sophisticated assembly of transmitter receptors and voltage-sensitive ion channel molecules. On the conceptual level, a key question that has not been answered is whether the hypothetical electrical isolation of synapses on spine

heads caused by a narrow spine neck is responsible for specific functions which are not supported by synapses on dendrites. The answer to that question is incomplete and controversial because it is mostly based on indirect evidence. To obtained direct evidence, we recently used a technique based on an electrochromic voltage-sensitive dye (JPW3028) applied selectively to individual neurons by intracellular injection. This lipophilic voltage probe binds to neuronal membrane and acts as a transmembrane optical voltmeter with a linear scale capable of recording simultaneously electrical signals from individual spines and parent dendrites. Our measurements demonstrated that synapses on spines of layer 5 pyramidal neurons from both mouse and rat somatosensory cortex are not electrically isolated by the spine neck to a significant extent. Electrically, they behave as if they are located directly on dendrites. Here, we report an extension of these studies to include spines from other neuronal types in the brain. To increase the accuracy of these measurements, we are currently working on improvements in the technique to minimize unwanted effects which result from the interaction of 720 nm light pulses from a femtosecond Ti:Saphire laser utilized to uncage glutamate with fluorescence of voltage-sensitive dye molecules excited by 532 nm light from a diode-pumped, continuous wave laser utilized to provide voltage-dependent fluorescent signals. We found that optical signal related to the EPSP could be contaminated by the stimulated emission depletion (STED) one-photon effect of 720 nm light reducing the intensity of fluorescence from the voltage probe. Additionally, if the uncaging spot is positioned too close to the spine head labelled by the voltage-sensitive dye, the intensity at the edge of the uncaging spot might be high enough to cause 2-photon excitation and subsequent bleaching of the voltage-sensitive dye. The unwanted signals related to one-photon STED effect and 2-photon excitation and bleaching of the voltage dye are in the range of 0% to 10% while the amplitude of the unitary EPSP optical signals are of the order of 1%. Thus, it is mandatory to minimize or eliminate the unwanted signals described above to avoid significant distortions of EPSP signals originating in the spine head.

Disclosures: J. Weng: None. D. Zecevic: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.01/L10

Topic: B.08. Synaptic Plasticity

Title: Inhibitory long term potentiation at parvalbumin expressing pallidal thalamic synapses.

Authors: *M. M. VAN SWIETEN, K. R. TAN;
Biozentrum, Univ. of Basel, Basel, Switzerland

Abstract: The basal ganglia play an important role in procedural learning and motor planning. One of the mechanisms proposed for information storage is long-term plasticity. Over the past two decades great efforts have been performed to describe synaptic plasticity in the basal ganglia. However, most studies have been focused on excitatory corticostriatal synapses, where both long-term potentiation and depression have been observed. Only recently, an interest in inhibitory synaptic plasticity arising from striatal medium spiny neurons has been developed. Nevertheless, synaptic plasticity at inhibitory synapses within and outside the basal ganglia is still underexplored. Synaptic plasticity plays a crucial role in learning and memory.
In the present study we investigated the synaptic properties of a recently identified projection from parvalbumin (PV) expressing neurons in the globus pallidus external segment to the parafascicular nucleus of the thalamus.
Using PV-cre mice with conditional expression of channelrhodopsin combined with in vitro whole-cell patch clamp recording, we showed that this projection is GABAergic and monosynaptic. Furthermore, sustained activation of this synapse (i.e high frequency stimulation) induced a strengthening of this synapse. This long-term potentiation (LTP) relied most probably on a postsynaptic mechanism, as the paired pulse ratio was unchanged. The induction of LTP was independent of NMDA-receptor activation but required a rise in intracellular calcium concentration and activation of the CAMKII signaling cascade. The expression of inhibitory LTP, which could be a result of an increased number of receptors on the cell surface, relied partially on protein trafficking mechanisms and receptors synthesis de novo.
The present study provides new insights in the physiological mechanisms involved at inhibitory plasticity. Moreover, this work is one of the first to investigate the functional synaptic properties of this synapse, which may play an essential role in the basal ganglia function in procedural learning and/or motor planning.

Disclosures: M.M. Van Swieten: None. K.R. Tan: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.02/L11

Topic: B.08. Synaptic Plasticity

Support: BBSRC Grant BB/L001977/1

NIH Grant R01MH060252

Title: Involvement of NMDAR subunits in the induction of STP and LTP in the GluN2D knockout mouse

Authors: *D. FERNANDEZ-FERNANDEZ[1], A. V. EAPEN[2], E. BURNELL[2], D. T. MONAGHAN[3], D. E. JANE[2], G. L. COLLINGRIDGE[2], A. VOLIANSKIS[4];
[1]MRC Ctr. for Synaptic Plasticity, Sch. of Physiology, Pharmacol. and Neuro, [2]Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [3]Dept. of Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE; [4]Ctr. for Neurosci. and Trauma, Queen Mary Univ. of London, London, United Kingdom

Abstract: The specific subunit composition of NMDA receptors (NMDARs) has a strong impact on their intrinsic functionality and therefore can directly determine their ability to induce long-term potentiation (LTP). Indeed, we have recently shown that different di- and tri-heteromeric subtypes of the NMDAR are responsible for the induction of the three temporal components of NMDAR-dependent potentiation in adult rat hippocampal slices, namely, a transient and biphasic short-term potentiation (STP1 decays with τ of ~ 7 min, STP2 with τ of ~ 16 min) that is followed by a sustained LTP phase. The development of UBP145, an antagonist that preferentially targets GluN2D subunit-containing NMDARs, has allowed us to reveal an unexpected role for these receptors in the induction of STP2. STP2 was also sensitive to the GluN2B antagonist, Ro 25-6981, suggesting that the receptor that mediates its induction might be a GluN2B/2D triheteromer (Volianskis et al 2013, 2015). Here, we describe for the first time the functional implications that knocking out the GluN2D subunit exert on the induction of STP and LTP. We find that both STP and LTP can be readily induced by theta-burst stimulation (TBS) in hippocampal slices from GluN2D knockout mice. Moreover, a similar STP and LTP pattern is found after a 30 min long interruption of stimulation following the TBS, showing that decay of STP can be halted during periods without stimulation despite the absence of this NMDAR subunit. In both scenarios, STP decay could be fitted by a monoexponential curve with a τ value of around 8 min, suggesting that STP2, the slower component of STP, is absent in hippocampal slices from GluN2D knockout mice. In support of that, the GluN2D preferring antagonist UBP145 (10 μM) had no effect on induction of STP or LTP in the GluN2D KO mice. This confirms the essential role of GluN2D in the induction of STP2. In addition, Ro 25-6981 (1 μM) had also no effect on STP and LTP in the GluN2D KO mouse. These data support the conclusion that STP2 is mediated by a GluN2B/D triheteromeric receptor and suggest that diheteromeric GluN2B containing receptors are not involved in the induction of LTP. This work was funded by the BBSRC grant number BB/L001977/1 and the National Institute of Mental Health of the National Institutes of Health under Award Number R01MH060252.

Disclosures: D. Fernandez-Fernandez: None. A.V. Eapen: None. E. Burnell: None. D.T. Monaghan: None. D.E. Jane: None. G.L. Collingridge: None. A. Volianskis: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.03/L12

Topic: B.08. Synaptic Plasticity

Support: QMUL

ABCAM

MRC

BBSRC (BB/L001977/1).

Title: Short-term potentiation in the ventral hippocampus

Authors: R. INGRAM[1], L. B. YSSENNAGGER[1], A. T. MICHAEL-TITUS[1], D. E. JANE[2], G. L. COLLINGRIDGE[2,3], *A. VOLIANSKIS[1,2];
[1]Barts and The London Sch. of Med. and Dent., Queen Mary Univ. of London, London, United Kingdom; [2]Ctr. for Synaptic Plasticity, Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [3]Dept. of Physiol., Univ. of Toronto and LTRI, Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Synaptic plasticity is most frequently studied in the CA1 area of dorsal hippocampal slices. Here, in response to high-frequency theta-burst stimulation (TBS), the Schaffer collaterals display two phases of potentiation, which are readily induced through activation of NMDA receptors. The initial phase of potentiation, commonly called short-term potentiation (STP), declines in response to low frequency synaptic activation, leading to a permanently enhanced level of synaptic transmission, long-term potentiation (LTP). We have recently shown that STP is not a unitary phenomenon, but consists of two forms, termed STP1 and STP2 (Volianskis et al., 2013; 2015). Induction of STP1 depends on activation of GluN2A and GluN2B containing NMDARs and in this way it appears to be similar to LTP, which in the adult hippocampal slices is induced through GluN2A/2B containing triheteromeric NMDARs. STP2, in contrast to STP1 and LTP, is induced through GluN2B and GluN2D subunit containing NMDARs. In response to stimulation, STP1 decays with a time course ($\tau \sim 7$ min) that is about 2 times faster than that of STP2 (~ 16 min).
STP and LTP can also be induced in slices from the ventral hippocampus. However, not much is known about the properties of STP in this part of the brain and it is generally assumed that the induction of LTP in the ventral hippocampus is less reliable than in the dorsal. We investigated the induction of NMDAR-dependent potentiation in ventral hippocampal slices and show that TBS induced LTP (~ 14%) was about 3 to 4 times smaller than that in the dorsal hippocampus.

Decay of ventral STP, similarly to that in dorsal hippocampus (Volianskis & Jensen, 2003), depended on synaptic stimulation. Ventral STP (~ 20%) decayed with a τ value of ~ 6 min and was very similar to STP1 in dorsal hippocampus. On the basis of these observations we suggest that induction of STP and LTP in ventral hippocampal slices might be mediated exclusively by GluN2A and GluN2B containing NMDARs.

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Volianskis A, Bannister N, Collett VJ, Irvine MW, Monaghan DT, Fitzjohn SM, Jensen MS, Jane DE & Collingridge GL (2013). Different NMDA receptor subtypes mediate induction of long-term potentiation and two forms of short-term potentiation at CA1 synapses in rat hippocampus in vitro. J Physiol (Lond) 591, 955–972.

Volianskis A, France G, Jensen MS, Bortolotto ZA, Jane DE & Collingridge GL (2015). Long-term potentiation and the role of N-methyl-D-aspartate receptors. Brain Res 1621, 5–16.

Supported by:

QMUL, ABCAM, the MRC and the BBSRC (BB/L001977/1).

Disclosures: R. Ingram: None. L.B. Yssennagger: None. A.T. Michael-Titus: None. D.E. Jane: None. G.L. Collingridge: None. A. Volianskis: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.04/L13

Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust

BBSRC

Title: Convergent metabotropic and cholinergic signaling in the facilitation of Hebbian synaptic plasticity

Authors: *C. M. TIGARET, M. C. ASHBY, J. R. MELLOR;
Sch. of Physiology, Pharmacol. & Neurosci., Univ. of Bristol, Bristol, United Kingdom

Abstract: Induction of spike timing-dependent plasticity (STDP) at Schaffer collateral (S/C)-CA1 synapses requires the sequential activation of postsynaptic NMDA receptors (NMDARs) and voltage-sensitive Ca^{2+} channels at dendritic spines(1, 2). NMDAR function and spine Ca^{2+}

signaling during induction of LTP are facilitated by metabotropic glutamate receptor ($mGluR_1$)-dependent inhibition of SK channels(2). In addition, muscarinic M1 receptors facilitate spine Ca^{2+} signalling and induction of theta burst LTP at S/C-CA1 synapses through a mechanism also involving SK channel inhibition(3-5). Here, we show in acute hippocampal slices from adult rats that the highly selective allosteric M1 agonist GSK-5 (1μM) (5) or direct inhibition of SK channels by apamin (100 nM) rescued STDP-LTP induction at S/C-CA1 synapses in the presence of mGluR1 selective antagonist YM 298198 (100 nM). STDP-LTP was induced with a train of 300 STDP stimulations at 5 Hz for 1 min, in whole-cell current clamp at 36°C under $GABA_A$ receptor inhibition (50 μM picrotoxin). Each stimulation consisted of one EPSP evoked in the stratum radiatum followed by two action potentials elicited by somatic current injection at 100 Hz. Using two-photon Ca^{2+} fluorescence imaging in dendritic spines of CA1 pyramidal neurons(2) we show that apamin enhanced both synaptically evoked EPSPs and spine Ca^{2+} transients (EPSCaTs). Spine Ca^{2+} imaging during STDP-LTP-inducing stimulus trains revealed that LTP induction requires a sustained sequence of spine EPSCaTs. GSK-5 amplified the sequence of EPSCaTs during LTP induction to an extent comparable to that of apamin. Furthermore, $mGluR_1$ blockade inhibited the EPSCaTs evoked during the LTP induction trains, and this effect was reversed in the presence of apamin or GSK-5. Our data indicate that SK channels are a common downstream target in a convergent signalling pathway underpinning the facilitation of LTP by metabotropic glutamate and cholinergic neuromodulation.

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Disclosures: C.M. Tigaret: None. M.C. Ashby: None. J.R. Mellor: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.05/L14

Topic: B.08. Synaptic Plasticity

Support: FAPESP Brazil 2012/06123-4

Title: Thalamo-prefrontal resonance of hippocampal inputs is plastic and attenuated by thalamic silencing

Authors: *L. S. BUENO-JUNIOR, R. N. RUGGIERO, J. E. PEIXOTO-SANTOS, D. B. MARQUES, M. A. V. AVILA, C. LOPES-AGUIAR, J. P. LEITE;
Med. Sch. of Ribeirao Preto, Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: The prefrontal cortex (PFC) receives overlapping terminals from CA1/subiculum (CA1/sub) and mediodorsal thalamus (MD). Because the PFC reciprocates its thalamic afferents, CA1/sub inputs could plastically reverberate in the PFC-MD loop, which we examined through unit activity and synaptic plasticity monitoring. Rats were implanted with electrodes in CA1/sub (electrical stimulation), MD and PFC (recording) for a chronic session with paired-pulse stimulation (0.1 Hz), and high-frequency stimulation (HFS, 250 Hz trains; 30 min baseline, 120 min post-HFS). Both PFC and MD firing responded to CA1/sub pulses with phasic increases (<200 ms), then a transient decrease (200-400 ms). Specifically in the PFC, we observed a delayed-onset increase (400-800 ms) that was potentiated after HFS. CA1/sub pulses elicited distinct field responses in PFC and MD, which underwent long-term potentiation. Moreover, electrophysiological responses were correlated with c-Fos and Zif-268 immunohistochemistry throughout the studied circuit. We further asked whether MD optogenetic hyperpolarization modulates the CA1/sub-PFC recruitment. A rat expressing green light-driven archaerhodopsins in the MD was implanted as above, except for an optrode into MD. When randomly paired with CA1/sub electrical pulses, MD light pulses attenuated PFC delayed-onset responses. Thus, hippocampal inputs seem to plastically resonate within the thalamo-prefrontal loop. These findings contribute to the systems-level understanding of limbic-prefrontal functions (e.g., working memory), and dysfunctions (e.g., psychoses and seizure amplification).

Disclosures: L.S. Bueno-Junior: None. R.N. Ruggiero: None. J.E. Peixoto-Santos: None. D.B. Marques: None. M.A.V. Avila: None. C. Lopes-Aguiar: None. J.P. Leite: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.06/M1

Topic: B.08. Synaptic Plasticity

Title: Identification of a novel PKA regulatory site in neuroligin-1.

Authors: *J. JEONG, M. A. BEMBEN, Y. LI, K. W. ROCHE;
NINDS, BUILDING 35, Bethesda, MD

Abstract: Neuroligin-1 is a member of the neuroligin gene family encoding a single-pass transmembrane postsynaptic cell adhesion molecule that is critical for excitatory synaptic assembly and function. The large extracellular domain of neuroligin-1 is responsible for transsynaptic binding with neurexin, a presynaptic cell adhesion molecule, and the short cytoplasmic tail of neuroligin-1 is exposed to a variety of intracellular regulation. Importantly, neuroligin-1 binds to PSD-95, a scaffolding protein at excitatory synapses, through the PDZ ligand in the cytoplasmic tail, which is important for neuroligin-1 clustering at excitatory synapses. In this study, we found protein kinase A (PKA) phosphorylates neuroligin-1, near the PDZ ligand, *in vitro* and in heterologous cells. We generated a phospho-specific antibody, which allows detection of PKA phosphorylated neuroligin-1 and is efficient for immunoprecipitation of native neuroligin-1. Interestingly, a phospho-mimetic mutation at the PKA site significantly reduced the interaction between neuroligin-1 and PSD-95 *in vitro* and *in situ*. Our results establish a novel molecular mechanism that regulates neuroligin-1 and PSD-95 binding in a PKA activity-dependent manner, which can provide insights into excitatory synaptic development and function.

Disclosures: J. Jeong: None. M.A. Bemben: None. Y. Li: None. K.W. Roche: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.07/M2

Topic: B.08. Synaptic Plasticity

Support: NIAAA Grant RO1AA021775

NINDS R21NS085502

Title: BDNF regains function in hippocampal long-term potentiation deficits caused by diencephalic damage.

Authors: L. C. VEDDER[1], *L. M. SAVAGE, Ph.D.[2];
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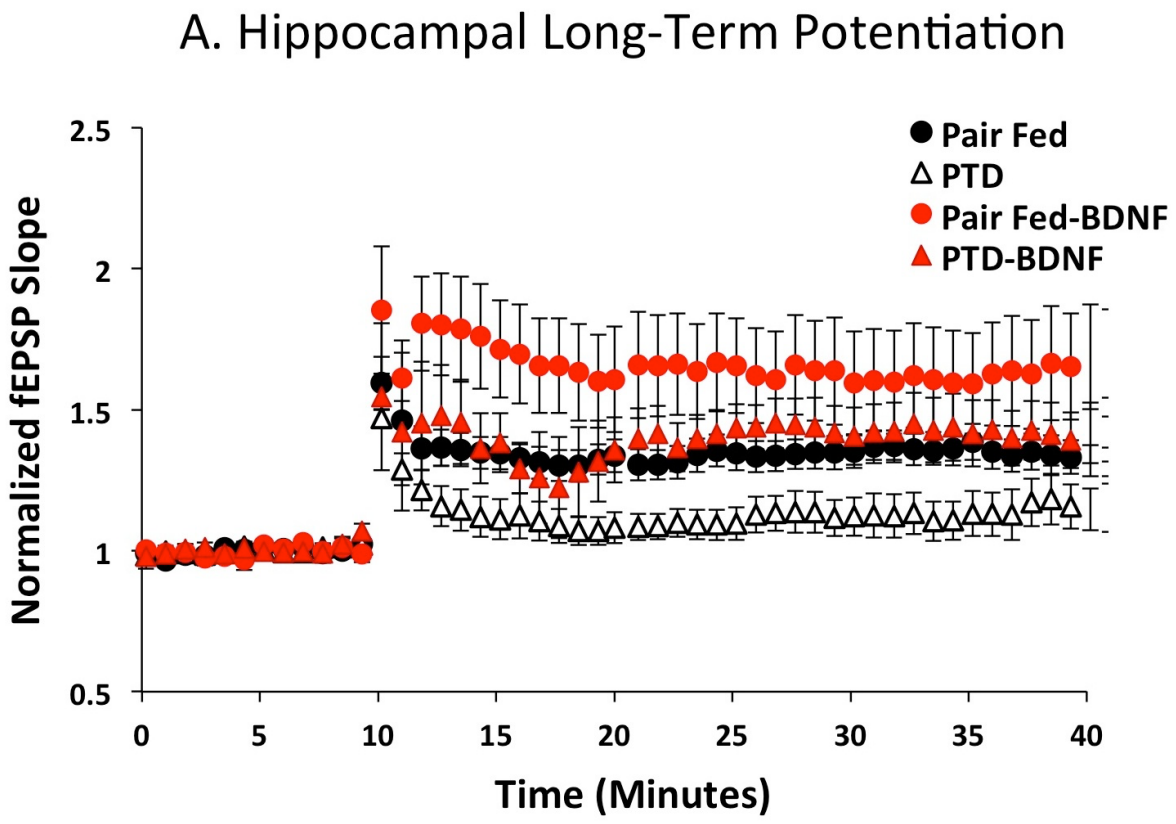
Abstract: Alcoholism is commonly associated with thiamine deficiency that leads to diencephalic damage, hippocampal dysfunction and spatial learning and memory deficits. We studied the effects of thiamine deficiency, in both male and female rats, on hippocampal synaptic plasticity and function. Using the pyrithiamine induced thiamine deficiency (PTD) model, along

with pair-fed controls, we found that following a prolonged recovery period from thiamine deficiency there were decreases in the magnitude of LTP (see A) and paired-pulse facilitation (PPF) at CA3-CA1 synapses in both male and female PTD rats. There were no sex differences in spatial working memory or measures of hippocampal plasticity. Thus, despite a lack of extensive hippocampal cell loss in the PTD model, diencephalic brain damage down-regulates synaptic plasticity within the hippocampus, likely contributing to impaired hippocampal-dependent behaviors. However, both measures of hippocampal plasticity (LTP, PPF) were restored with a BDNF application (20 ng/mL), revealing an avenue for neural and behavioral recovery following diencephalic damage.



Disclosures: **L.C. Vedder:** None. **L.M. Savage:** A. Employment/Salary (full or part-time): Binghamton University State University of New York. 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; NIAAA (RO1AA021775) and the NINDS (R21NS085502) to LMS.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.08/M3

Topic: B.08. Synaptic Plasticity

Title: Investigating the roles of atg1 and atg8a in glutamate receptor localization

Authors: *K. BERNARD[1], F. LIEBL[2];
[1]Biol. Sci., Southern Illinois Univ. Edwardsville, Edwardsville, IL; [2]Southern Illinois University-Edwardsville, Edwardsville, IL

Abstract: Glutamate is the most abundant excitatory neurotransmitter in the central nervous system. It is implicated in synaptic plasticity including learning and memory and long term potentiation, which are characterized by changes in the number and localization of glutamate receptors (GluRs) in the postsynaptic cell. We have found that proteins important for autophagy, a cellular process that degrades cellular components for reuse, affect the synaptic localization of GluRs at the *Drosophila* neuromuscular junction. Interestingly, mutations in *atg1*, *atg13*, and *atg8a* lead to a reduction in synaptic GluRs suggesting that they either indirectly regulate GluR localization or signal independent of autophagy. In support of the latter, loss of *atg8a* function leads to a more severe reduction in synaptic GluRs than loss of *atg1* function. To examine the effects of Atg8a on glutamate receptor distribution, we preformed tissue specific overexpression and knockdown experiments and are examining the possibility that Atg8a signals downstream of the insulin receptor pathway to regulate GluR localization.

Disclosures: K. Bernard: None. F. Liebl: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.09/M4

Topic: B.08. Synaptic Plasticity

Support: Grants-in-Aid for Young Scientists

Meiji Yasuda Life Foundation of Health and Welfare

Grants-in-Aid from the Nakatomi Foundation

Title: Motor training promotes both synaptic and intrinsic plasticity of layer II/III pyramidal neurons in the primary motor cortex

Authors: *H. KIDA, Y. TSUDA, Y. YAMAMOTO, Y. OWADA, N. ITO, D. MITSUSHIMA; Yamaguchi university Sch. of Med., Ube, Yamaguchi, Japan

Abstract: Motor skill training induces structural plasticity at dendritic spines in the primary motor cortex (M1). To further analyze both synaptic and intrinsic plasticity in the layer II/III area of M1, we subjected rats to a rotor rod test and then prepared acute brain slices. Motor skill consistently improved within two days of training. Voltage clamp analysis showed significantly higher AMPA/NMDA ratios and miniature EPSC amplitudes in 1-day trained rats compared to untrained rats, suggesting increased postsynaptic AMPA receptors in the early phase of motor learning. Compared to untrained controls, 2-days trained rats showed significantly higher miniature EPSC amplitude and frequency. Paired-pulse analysis further demonstrated lower rates in 2-days trained rats, suggesting increased presynaptic glutamate release during the late phase of learning. 1-day trained rats showed decreased miniature IPSC frequency and increased paired-pulse analysis of evoked IPSC, suggesting a transient decrease in presynaptic GABA release. Moreover, current clamp analysis revealed lower resting membrane potential, higher spike threshold, and deeper afterhyperpolarization in 1-day trained rats—while 2-days trained rats showed higher membrane potential, suggesting dynamic changes in intrinsic properties.
Our present results revealed dynamic changes in synaptic plasticity and neural properties after motor training within M1. In the 1-day trained rats, motor training strengthened AMPA receptor-mediated excitatory synapses and drastically reduced presynaptic GABA release probability. In the 2-days trained rats, motor training further strengthened AMPA receptor-mediated excitatory synapses together with NMDA receptors and increased presynaptic glutamate release, while increasing the presynaptic GABA release probability. Based on our findings, we conclude that motor training promotes synaptic plasticity at both excitatory and inhibitory synapses, as well as changes neural properties, such as resting membrane potential and threshold of M1 layer II/III neurons.

Disclosures: H. Kida: None. Y. Tsuda: None. Y. Yamamoto: None. Y. Owada: None. N. Ito: None. D. Mitsushima: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.10/M5

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH060387

CIHR Operating Grant MOP 340328

Title: Different PKM isoforms in the postsynaptic target neuron contribute to different forms of persistent synaptic plasticity expressed at convergent presynaptic inputs

Authors: *S. SCHACHER[1], K. ADLER[2], M. HASTINGS[3], W. SOSSIN[4], J. HU[2];
[2]Neurosci., [1]Columbia Univ. & NYSPI, New York, NY; [3]Psychology, [4]Neurol. and Neurosurg., McGill University-Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Synaptic tagging and capture is a potential cellular mechanism mediating some forms of behavioral generalization. Weak stimuli, insufficient to produce a long-term change, evoke long-term memory or synaptic plasticity when temporally paired with strong stimuli that produce long-term memory or synaptic plasticity at a convergent pathway. At the cellular level it is not clear whether persistent plasticity (e.g. facilitation) expressed at each input share the same properties. Here we used the behaviorally relevant sensorimotor synapse of *Aplysia* to examine whether convergent sensory neuron inputs onto a common postsynaptic target (motor neuron L7) use different mechanisms to sustain persistent long-term facilitation (LTF). Two sensory neurons were plated with a single L7 in culture and allowed to form stable synaptic connections. One sensory neuron input received activity paired with a bath application of serotonin (5-HT; strong stimuli), while the other sensory neuron input received the bath application of 5-HT alone (weak stimuli). Two pairings of activity plus 5-HT to the same sensory neuron input (one pairing each day) resulted in persistent LTF (lasting more than 6 days) expressed at both sensory neuron synapses. Two unpaired stimuli presented in the same manner (one set of stimuli per day) failed to induce any form of LTF at either sensory neuron synapse. The persistent LTF evoked at each synapse had different properties. The sensory neuron input that received the paired stimuli expressed a plasticity characteristic of persistent associative LTF, while the other input that received the weak stimuli of two brief applications of 5-HT expressed a plasticity characteristic of persistent non-associative LTF. Selectively blocking the activity of PKM Apl III, derived from the atypical PKC Apl III, in L7 only reversed the persistent plasticity expressed at the sensory neuron input that received the paired stimuli. In contrast, selectively blocking the activity of PKM Apl I, derived from the classical PKC Apl I, in L7 reversed the persistent plasticity expressed at the other input that received the weak stimuli. Thus convergent inputs onto a common target neuron can use different mechanisms initiated in the postsynaptic partner

to maintain persistent LTF. Selective manipulations in the postsynaptic neuron enabled reversals in an input-specific manner.

Disclosures: S. Schacher: None. K. Adler: None. M. Hastings: None. W. Sossin: None. J. Hu: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.11/M6

Topic: B.08. Synaptic Plasticity

Support: R01MH104319

R01MH095980

R01 EB002170

BRF SIA-2014-01

Howard Hughes Medical Institute

NIH NS21184

NIH MH095980

Title: Quantifying information storage in the hippocampus: Lessons from the dentate gyrus

Authors: *C. BROMER[1,2], T. M. BARTOL[1], W. C. ABRAHAM[3], J. BOWDEN[4], P. GONZALEZ[4], D. HANKA[4], D. HUBBARD[4], M. KUWAJIMA[4], J. MENDENHALL[4], P. PARKER[4], T. J. SEJNOWSKI[1], K. HARRIS[4];
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Abstract: Understanding how the amount of information stored at the synapse differs across the hippocampus has profound implications for memory. Our previous work (Bartol et al. eLife, 4:e10778, 2016) demonstrated a tight correlation in size and strength of dendritic spines with a shared presynaptic and postsynaptic history in a 3D reconstruction of hippocampal neuropil from rat CA1 stratum radiatum. In this brain region, when two spines are located on the same dendritic branch and receive input from a single axon, the coefficient of variation (CV) in the size of the

two spines (spine head volume) is 0.083. Using the observed distribution of spines in the sample, the calculated CV and standards from signal detection theory, we predicted this tight level of control over synaptic strength to correspond to ~4.7 bits of information stored at the synapse. In our current study, we examined pairs of spines with a shared presynaptic and postsynaptic history in three layers of dentate gyrus in rat neuropil (inner molecular layer, middle molecular layer and outer molecular layer) under control and long-term potentiation (LTP) conditions. We were not only able to examine differences in information processing across the hippocampus (the dentate gyrus being a region typically perceived as "upstream" of CA1), but also to make predictions about how the high correlation between spine pairs changes in response to activity (in the LTP condition). In electron microscopy images taken 30 minutes after induction, where the contralateral hippocampus (not receiving stimulation) serves as a control, potentiation in the middle molecular layer and concurrent long-term depression (cLTD) in the inner and outer molecular layers provides an opportunity to parse bidirectional changes in synaptic weight relative to control. In particular, the correlation in sizes of spines sharing a presynaptic and postsynaptic history is altered under a stimulation protocol.

Disclosures: C. Bromer: None. T.M. Bartol: None. W.C. Abraham: None. J. Bowden: None. P. Gonzalez: None. D. Hanka: None. D. Hubbard: None. M. Kuwajima: None. J. Mendenhall: None. P. Parker: None. T.J. Sejnowski: None. K. Harris: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.12/M7

Topic: B.08. Synaptic Plasticity

Support: Canadian Institute of Health Research (CIHR) Operating grant MOP 340328

Title: KIBRA is an isoform-specific PKM stabilizer in *Aplysia* sensory neurons

Authors: *L. FERGUSON, N. BENFEY, T. DUNN, W. SOSSIN;
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Abstract: The persistent activity of PKMs has been proposed to be critical to the maintenance of transcription-dependent memory, despite the fact that no links have been established between transcription and PKM maintenance. We have been studying the role of PKM in maintaining memories in the sensory-motor reflex of the marine mollusk, Aplysia californica. In this system, PKM formation is mediated through transcription-independent calpain-mediated cleavage of PKC into PKM. Indeed, PKMs are important in the maintenance of several forms of

transcription-independent intermediate facilitation (Sutton et al, 2004; Bougie et al, 2012). Nevertheless, similar to vertebrates, inhibitors of PKM can erase transcription-dependent forms of memory and synaptic facilitation, suggesting that transcriptional events can prolong the lifetime of PKM activity (Cai et al, 2011). We are examining the kidney-brain adaptor protein, KIBRA, as a possible link between transcription and PKM. KIBRA has been implicated in human episodic memory and has been shown to stabilize PKMs in rodent hippocampus and to be required for long-term memory formation (Vogt-Eisele et al., 2014). We have cloned the Aplysia orthologue of KIBRA and found that the PKC/PKM-binding domain (as well as the WW and C2 domains) is highly conserved between mammalian and Aplysia KIBRA. Quantitative PCR experiments demonstrate that KIBRA mRNA is upregulated during learning (Ferguson et al., SfN 2015 poster). We show that removing the putative PKM binding domain of KIBRA abolishes stabilization of PKM Apl III, but surprisingly increases stabilization of PKM Apl I, whose levels are not increased by expression of wild-type KIBRA. This suggests distinct KIBRA domains required for stabilization of different PKMs and competition in cells for KIBRA binding. Additionally, KIBRA lacking the PKM binding domain acts as a dominant negative that destabilizes PKM produced through cleavage of overexpressed PKC Apl III. We will determine whether this differential stabilization is due to differential binding of PKM isoforms, and whether KIBRA overexpression in the postsynaptic neuron of Aplysia sensory-motor neuron co-cultures is sufficient to prolong intermediate-term facilitation. We will also investigate KIBRA's role in localizing PKM to its synaptic targets by exploring KIBRA's colocalization with postsynaptic AMPA receptors. Confirming KIBRA's role in prolonging PKM's half-life in the potentiated synapse will broaden our understanding of the relationship between transcription-independent PKM formation and transcription-dependent synaptic facilitation.

Disclosures: L. Ferguson: None. N. Benfey: None. T. Dunn: None. W. Sossin: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.13/M8

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH060387

CIHR Operating Grant MOP 340328

Title: Cell-specific PKM isoforms are required for maintaining different forms of persistent synaptic plasticity at a behaviorally relevant synapse

Authors: *J. HU[1], K. ADLER[1], C. A. FARAH[2], M. HASTINGS[3], W. SOSSIN[2], S. SCHACHER[1];
[1]Dept. of Neurosci., Columbia Univ. Med. Ctr. & NYSPI, New York, NY; [2]Neurol. and Surgery, [3]Psychology, McGill University-Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Long-term changes in synaptic strength contribute to long-term memory. There is little information about the role of specific molecules in both the presynaptic and postsynaptic partner in maintaining persistent plasticity. The sensorimotor synapse of *Aplysia* expresses both non-associative and associative forms of persistent long-term facilitation (LTF) that are cellular analogs of long-term sensitization and classical conditioning, respectively. Although several kinases contribute to the expression of different forms of LTF, constitutive PKC activity is required for maintaining both non-associative and associative LTF. *Aplysia* neurons express three isoforms of PKC - Apl I (classical), Apl II (novel) and Apl III (atypical) – that are also cleaved in a calpain-dependent manner into constitutively active forms PKM Apl I, PKM Apl II and PKM Apl III, respectively. We examined the cell-specific roles of the PKMs in maintaining persistent non-associative and associative LTF by overexpressing dominant negative forms of each PKM In either the sensory neuron or the motor neuron L7. Constructs were injected into the cell 48 hours after the last stimuli, and synaptic strength was monitored over the next 3-4 days to determine the consequences of the cell-specific overexpression of each dominant negative construct on control synaptic baseline and on the maintenance of persistent LTF evoked by the different stimuli. PKM activities were not required for maintaining control synaptic baseline. However, the maintenance of persistent non-associative LTF selectively required PKM Apl II and PKM Apl III activities (but not PKM Apl I) in sensory neurons and PKM Apl I activity (but not PKM Apl II and PKM Apl III) in motor neuron L7. In contrast, the maintenance of persistent associative LTF selectively required PKM Apl II and PKM Apl III activities (but not PKM Apl I) in motor neuron L7 and PKM Apl II activity (but not PKM Apl I and PKM Apl III) in sensory neurons. The classical calpain in L7, one isoform of calpain known to cleave the PKC isoforms into the PKMs, was not required for maintaining either form of persistent plasticity. These results indicate that different stimuli inducing different forms of persistent synaptic plasticity regulate the cell-specific activation of different PKM isoforms for maintaining different forms of persistent plasticity.

Disclosures: J. Hu: None. K. Adler: None. C.A. Farah: None. M. Hastings: None. W. Sossin: None. S. Schacher: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.14/M9

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS081786

Title: Increase in metabolic efficiency underlies Hebbian hippocampal long term potentiaiton

Authors: *P. MIRANDA[1], H.-A. PARK[1], P. LICZNERSKI[1], C. PEQUIGNOT[2], S. SACCHETTI[1], K. ALAVIAN[3], H. LI[1], H. IMAMURA[4], H. NOJI[5], J. SHEPHERD[6], A. E. CHAVES[7], R. S. ZUKIN[7], E. A. JONAS[1];
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Abstract: Long-term potentiation (LTP) and depression (LTD) are the mechanisms by which neurons modulate their inherent synaptic plasticity and support the storage and recovery of memories in the mammalian brain. The ability to potentiate a synapse declines significantly in neurodegenerative disorders and is related to deficiencies in LTP. In addition to deficiencies in synaptic plasticity, degenerating neurons display acute and chronic mitochondrial dysfunction, suggesting that dysregulated mitochondria play a hand in synaptic dysfunction in addition to their known role in apoptotic death. Our previous work shows that the anti-apoptotic protein Bcl-xL not only prevents somatic cell death, but it also enhances synaptic responses. Here, we show that Bcl-xL is responsible for dramatic changes in ATP levels at synapse-specific sites in hippocampal neurons. Using fluorescent imaging of an ATP-FRET construct in living cells, we find that LTP induction in isolated hippocampal neurons causes a short decrease in ATP levels followed by a persistent long term increase in ATP production. This suggests that after high frequency or intense synaptic stimulation, neurons may become metabolically more active and possibly more efficient; oxygen consumption rates during LTP are now being performed to confirm or refute the proposed change in efficiency. The long-term increase in ATP levels in LTP-stimulated synapses is blocked when we inhibit Bcl-xL and when ATP synthase activity is blocked with oligomycin, suggesting that mitochondria are required to produce the increased ATP levels. Bcl-xL inhibition also prevents a long-term increase in surface glutamate receptor insertion. We are completing studies to see what effect blocking ATP synthase activity has on glutamate receptor insertion. In hippocampal slice recordings, inhibition of Bcl-xL greatly impairs early stage LTP and prevents late stage LTP. We suggest that long term changes in mitochondrial efficiency brought on by activity-dependent translocation of Bcl-xL to mitochondria are required for LTP. Our studies shed light on the role of changes in mitochondrial metabolic efficiency in the acute induction and long term maintenance of learning and memory storage. If such mitochondrial changes fail to occur, synaptic dysfunction associated with neurodegeneration may ensue. Our study places mitochondria and Bcl-x_L at the center of synaptic metabolism and synaptic plasticity.

Disclosures: P. Miranda: None. H. Park: None. P. Licznerski: None. C. Pequignot: None. S. Sacchetti: None. K. Alavian: None. H. Li: None. H. Imamura: None. H. Noji: None. J. Shepherd: None. A.E. Chaves: None. R.S. Zukin: None. E.A. Jonas: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.15/M10

Topic: B.08. Synaptic Plasticity

Support: NMRC/CBRG/0041/2013

Title: Metabotropic glutamate receptor 4 (mGluR4) determines plasticity and associativity in hippocampal area CA2

Authors: *A. DASGUPTA, L. YU JIA, S. SREEDHARAN;
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Abstract: Mammalian brain is well known for its 'plasticity' i.e. the ability of an experience generated neural activity to alter the strength and efficacy of its neural circuits and has been widely studied in hippocampal area CA1. However, area CA2 is starting to garner attention in recent years, due to better characterization of the boundaries of CA2 and greater understanding of its unique physiological, molecular and genetic properties that differentiate it from CA1 and CA3.
Entorhinal cortex projections on CA2 (EC→CA2) express robust Long-Term Potentiation (LTP) on high-frequency stimulation whereas Schaffer collateral synapses (SC→CA2) on this area lack this activity dependent plasticity under the normal high-frequency stimulation protocols. However, not much about the mechanisms and pathways involved in restricted plasticity within CA2 has been elucidated. Presence of a plasticity regulating protein RGS 14 and excessive calcium buffering could be the attributing factors. The metabotropic glutamate receptor 4 (mGluR4) is highly expressed in area CA2 compared to other regions of the hippocampus. In this study, we hypothesize that over-expression of mGluR4 could be one of those regulating factors restricting plasticity at Schaffer collateral synapses. Thus, inhibition of mGluR4 might help in restoring plasticity in this area. Therefore, the pharmacological blockade of mGluR4 on induction of LTP in Schaffer collateral CA2 synapses is investigated using electrophysiological techniques. Inhibition of mGluR4 results in significant late-LTP when a strong tetanus is given. This LTP is N-methyl-D-aspartate (NMDA) receptor-dependent and has a late-phase which is reliant on protein synthesis. The induced LTP also shows preliminary support for synaptic tagging and capture.
Hence, it can be concluded that the identification of mGluR4 as a player in the regulation of synaptic plasticity helps bridge the gap in understanding the plasticity regulation in area CA2.

Disclosures: A. Dasgupta: None. L. Yu Jia: None. S. Sreedharan: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.16/M11

Topic: B.08. Synaptic Plasticity

Support: KAKENHI

Takeda Foundation

Uehara Foundation

Novartis Foundation

Title: Phosphoinositide responsive Phldb2 regulates synaptic plasticity

Authors: *M. XIE[1,2,3,4], H. YAGI[5], T. IGUCHI[6], Y. OKA[4,6], Y. FUKAZAWA[1,3], H. MATSUZAKI[2,4], K. IWATA[2,4], Y. ISHIKAWA[7], M. SATO[2,4,6];
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Abstract: Phosphoinositides, such as phosphatidylinositol 3,4,5-triphosphate (PIP_3) and phosphatidylinositol 4,5-bisphosphate (PIP_2), are essential regulators for diverse range of biological events. For synaptic plasticity, involvement of PIP_3 and/or PIP_2 for LTP as well as LTD is reported, yet how they regulate the subsequent molecular machineries mostly remains elusive. We show here that Phldb2, of which PH domain is one most sensitive to PIP_3, controls synaptic plasticity responding to membrane phosphoinositides. Phldb2 kept an interaction between NMDA receptor and CaMKII last long, which is critical for LTP induction and maintenance, and contributed to AMPA receptor accumulation at the postsynaptic membrane responding to PIP_3. Indeed, LTP was impaired, from an early phase to the late phase (memory formation), in the Phldb2 knockout mice. Although Phldb2 was unstable in the presence of activated CaMKII, it was more stable in the presence of GluA1 or GluA2, suggesting a crucial role of AMPA receptor for long-lasting LTP. Furthermore, Phldb2 was essential for LTD: Phldb2 bound to AP2 for receptor endocytosis as well as to PSD-95. In conclusion, Phldb2 is a versatile, but is critical mediator for synaptic plasticity and its function, responding to dynamic membrane phosphoinositides.

Disclosures: M. Xie: None. H. Yagi: None. T. Iguchi: None. Y. Oka: None. Y. Fukazawa: None. H. Matsuzaki: None. K. Iwata: None. Y. Ishikawa: None. M. Sato: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.17/M12

Topic: B.08. Synaptic Plasticity

Support: NRW-Rückkehrerprogramm

Human Frontiers Science Program RGY-0084/2012

UCL Excellence Fellowship

German Research Foundation SFB1089 B03

German Research Foundation SPP1757

Title: Extracellular matrix heparan sulfates contribute to pyramidal cell excitability and synaptic long-term potentiation

Authors: *D. MINGE[1], O. TIKHOBRAZOVA[2], G. KOCHLAMAZASHVILI[2], A. DITYATEV[2], C. HENNEBERGER[1,3,4];
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Abstract: Heparan sulfates (HS) are a heterogeneous group of extracellular glycosaminoglycans consisting of sulfated disaccharide repeats. Within the extracellular matrix (ECM) of the adult central nervous system, the majority of HS are attached to a variety of core proteins and form the complex group of heparan sulfate proteoglycans (HSPG). HSPGs have been suggested to play a pivotal role in maintaining various aspects of hippocampal neuron function. Therefore, acute loss of HS/HSPG is expected to modify synaptic and intrinsic properties of neurons within the hippocampus and also their long-term plasticity. This was tested by treating acute hippocampal slices obtained from five to seven week old C57BL/6 mice with *Heparinase I*, an enzyme that specifically cleaves HS, and combining electrophysiology and two-photon excitation fluorescence intensity and lifetime microscopy. Enzyme treatment led to an impairment of long-term potentiation (LTP) at CA3-CA1 Schaffer collateral synapses induced by theta burst

stimulation (TBS). This impairment persisted when GABAergic inhibition was pharmacologically blocked and did not result from altered excitatory synaptic transmission. Nonetheless, individual CA1 pyramidal neurons showed decreased dendritic and spine Ca^{2+} influx during TBS. Further experiments revealed, that Ca^{2+} entry evoked by back-propagating actions potentials (b-APs) and direct depolarization in voltage clamp and also resting Ca^{2+} concentrations were not affected by heparinase treatment. In contrast, the number of APs generated by CA1 pyramidal cells during TBS was significantly lower after heparinase treatment. Therefore, heparinase treatment impairs LTP by reducing CA1 pyramidal cell excitability during TBS with no detectable effect on voltage-dependent Ca^{2+} influx at spines/dendrites. Somatic current injections mimicking TBS also resulted in fewer APs in heparinase treated slices suggesting that HS maintain pyramidal cell excitability. Analysis of spiking behavior revealed a selective effect of heparinase treatment on the threshold of spikes evoked by strong, repetitive stimuli. In contrast, various other parameters of neuronal excitability like sag potential and afterhyperpolarization were not affected. This suggests that heparinase treatment may reduce axonal excitability. Thus, heparan sulfates support high-frequency firing of CA1 pyramidal cells. They indirectly promote Ca^{2+} influx during induction of synaptic plasticity using TBS and thereby support synaptic long-term plasticity.

Disclosures: D. Minge: None. O. Tikhobrazova: None. G. Kochlamazashvili: None. A. Dityatev: None. C. Henneberger: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.18/M13

Topic: B.08. Synaptic Plasticity

Support: NIH GRANT R01 MH104602-01

Title: Input-timing dependent plasticity provides a general synaptic learning rule in hippocampal CA1 and CA2 regions

Authors: *F. LEROY, D. H. BRANN, S. A. SIEGELBAUM;
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Abstract: Classical hippocampal long-term synaptic plasticity is homosynaptic, induced by tetanic stimulation of a single input pathway. In contrast, input-timing dependent plasticity (ITDP) is a circuit-based form of heterosynaptic plasticity induced by low-frequency paired stimulation of the direct entorhinal cortex (EC) inputs to CA1 pyramidal neurons (PNs) precisely

20 ms prior to activating their CA3 Schaffer collateral (SC) inputs. This pairing results in the long-term depression of SC-evoked feedforward inhibition (iLTD) mediated by cholecystokinin (CCK)-expressing basked cells through the activation of CB1 endocannabinoid receptors. As CA2 PNs also receive direct EC and SC inputs, we asked whether ITDP may represent a general synaptic learning rule. Paired activation of EC and SC inputs at the 20 ms pairing interval also induced a robust ITDP in CA2 PNs as a result of iLTD. However, contrary to CA1, the EC-evoked PSP also displayed a moderate but significant increase. Furthermore, ITDP in CA2 relies on δ-opioid receptor activation rather than CB1 receptors, suggesting that the iLTD was mediated by parvalbumin-expressing interneurons. Additionally we show that pairing of electrical stimulation of the SC inputs with optogenetic stimulation of defined EC inputs is sufficient to induce ITDP in CA2 PNs, confirming the importance of the EC inputs in ITDP. Thus, precise temporal pairing of EC and SC inputs may provide a general synaptic learning rule whose precise cellular ad molecular mechanisms are tuned to the specific local inhibitory circuitry.

Disclosures: F. Leroy: None. D.H. Brann: None. S.A. Siegelbaum: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.19/M14

Topic: B.08. Synaptic Plasticity

Support: CIHR

NSERC

Title: Sleep slow-wave activity induces long-term potentiation of thalamocortical responses and increase memory consolidation in mice

Authors: *I. V. TIMOFEEV, B. LOBO DE FRANCA, A. OZUR, J. SEIGNEUR, S. CHAUVETTE;
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Abstract: It is well known that sleep contributes to consolidation of different forms of memory. It is apparent that synaptic reorganization plays a role in sleep-dependent memory consolidation. The proposed patterns of cortical synaptic reorganization spanned from sleep-dependent synaptic down scaling to sleep-dependent synaptic facilitation. We previously demonstrated that sleep slow waves induce potentiation of intracortical synapses. Here we ask: Does slow-wave sleep

potentiate thalamocortical synapses and does an increase in slow-wave activity facilitate memory formation in mice? To test these hypotheses, we used B6Cg-Tg(Thy1-COP4/EYFP)18Gfng/J mice, which express channelrhodopsin 2 channels in the vast majority of cortical and thalamic neurons. We used cortical (frontal or occipital cortex) or thalamic (MD nucleus) LED stimuli delivered via a fiber-optic microcannula and recorded neuronal or field potential responses in either anesthetized or behaving animals. To test the memory formation, we used the novel object recognition test because it represents a model of declarative memory, which does not have spatial (hippocampal) component. MD thalamic stimulation induced short-latency spikes in the vicinity of stimulating fiber-optic microcannula and 3-5 ms later it triggered evoked potential in the frontal cortex. The onset of neuronal depolarization in different neurons corresponded to different components of evoked potential. The amplitude of the earliest depth-negative component of evoked potential was always enhanced after a period of slow-wave sleep, suggesting synaptic potentiation. Optogenetic stimulation of frontal cortex induced local excitation followed by a slow wave that propagated to motor, somatosensory, visual, and retrosplenial cortices. We used rhythmic blue light stimulations for 4 hours of the frontal cortex at the onset of light period with a frequency mimicking slow-wave sleep (1 Hz), which significantly enhanced LFP delta power. Novel object recognition test done at the end of dark cycle shows that both stimulated and not stimulated mice spent much more time exploring the novel object than the old one. However, mice from the stimulated group spent much less time exploring the old object, suggesting that they remembered more the old object than the sham group of mice. These results show that slow-wave sleep induces synaptic potentiation of thalamocortical synapses and enhanced slow-wave activity increases memory consolidation.

Disclosures: I.V. Timofeev: None. B. Lobo de Franca: None. A. Ozur: None. J. Seigneur: None. S. Chauvette: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.20/M15

Topic: B.08. Synaptic Plasticity

Title: The synaptic role of GSK3 isoforms in adult mouse brain synapticity

Authors: *T. MIYATA[1,2], A. AVILA[1], J. GEORGIOU[1], G. L. COLLINGRIDGE[1,3], J. WOODGETT[1,2];
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Abstract: Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase implicated in normal brain development and a variety of brain and other disorders. For example, GSK3 is required for proper formation of axons, the signalling arms of neurons (Garrido et al., 2007). Moreover, the most commonly prescribed mood-stabilizing drug for patients with bipolar disorder, lithium, directly inhibits GSK3 activity (Freland and Beaulieu, 2012). However, the mechanisms by which GSK3 orchestrates its effects on brain function and dysfunction in psychiatric and cognitive disorders are poorly understood. This study was designed to investigate the role of the two mammalian isoforms of GSK3 (GSK3α and β) by selective inhibition of their expression in the adult brain of mice. To evaluate the effects of inactivation of each isoform individually or together, the genes for GSK3α and GSK3β were inactivated using a Cre-LoxP strategy; in these GSK3-Cre knockout (KO) mice, Cre recombinase expression is driven in the adult forebrain by the calmodulin-dependent protein kinase II promoter (CamMK2). Phenotypes of GSK3-Cre KO and control mice were compared at around P50 using electrophysiological measures of hippocampal synaptic function and plasticity. Preliminary findings from electrophysiology experiments revealed that the effect of GSK3 suppression on synaptic plasticity was isoform specific such that hippocampal brain slices from GSK3α-Cre KO mice, but not GSK3β-Cre KO, exhibited impaired maintenance of long-term potentiation (LTP) and also long-term depression (LTD), as compared to control slices. These findings illustrate the unique function of GSK3 isoforms on adult hippocampal synaptic plasticity in an adult excision model where early neurodevelopmental effects are not likely to confound the conclusions. The results exemplify the utility of combining genetic approaches with electrophysiological approaches for investigating the molecular mechanisms of disorders that affect cognitive function. Future work will include pharmacological and behavioural analysis to elucidate drug actions according to gene dosage.

Disclosures: T. Miyata: None. A. Avila: None. J. Georgiou: None. G.L. Collingridge: None. J. Woodgett: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.21/M16

Topic: B.08. Synaptic Plasticity

Support: NIH Grant Z01 ES100221

Title: Disentangling negative regulators of plasticity in CA2 pyramidal neurons

Authors: *K. CARSTENS[1], D. LUSTBERG[2], S. M. DUDEK[2];
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Abstract: CA2 pyramidal neurons are molecularly distinct from neighboring CA1 and CA3 subfields as early as embryonic development in the mouse. Interestingly, glutamatergic synapses in stratum radiatum (SR) of CA2 are resistant to some forms of synaptic plasticity, specifically to the induction of long-term potentiation (LTP). Perhaps not coincidently, during early postnatal development, several negative regulators of synaptic plasticity appear in CA2 pyramidal cells but not in CA1 or CA3. CA2 synapses still however have the capacity for LTP, for example, a knock-out of regulator of G-protein signaling 14 (RGS14), a negative plasticity regulator in CA2, enables LTP in CA2. We studied the developmental expression pattern of several negative plasticity regulators in CA2 and whether they may function together to dampen plasticity in CA2 later in development. Recently, we identified another negative plasticity regulator in CA2, the specialized extracellular matrix perineuronal nets (PNNs), that also suppresses LTP at CA2 excitatory synapses. Degradation of PNNs in acute hippocampal slices between postnatal day 14 (PN14) and PN18 was sufficient to enable LTP in CA2. Immunohistochemical analysis indicates that the PNN component, aggrecan, first appears in CA2 at PN14 and is absent at PN11; however, other negative plasticity regulators are present at PN11, such as RGS14 and Purkinje cell protein 4 (Pcp4). To disentangle which negative plasticity regulators are necessary to block LTP induction in CA2, we tested LTP in CA2 at younger ages when PNNs are absent. We found that LTP is expressed at CA2 SR synapses between PN8-11, suggesting that RGS14 is not sufficient to suppress LTP at younger ages. Finally, we investigated how PNNs and other negative plasticity regulators may be altered during early development in a mouse model of Rett Syndrome (MECP2 KO mice). We found that PNNs are increased in the MECP2 KO mouse surrounding excitatory CA2 pyramidal cells from PN14 to adulthood and that PNNs develop abnormally early in CA2 compared to control littermates, suggesting that synapse stabilization and synaptic plasticity may be prematurely dysregulated in CA2 during critical windows of development. Taken together, these results may reveal a mechanism behind the severe hippocampal-dependent learning impairments that emerge during postnatal development in Rett syndrome infants.

Disclosures: K. Carstens: None. D. Lustberg: None. S.M. Dudek: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.22/M17

Topic: B.08. Synaptic Plasticity

Support: Cure Alzheimer's Fund

NIH P50MH086403

NIH 845098274057

NIH T32 NS 7280-27

NIH T32 DA 35165-2

Title: Investigating the role of the retromer, an endosomal sorting complex, in long-term potentiation in the adult mouse hippocampus

Authors: *P. TEMKIN, W. MORISHITA, D. GOSWAMI, R. MALENKA;
Psychiatry and Behavioral Sci., Stanford, Stanford, CA

Abstract: The retromer is an endosomal sorting complex that has been shown to be involved in several regulated and specialized endosome to plasma-membrane trafficking pathways. Furthermore, malfunction of the retromer has been linked to two prominent neurodegenerative diseases, Alzheimer's and Parkinson's disease. In this study we investigated the role of the retromer complex in the function of excitatory synapse in adult mouse hippocampus. To do so we made whole-cell voltage-clamp recordings from CA1 pyramidal cells in acutely prepared hippocampal slices from adult mice that, 2-3 weeks previously, had received injections of lentivirus containing shRNA against VPS35, a key component of the retromer complex. We find that VPS35 knockdown (KD) severely impairs NMDA receptor-dependent long-term potentiation (LTP), without altering basal synaptic properties or other NMDA receptor mediated forms of synaptic plasticity. Experiments in knockout mice lacking the amyloid precursor protein (APP) reveal that the impairment of LTP due to VPS35 KD is independent of the retromer's influence on APP processing. We also find that in vivo expression of shRNA-resistant wildtype VPS35 rescues the LTP deficit caused by VPS35 KD, while expressing VPS35 containing a familial Parkinson's mutation does not rescue this phenotype. Lastly, live imaging experiments, performed on cultured hippocampal neurons expressing superecliptic-GFP tagged GluA1, show that VPS35 KD prevents insertion of AMPA receptor after induction of chemical LTP. These results suggest that retromer function is necessary to support AMPA receptor trafficking during LTP. Further experiments will be necessary to determine whether this novel retromer-dependent membrane trafficking pathway contributes to its pathological role in Alzheimer's and Parkinson's diseases.

Disclosures: P. Temkin: None. W. Morishita: None. D. Goswami: None. R. Malenka: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.23/M18

Topic: B.08. Synaptic Plasticity

Title: The role of schizophrenia risk gene dysbindin in stress induced anxiety and aggression

Authors: *Q. GU;
NIH, Bethesda, MD

Abstract: Schizophrenia is a serious psychiatric disorder which affects how people feel, think and act. It has 1% prevalence in the population. Many schizophrenia risk genes have been identified, but their effect sizes are small. Environmental factors, such as stress, also contribute to the onset of schizophrenia. However, it is not clear whether schizophrenia risk genes also confer vulnerability to environmental factors. Sandy mice are mutant mice null for the schizophrenia risk gene dysbindin-1. They have working memory impairment but do not exhibit anxiety or abnormal social behavior. Here we tested whether sandy mice are vulnerable to traumatic stress by shocking with 14 strong electric-shocks in variable intervals during an 85-min period. One week after foot-shock, we tested the mice for locomotion, anxiety, social interaction, aggressive behavior and depression. Interestingly, we found that stressed sandy mice are anxious and aggressive. Because amygdala is a structure which is involved in anxiety, emotion and aggressive behavior, we hypothesize that dysbindin is involved in the stress response of the amygdala. To test this hypothesis, we use electrophysiology, calcium imaging and optogenetics to study the synaptic function and neuronal circuit in amygdala, aiming to uncover the cellular mechanism underlying the behavior changes induced by traumatic stress.

Disclosures: Q. Gu: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.24/N1

Topic: B.08. Synaptic Plasticity

Support: NIH DA036569

Title: N-Acetylcysteine inhibits cue-induced nicotine seeking and relapse-associated rapid, synaptic plasticity

Authors: *G. L. POWELL[1,2], Y. M. KUPCHIK[3], S. SPENCER[4], C. GARCIA-KELLER[4], N. STANKEVICUITE[4], D. SCHWARTZ[4], A. DEL FRANCO[1,2], J. GOENAGA[1], C. D. GIPSON[1]; [1]Dept. of Psychology, [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [3]Dept. of Med. Neurobiology, Fac. of Med., Hebrew Univ. of Jerusalem, Jerusalem, Israel; [4]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Cigarette smoking is a leading cause of preventable death, and addiction to nicotine produces long-lasting changes in brain synaptic physiology that potentially contributes to a vulnerability to relapse. Targeting glutamatergic signaling has shown somewhat effective in preventing cocaine relapse, yet there is limited utilization of compounds targeting the dysregulation of glutamatergic signaling in promoting smoking cessation. Additionally, existing smoking cessation treatments are insufficient as relapse rates remain high within the population. Cues associated with nicotine can precipitate nicotine-seeking behavior, and recently we showed that cue-induced nicotine seeking is associated with rapid, transient synaptic plasticity in the nucleus accumbens core (NAcore). It was unknown, however, if N-Acetylcysteine (NAC), an antioxidant and cystine prodrug that has shown promise as a pharmacotherapy in the treatment of various mental health disorders, could restore nicotine-induced alterations in glutamatergic signaling and synaptic plasticity thought to underlie relapse vulnerability. Chronic administration of NAC (100 mg/kg, i.p., across five sessions) during extinction from nicotine self-administration caused a significant reduction in cue-induced nicotine seeking using a preclinical model of relapse (reinstatement) after 15 min (T (time) = 15). Additionally, NAC inhibited the rapid cue-induced increase in NAcore AMPA/NMDA ratio and dendritic spine head diameter compared to vehicle treated rats. NAC also inhibited the increase in NMDA current decay associated with cue-induced nicotine seeking at T=15. As withdrawal from nicotine self-administration has been associated with a decrease in NAcore expression of glial glutamate transporter (GLT-1), we examined the ability of a lower dose of NAC (30 mg/kg) to restore sodium-dependent glutamate uptake as well as expression of GLT-1 protein. Although a significant decrease in GLT-1 function was found in nicotine-withdrawn animals compared to yoked saline, the lower dose of NAC did not restore GLT-1 protein expression or function perhaps due to bioavailability issues which have been observed in humans. We will next determine if a possible NAC-induced GLT-1 restoration is dose-dependent.

Disclosures: G.L. Powell: None. Y.M. Kupchik: None. S. Spencer: None. C. Garcia-Keller: None. N. Stankevicuite: None. D. Schwartz: None. A. del Franco: None. J. Goenaga: None. C.D. Gipson: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.25/N2

Topic: B.08. Synaptic Plasticity

Support: NSF GRFP

Title: Activity-dependent trafficking of lysosomes in dendrites

Authors: ***M. GOO**, L. SANCHO, B. L. BLOODGOOD, G. N. PATRICK;
UCSD, San Diego, CA

Abstract: In eukaryotic cells, lysosomes handle a large majority of membrane protein turnover. In neurons, the lysosome has been primarily shown to exist in somatic and axonal compartments, with little understanding of lysosomal trafficking and function in distal dendrites. However, tight control of protein stability and turnover in these distinct compartments is crucial to normal synaptic function. How then are membrane proteins degraded by the lysosome in distal dendrites? Using a combination of static and live confocal microscopy and electron microscopy, we show that lysosomes are found in distal dendrites and dendritic spines and that perturbing actin or microtubule networks affect lysosomal distribution and trafficking. Moreover, we find that lysosomes can traffic to dendritic spines in response to synaptic activity. Strikingly, 2-photon glutamate uncaging at a single spine recruits a lysosome to that spine. Our findings provide the first evidence that synaptic activity can promote the trafficking and recruitment of lysosomes into dendritic spines.

Disclosures: M. Goo: None. L. Sancho: None. B.L. Bloodgood: None. G.N. Patrick: None.

Poster

222. Long-Term Potentiation: Pre- and Postsynaptic Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 222.26/N3

Topic: B.08. Synaptic Plasticity

Support: ERC Advanced Grant 268548

Austrian Science Fund P24909-B24

Title: Symmetric spike timing-dependent plasticity at recurrent synapses optimizes storage and recall in autoassociatve CA3 networks

Authors: R. K. MISHRA[1], S. KIM[2], S. J. GUZMAN[1], *M. FROTSCHER[3], P. JONAS[1];
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Abstract: Hippocampal CA3 pyramidal neurons play a critical role in recall of memories by incomplete or degraded cues, a process termed pattern completion. Because genetic ablation of N-methyl-D-aspartate receptors (NMDARs) in CA3 neurons impairs recall of associative memories, it is suggested that plasticity at CA3-CA3 synapses play a key role in pattern completion. However, the conditions and mechanisms necessary to modify recurrent collateral CA3 synapses are largely unknown. Thus, without knowledge of plasticity rules between CA3 neurons, it is not possible to evaluate how effective the storage of memories in autoassociative memory models of the CA3 network is.
We examined the induction rules of synaptic plasticity by performing patch-clamp recordings on CA3 pyramidal neurons and by stimulating the recurrent collateral synapses in an acute hippocampal slice preparation. We found that low frequency (1 Hz) pairing of presynaptic activity (i.e. excitatory postsynaptic potentials, EPSPs) followed by postsynaptic action potentials (APs) induced long-term potentiation (LTP) of the recurrent CA3 synapses (178.0 ± 22.1% of control). Interestingly, reversing the pairing sequence (i.e. pairing APs with EPSPs) induced LTP of comparable magnitude (159.3 ± 11.5%). LTP magnitude was reduced when the delay between EPSP and AP was longer. This led to a symmetric relationship between the LTP magnitude and the time between pre- and postsynaptic activity, characterized by a Gaussian function with a half-width of ~150 ms. Induction of LTP was mechanistically identical in the EPSP-AP and the AP-EPSP sequence. It required activation of NMDARs, since LTP was prevented in the presence of 20 μM D-APV (101.2 ± 5.2% for the EPSP-AP sequence and 100.5 ± 4.2 % for the AP-EPSP sequence). Additionally, dialysis of CA3 cells with 20 mM EGTA prevented LTP (101.8 ± 7.2% and 103.0 ± 9.0% for pre-post and post-pre, respectively) suggesting that a rise in $[Ca^{2+}]_i$ was necessary. Accordingly, we found supralinear $[Ca^{2+}]_i$ transient responses in CA3 spines when pairing AP-EPSP and EPSP-AP events. In both cases, supralinear $[Ca^{2+}]_i$ summation decreased when increasing the time between pre- and postsynaptic events. In an autoassociative network model of 3000 integrate-and-fire cells, storage of binary memory patterns was more efficient with the symmetric rule when compared with the conventional asymmetric rule containing long-term depression (LTD). Accordingly, recall of patterns upon incomplete cues was better and the capacity of the network was greatly enhanced. Thus, a symmetric plasticity rule performs better storage and recall of memories in autoassociative networks.

Disclosures: R.K. Mishra: None. S. Kim: None. S.J. Guzman: None. M. Frotscher: None. P. Jonas: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.01/N4

Topic: B.08. Synaptic Plasticity

Support: World Class University Grant R32-10142

MRC grant MR/K023098/1

Title: Correlation between AMPAR trafficking and probability of neurotransmitter release during DHPG-LTD at CA1 hippocampal synapses

Authors: *T. M. SANDERSON[1,2], S. J. KIM[2], G. L. COLLINGRIDGE[1,2,3,4];
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Abstract: AMPA receptor (AMPAR) trafficking is an important mechanism by which the strength of synaptic connections is modulated. One highly heterogeneous property of synapses is their probability of neurotransmitter release (P(r)). How this property is related to AMPAR trafficking in synaptic plasticity is unknown. Here we measured P(r) using the styryl dye FM 4-64 and AMPAR trafficking using a version of the AMPAR subunit GluA2 tagged with the pH sensitive probe super ecliptic pHluorin (SEP). We studied a form of long-term depression that is induced by activating group I metabotropic glutamate receptors (mGluRs) with the agonist dihydroxyphenylglycine (DHPG-LTD).
In organotypic slices DHPG-LTD resulted in responses of 45 ± 8 % of baseline (n = 8). It was blocked by the selective mGluR1 antagonist YM 298198 (2 μM; 92 ± 12 %; n = 7; $p < 0.0001$), but not by the selective mGluR5 antagonist MPEP (10 μM; 55 ± 9 %; n = 6; $p > 0.05$). This form of plasticity did not result in a change in P(r), as PPF was unchanged following DHPG application (99 ± 11 % of baseline; n = 6). Analysis of changes in SEP-GluA2 fluorescence at synapses revealed that DHPG resulted in a leftward shift in a cumulative probability plot with respect to untreated control cells, indicating a decrease in GluA2 containing AMPARs. Comparing these changes to the P(r) of the synapses under study revealed a correlation such that AMPAR internalization was greater at low P(r) synapses (Spearman's rs, 0.48, $p < 0.001$, n = 58 spines from 8 cells).
These findings suggest that synapses are not equal in their potential for undergoing a postsynaptic form of synaptic plasticity, but are differentially susceptible depending on their P(r).

Disclosures: T.M. Sanderson: None. S.J. Kim: None. G.L. Collingridge: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.02/N5

Topic: B.08. Synaptic Plasticity

Support: World-Class University program in Korea (R32-10142)

UK Medical Research Council in the UK (MR/K023098/1)

European Research Council (ERC) - 341089

BBSRC BB/K019899/1

Title: Inhibition of GSK-3 inhibits NMDAR-LTD *In vivo* and enhances accuracy of spatial memory in mice

Authors: *Y. LEE[1,2], Z. BORTOLOTTO[1], B.-K. KAANG[2], G. COLLINGRIDGE[1,2,3,4]; [1]Univ. of Bristol, Bristol, United Kingdom; [2]Cognitive and Brain Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [4]Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada

Abstract: Synaptic plasticity is a property of synapses in which connections get stronger or weaker in response to activity. Synaptic plasticity appears in several different forms and it is widely thought to be the main mechanism underlying memory formation. In the hippocampus where spatial information is processed, dysregulation of synaptic plasticity could lead disruption of spatial learning and memory. Among a large number of ser/thr kinases, glycogen synthase kinase 3 (GSK-3), which is constitutively active and abundant in the CNS, was found to be the only ser/thr kinase involved in N-methyl-D-aspartate receptor-dependent long-term depression (NMDAR-LTD) in the hippocampal CA1 region *in vitro*. Here, we have examined the effect of the GSK-3 inhibitor, CT99021, on NMDAR-LTD *in vivo* and on spatial memory using the Morris water maze test (MWM) in C57BL/6J mice. We found that CT99021 (25mg/kg, i.p), blocked the induction of NMDAR-LTD *in vivo* in a reversible manner. In the MWM it decreased the path length to the hidden platform during training session. In a probe test CT99021 increased the time spent near the platform and the number of crossing. In contrast, contextual fear memory, spatial working memory and reversal learning were unaffected. Taken together, these data raise the possibility that activation of GSK-3, possibly due to its involvement in NMDAR-LTD, may impede the rate of spatial learning and the accuracy of spatial memory.

Disclosures: Y. Lee: None. Z. Bortolotto: None. B. Kaang: None. G. Collingridge: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.03/N6

Topic: B.08. Synaptic Plasticity

Support: CDMRP grant # W81XWH-08-2-0136 to MJF

Title: Stimulus regularity differentially influences synaptic plasticity outcome in control rats vs rats with mild traumatic brain injury

Authors: *Q. S. FISCHER, D. KALIKULOV, M. J. FRIEDLANDER;
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Abstract: Typical protocols for inducing synaptic plasticity use stimulus patterns with constant interstimulus intervals (ISIs), while neurons *in vivo* receive synaptic input with irregular ISIs. A study of more physiologically salient stimulation patterns is important to understand the role that stimulus temporal pattern may play in synaptic plasticity induction. Here we evaluate how stimulus regularity influences synaptic plasticity induction in the visual cortex of 10-12 week old normal rats and rats which received a mild traumatic brain injury (mTBI) 2-3 weeks prior. We applied a defined pattern of stimulation to layer 4 in acute slices and made whole cell patch recordings of evoked postsynaptic potentials (PSPs) from 122 layer 2/3 pyramidal cells. Conditioning stimulation consisted of 900 pulses, at a mean frequency of 1Hz, with 1 of 3 different patterns of ISI regularity defined by the coefficient of variation (CV): regular (CV=0), slightly irregular (CV=0.2), or highly irregular (CV=1). PSP peak amplitudes evoked by 0.1Hz stimulation were measured before and after conditioning stimulation, and the post- to pre-conditioning ratio was used to assess plasticity. Plasticity outcome distributions differed significantly between mTBI and control rats for CV=1 (1.21±0.15 vs 0.85±0.07; P=0.03, t-test), but not CV=0 (0.88±0.07 vs 0.87±0.06; P=0.94, t-test) or CV=0.2 stimulation (0.73±0.06 vs 0.75±0.05; P=0.85, t-test). For CV=1 stimulation, this reflected an increase in the incidence of individual neurons that underwent long-term potentiation (LTP; 38% vs 16%), and a decrease in the incidence of neurons that underwent long-term depression (LTD; 47% vs 31%) for mTBI vs control rats. Moreover, PSP amplitudes at the beginning (first 90 sec) and the end (last 90 sec) of the conditioning stimulation period were both significantly increased in mTBI (123±10% and 107±14% of baseline, respectively) vs control rats (93±6% and 71±5% of baseline, respectively; P<0.03, t-tests). In addition to the peak amplitudes, we also compared PSP waveform characteristics including half width, rise time, decay time, and latency before and after CV=1 conditioning stimulation. In mTBI rats, PSP half-width and decay time increased only for cells expressing LTP (P<0.05, t-tests). In contrast, for control rats PSP half-width and latency decreased only for cells expressing LTD (P<0.05, t-tests). Finally, preconditioning somatic

calcium levels were significantly increased in mTBI vs control rats (91.2±5.5 nM vs 63.2±3.2 nM, P<0.001, t-test). These results suggest that stimulus regularity modifies the efficacy of synaptic plasticity induction and that mTBI can alter this interaction.

Disclosures: Q.S. Fischer: None. D. Kalikulov: None. M.J. Friedlander: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.04/N7

Topic: B.08. Synaptic Plasticity

Support: CDMRP grant#W81XWH-08-2-0136 to MJF

Title: Role of the temporal pattern of 10 Hz synaptic conditioning stimulation on induction of synaptic plasticity in visual cortex after mild traumatic brain injury

Authors: *D. KALIKULOV, Q. S. FISCHER, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Deep brain stimulation (DBS) is an effective treatment for a variety of disorders. However, the most effective therapeutic stimulation parameters, including specific combinations of stimulation frequency and the temporal pattern of stimulating pulses for DBS are not well delineated. We investigated several temporal patterns of synaptic conditioning stimulation to evaluate their effects on synaptic plasticity in visual cortex in control (normal) rats, sham operated rats, and rats with mild traumatic brain injury (mTBI). Whole cell recordings were made at 10-12 weeks, with sham or mTBI treatments performed 2-3 weeks prior. Highly irregular conditioning stimulation consisted of an interstimulus interval distribution coefficient of variation of 1.0 with either a continuous train of 900 pulses at 10 Hz for 90 sec, or a discontinuous train of 9 bursts of 100 pulses each at 10 Hz with a pause between each burst. Baseline postsynaptic potential (PSP) responses were evoked at 0.1 Hz for 10 minutes preconditioning and for 20 minutes postconditioning. We successfully recorded from 38 control, 34 sham, and 38 mTBI cells**.** For continuous conditioning, controls showed a net LTD (PSP amplitude post-/pre- conditioning = 0.86±0.04; n=19, while individual cells either underwent statistically significant LTD, no significant change (NC) or LTP (0% LTP, 42% NC, 58% LTD); shams, exhibited no net change (0.96±0.04; n=20; 15% LTP, 55% NC, 30%LTD), but mTBI rats showed a net LTP (1.26±0.11; n=20; 50% LTP, 30% NC, 20% LTD). For discontinuous conditioning, controls showed net LTD (0.63±0.07; n=19; 5% LTP, 5% NC, 90% LTD), shams also showed net LTD (0.72±0.07; n=14; 7% LTP, 14%NC, 79%LTD), but mTBI rats showed net

LTP (1.17±0.12; n=18; 50% LTP, 22% NC, 28% LTD). PSP waveform analyses show that for continuous stimulation in cells from mTBI rats that underwent LTD there was a significant increase in PSP latency ($P<0.01$, t-test). For discontinuous stimulation, control cells that expressed LTD showed a significant decrease in PSP half width ($P<0.05$, t-test). Additionally, for continuous conditioning, we analyzed the changes in PSP amplitude during the course of the conditioning train. Those amplitudes (averaged over the last 9 sec of conditioning as compared to the preconditioning baseline) showed a significant decrease for control or sham vs mTBI rats ($P<0.01$, t-test), with no difference between control and sham rats. Thus, a highly irregular stimulus pattern shifts a net LTD in cells from control rats to a net LTP in cells from mTBI rats and should be considered in selecting therapeutic approaches to chronic brain stimulation to potentially rebalance weights of altered synaptic circuits after mTBI.

Disclosures: D. Kalikulov: None. Q.S. Fischer: None. M.J. Friedlander: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.05/N8

Topic: B.08. Synaptic Plasticity

Support: IIAA number AOD12059-0001-00

Title: Soman-induced alterations in plasticity at the hippocampal ca1-schaffer collateral synapse following acute nerve agent exposure

Authors: *K. M. HOFFMAN, M. R. NELSON, D. L. NGUYEN, M. R. EISEN, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Organophosphorus nerve agents (NAs) irreversibly block acetylcholinesterase function, causing accumulation of excess acetylcholine in cholinergic synapses. If untreated, the resulting overstimulation of cholinergic receptors elicits prolonged seizures known as *status epilepticus* (SE), which result in brain damage and neuropsychiatric deficits in survivors. Although central nervous system responses to NA have been studied in animal models using electrical recordings of whole brain activity, the acute changes in synaptic physiology that are the basis for these pathological circuit dysfunctions have not been described. Therefore to better understand the acute synaptic response to NA exposure, we conducted field recordings at the Schaffer collateral-CA1 synapse in hippocampal coronal slices following soman application. Perfusion of soman (10 μM) rapidly elicited a stable reduction of field excitatory post-synaptic

potential (fEPSP) amplitudes that persisted after wash-out. The concomitant increase in paired-pulse ratios (PPR; n = 6 for each, $p \leq 0.01$) suggests that soman-induced long-term depression (s-LTD) results from decreased pre-synaptic release probabilities. s-LTD was blocked by co-administration of the non-selective muscarinic receptor (mAChR) antagonist atropine (n = 7, ns versus control) or the M1 mAChR-selective VU-255035 (n = 5, ns versus control), confirming the role of cholinergic overstimulation in s-LTD. To further investigate mechanisms responsible for s-LTD, we pharmacologically dissected pre- and post-synaptic signaling pathways characteristically involved in receptor-mediated plasticity. s-LTD was refractory to APV treatment, demonstrating that NMDAR currents are not required for s-LTD. Alternatively, s-LTD was completely blocked by AM-251, which is a reverse agonist of the cannabinoid type 1 receptor (CB1R). These findings suggest that soman activation of M1 mAChR evokes retrograde endocannabinoid signaling that reduces pre-synaptic release probability via CB1R. Based on these data, we hypothesize that endocannabinoid signaling represents a potential therapeutic modality to mitigate acute and persistent neurological responses to NA exposure. We are further exploring the endocannabinoid signaling system as a target for pharmacological intervention to delay seizure onset or increase survival.

Disclosures: K.M. Hoffman: None. M.R. Nelson: None. D.L. Nguyen: None. M.R. Eisen: None. P.M. McNutt: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.06/N9

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY12782 to MJF

Title: Regular and irregular (Poisson) conditioning trains induced synaptic plasticity between individual neurons in layer 4 of mouse visual cortex

Authors: *J. WU, M. J. FRIEDLANDER;
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Abstract: Visual information is transmitted from retina through LGN_d to primary visual cortex (V1). The majority of geniculocortical axon fiber terminals project to V1 Layer 4 (L4). Information is initially processed in L4 and then routed to supra- and infragranular cortical layers. Within L4, ~45% of neurons receive synaptic input from their neighboring neurons, such that local synaptic integration of early visual information processing occurs in L4 that has been

hypothesized to act as an amplifier of thalamocortical inputs. To better understand the plasticity of synaptic connections between L4 neurons and the effect of different temporal patterns of synaptic input on plasticity, we performed dual and triple whole cell patch clamp recording from L4-L4 pairs or triplets in visual cortical slices from P25~35 C57B1/6 mice. A pair of action potentials was repeatedly elicited in one L4 presynaptic neuron with two square depolarizing pulses at 0.1Hz. The uEPSCs were recorded from the postsynaptic neuron. Two different protocols were used to induce synaptic plasticity: a 15min 10Hz Poisson distributed spike train of interstimulus intervals having a coefficient of variation (CV) = 1.0 and a 15min 10Hz regular spike train protocol (CV=0). A total of 27 pairs of synaptically coupled neurons were recorded. The synaptic strength, failure rate and paired pulse ratio (PPR) were: 14.1 ±3.5pA, 31±6% and 0.91±0.06, respectively. Short term plasticity was heterogeneous: 7 pairs exhibited PPF, 10 pairs exhibited PPD and 10 pairs did not exhibit any short term plasticity (NC). Among the 27 pairs, 22 underwent the Poisson protocol, the net synaptic strength and PPR decreased, while the failure rate increased. One pair switched from NC to PPF; two pairs switched from PPF to either PPD or NC; two pairs remained unchanged; for the remaining 17 pairs, PPD increased. Among the 22 pairs, 16 underwent LTD, 2 remained unchanged , and 4 displayed LTP. Another 5 pairs of connection underwent the regular protocol, the net synaptic strength and PPR decreased and the failure rate increased. One pair switched from NC to PPF; one pair from NC to PPD and in the other 3 pairs, PPD increased. All the 5 pairs of connections exhibited LTD. In summary, both conditionings increased synaptic failure rate and decreased synaptic strength. For short term plasticity, both augmented PPD in ≥60% of pairs, while in the other 40% pairs, short term plasticity either remained unchanged or switched from PPD to PPF or NC. For long term plasticity, Poisson conditioning induced ~70% pairs to undergo LTD, ~20% pairs to undergo LTP and ~10% pairs remained unchanged, while the regular conditioning induced all recorded pairs to undergo LTD.

Disclosures: J. Wu: None. M.J. Friedlander: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.07/N10

Topic: B.08. Synaptic Plasticity

Support: NIH Grant 5R01HD052731-08

Title: Activity and transcription-dependent priming of mGluR-LTD: Mechanisms and alterations in a Fragile X Syndrome mouse model

Authors: *K. KIM[1], J.-Y. JOO[2], T.-K. KIM[2], K. M. HUBER[2];
[2]Neurosci., [1]UT Southwestern Med. Ctr., Dallas, TX

Abstract: Brief novelty experience or neuronal activity rapidly induces the immediate-early gene *Arc* in hippocampal CA1 neurons. Arc protein stimulates endocytosis of AMPA-type glutamate receptors and is required for a form of long-term synaptic depression (LTD) induced by metabotropic glutamate receptors (mGluR1/5). This suggests that brief novelty experience or activity-patterns that induce *Arc* may cause synaptic weakening or LTD. To test this idea, we used optogenetics to stimulate CA1 neurons in organotypic hippocampal slice cultures to fire in a pattern mimicking that observed during exploration of a novel environment. CA1 neurons in slice cultures, were sparsely (<1%) transfected with ChETA, and stimulated with blue light to fire action potentials in brief bursts of high-frequency action potentials, or Burst Photo-Stimulation (BPS). Although BPS activated an *Arc* transcriptional reporter (SARE-GFP) in CA1 neurons, it did not weaken excitatory synaptic transmission, as measured by evoked and miniature (m) EPSCs. However, neurons that received BPS displayed enhanced LTD magnitude in response to subsequent synaptic or pharmacological (DHPG) activation of mGluR1/5. BPS-induced priming of LTD required transcription as well as translation of *Arc*. BPS induced *Arc* enhancer (e) RNA (Kim et al., Nature, 2010) and mRNA, as measured with qPCR in dissociated hippocampal cultures, but did not increase Arc protein. DHPG treatment of cultures after BPS induced a robust increase in Arc protein, in comparison to control cultures. These results suggest that *Arc* mRNA, induced in response to brief neuronal activity, is translationally-suppressed until stimulated by mGluR1/5. To test the role of translational suppression of Arc, we examined BPS-induced priming of LTD in the mouse model of Fragile X Syndrome, *Fmr1* KO, which encodes an RNA binding protein and translational suppressor, FMRP. In slice cultures of *Fmr1* KO mice, LTD magnitude is robust regardless of prior BPS, indicating that *Fmr1* KO synapses are insensitive to the activity history of the neuron. These results suggest a synaptic mechanism by which novelty-induced *Arc* promotes plasticity and learning of new environments and reveals a deficit in novelty associated plasticity in a cognitive disorder.

Disclosures: K. Kim: None. J. Joo: None. T. Kim: None. K.M. Huber: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.08/N11

Topic: B.08. Synaptic Plasticity

Support: CIHR

NSERC

Title: The developmental regulation of cofilin in hippocampal long-term depression

Authors: *F. CAO[1,2,3], Z. ZHOU[1,2,3], W. XIE[3], Z. JIA[1,2];
[1]Neurosci. and Mealth Hlth., The Hosp. For Sick Children, Toronto, ON, Canada; [2]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [3]Inst. of Life Sci., Southeast Univ., Nanjing, China

Abstract: Long-lasting synaptic plasticity, a key mechanism for memory, involves both structural and functional changes, but how these changes are coordinated to achieve input-specific, persistent plasticity remains elusive. Cofilin is an actin-binding protein critical for the regulation of the actin reorganization, a key process essential for the structural plasticity of the synapse. We have previously shown that cofilin-mediated actin changes are required for mGluR-dependent long-term depression (LTD), but whether the involvement of cofilin in plasticity is developmentally regulated remains unknown. In this study, we systematically investigated the role of cofilin in LTD in both young (2-3 weeks) and mature (2-3 months) mice by using electrophysiological recordings and biochemical assays. We found that the expression of cofilin in the brain is developmental regulated, being much higher in young than mature mice. Interestingly, the inhibition of cofilin blocks LTD in mature but not young mice. These results reveal a developmental switch in the molecular mechanisms underlying hippocampal LTD, which may play a key role in coordinating structural and functional changes during synaptic plasticity.

Disclosures: F. Cao: None. Z. Zhou: None. W. Xie: None. Z. Jia: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.09/N12

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU Mentoring Environment Grant

BYU Graduate Research Fellowship Award

Title: The expression of mGluR5 predicts interneuron plasticity in the stratum radiatum

Authors: *T. M. NUFER[1], C. MERILL[3], L. FRIEND[1], Z. HOPKINS[4], J. EDWARDS[2]; [1]Physiol. and Developmental Biol., [2]Brigham Young Univ., Provo, UT; [3]Univ. of California Irvine, Irvine, CA; [4]Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: Changes in synaptic strength in hippocampal CA1 pyramidal cells are thought to be responsible for the acquisition and retention of short-term memory. This plasticity is modulated by feedforward inhibitory interneurons in the stratum radiatum which are composed of many subtypes including, among others, parvalbumin-containing axo-axonic cells, calretinin-containing interneuron-selective cells, and cholecystokinin/calbindin positive basket cells. While radiatum interneurons exhibit long-term depression (LTD), short-term depression (STD), or lack of plasticity (McMahon & Kauer, 1997, Neuron), it is not known whether these types of plasticity correlate to specific interneuron subtypes. Using whole cell electrophysiology and real time quantitative PCR, we characterized the plasticity expressed by different hippocampal interneuron subtypes in correlation with their mRNA expression patterns to determine cell subtype using calcium binding proteins and neuropeptide CCK. We also assessed the expression of endocannabinoid (eCB) biosynthetic enzymes including diacylglycerol lipase α, N-acyl-phosphatidylethanolamine-specific phospholipase D, and 12-lipoxygenase, as well as metabotropic glutamate receptor subunits known to mediate plasticity. Cells exhibiting LTD tended to express mRNA for at least one of the eCB biosynthetic enzymes (4 of 5 cells) and the metabotropic glutamate receptor subunit mGluR5 (3 of 5 cells). mGluR5 was not expressed by cells exhibiting STD or no plasticity (0 of 10 cells). Cells that exhibited short-term depression tended to express mRNA for at least one of the eCB biosynthetic enzymes (4 of 6 cells), but not mGluR5 (0 of 6 cells). This suggests that stratum radiatum interneuron plasticity can be predicted based on cell subtype and mGluR expression, and that these different types of plasticity may have some importance in hippocampal function.

Disclosures: T.M. Nufer: None. C. Merill: None. L. Friend: None. Z. Hopkins: None. J. Edwards: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.10/N13

Topic: B.08. Synaptic Plasticity

Support: NRSA NS080605 to KG

NIH Grant #NS44421 to PS

Karolinska Institute Fellowship to MI

Title: The interaction of Gβγ and SNAP-25 promotes the expression of presynaptic long-term depression (LTD) of synaptic transmission

Authors: *K. R. GOPAUL[1], M. IRFAN[2,1], O. MIRY[1], G. SUBAH[1], L. R. VOSE[1], X.-L. ZHANG[1], C. BARK[2], P. K. STANTON[1];
[1]Cell Biol. and Anat., New York Med. Col., Valhalla, NY; [2]Dept. of Mol. Med. and Surgery, Karolinska Inst., Stockholm, Sweden

Abstract: Episodic and spatial memories are thought to be encoded by long-term potentiation (LTP) and LTD at hippocampal and neocortical synapses. Multiple receptors and messengers have been suggested to mediate synapse-specific alterations in synaptic transmission that contribute to LTP and LTD at both presynaptic and postsynaptic terminals. Mechanisms involved in presynaptic expression of LTD are much less studied than postsynaptic alterations. Two receptors involved in induction of presynaptic LTD are group II metabotropic glutamate receptors (mGluRII), and N-methyl-D-aspartate receptors (NMDAR). The G protein-coupled receptor (GPCR), mGluRII, reduces neurotransmitter release from presynaptic vesicles through a cascade requiring dissociation of Gα and Gβγ subunits. Activation of NMDARs also elicits presynaptic LTD by modifying release of glutamate via postsynaptic production of the intercellular messenger nitric oxide, which drives presynaptic synthesis of cyclic guanosine monophosphate. We have previously shown that Gβγ, released from a GPCR, such as mGluRII, binding to the C-terminus of the 25kDa synaptosomal-associated, SNAP REceptor (SNARE), protein SNAP-25, is necessary for the induction of presynaptic LTD. SNAP-25 is alternatively spliced to adolescent (SNAP-25a) and adult (SNAP-25b) isoforms. Our hypotheses are: 1) differential interaction of the SNARE complex with SNAP-25 splice variants and Gβγ during development alters the expression of LTD of transmitter release at glutamatergic synapses, and perhaps GABAergic synapses, and 2) mGluRII and NMDAR have a final common pathway that mediates presynaptic LTD of vesicular release. Using mutant mice expressing only SNAP-25a, we examined how isoforms of SNAP-25 differentially affect plasticity of vesicular release. The presence of only SNAP-25a reduced neurotransmitter release and altered paired-pulse facilitation, compared to wildtype. These SNAP-25a mice showed impaired LTP, but enhanced LTD, of synaptic strength that varied with age. SNAP-25a mice exhibited both mGluRII- and NMDAR-dependent forms of LTD. We elucidate the functional roles of SNAP-25 isoforms by comparing behavioral phenotypes (locomotion, anxiety, learning and memory) of mice expressing SNAP-25a versus wildtype controls. Our findings suggest that the balance of presynaptic LTP and LTD, important to learning and memory, are functionally altered by isoforms of SNAP-25. These results give insight on how long-term regulation of neurotransmitter release contributes to cognition, learning and memory, and the potential of SNAP-25 as a regulatory target in diseases involving defects in synaptic transmission.

Disclosures: K.R. Gopaul: None. M. Irfan: None. O. Miry: None. G. Subah: None. L.R. Vose: None. X. Zhang: None. C. Bark: None. P.K. Stanton: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.11/N14

Topic: B.08. Synaptic Plasticity

Support: R01 NS076312

F31 NS095568

Title: Increasing O-GlcNAcylation induces AMPAR internalization at CA3-CA1 synapses

Authors: *L. T. STEWART[1], A. U. KHAN[2], M. L. OLSEN[2], J. C. CHATHAM[3], L. L. MCMAHON[2];
[2]Cell, Developmental and Integrative Biol., [3]Dept. of Pathology, [1]Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Regulated trafficking of AMPARs at mammalian excitatory synapses is a fundamental mechanism required for acquisition and storage of new information. Evidence suggests that AMPARs containing GluA2/3 subunits undergo constitutive recycling at hippocampal synapses, while those containing GluA1/2 subunits undergo activity dependent insertion or removal during many forms of long-term plasticity required for learning and memory. This activity dependent trafficking is modulated by serine/threonine phosphorylation of AMPAR subunits. We recently reported that another dynamic post-translational modification at serine/threonine residues involving the O-linkage of β-*N*-acetylglucosamine, termed O-GlcNAcylation, induces a novel form of LTD at CA3-CA1 synapses. In recent studies, we found that acute increases in O-GlcNAcylation also dampen hyperexcitability in vitro and in vivo. We previously showed that GluA2, but not GluA1 subunits are O-GlcNAc modified, that O-GlcNAc transferase co-immunoprecipitates with GluA2 subunits, and O-GlcNAc LTD is absent in GluA2 KO mice. Because serine phosphorylation of GluA2 subunits drives AMPAR endocytosis during expression of NMDAR-dependent LTD, we investigated whether expression of O-GlcNAC LTD is due to AMPAR endocytosis and used the endocytosis blocking peptide, TatGluR23Y. In preliminary experiments we find that O-GlcNAc LTD at CA3-CA1 synapses is inhibited in the presence of the peptide but is normal in the presence of the scrambled peptide, suggesting O-GlcNAcylation of GluA2 subunits drives endocytosis similar to phosphorylation. Additional experiments will test the requirement of AMPAR GluA2 subunit in O-GlcNAc mediated dampening of neuronal hyperexcitability. Based on our current and previously published work, the hexosamine biosynthetic pathway is an important mechanism in the modification of synaptic efficacy at hippocampal synapses, via targeted O-GlcNAcylation of synaptic protein substrates.

Disclosures: L.T. Stewart: None. A.U. Khan: None. M.L. Olsen: None. J.C. Chatham: None. L.L. McMahon: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.12/N15

Topic: B.08. Synaptic Plasticity

Support: Computational Neuroscience Unit, OIST

Title: A unified molecular mechanism of bidirectional plasticity at cerebellar parallel fiber-Purkinje cell synapses

Authors: *A. R. GALLIMORE, E. DE SCHUTTER;
Computat. Neurosci. Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: The expression of postsynaptic long-term depression (LTD) and long-term potentiation (LTP) in cerebellar Purkinje cells results from the internalisation or insertion, respectively, of postsynaptic AMPA receptors (AMPAR) [1,2]. LTD is induced by concurrent parallel fiber and climbing fiber stimulation of Purkinje cells, and is regulated by a complex intracellular signalling network that suppresses phosphatase activity leading to activation of a positive feedback loop that maintains PKC activity for at least 30 minutes [3]. LTP is dependent on nitric oxide [4], produced during parallel fibre stimulation [5,6], which nitrosylates N-ethylmaleimide-sensitive factor (NSF) and promotes exocytosis of AMPARs by actively disrupting the interaction between AMPAR-GluR2 and protein interacting with C-kinase 1 (PICK-1) [7,8]. Using a new differential equation model of Purkinje cell signalling and AMPAR trafficking, we have developed the first unified molecular mechanism of bidirectional plasticity at PF-PC synapses. Using this model, we explain the induction of both LTP and LTD under low and high calcium conditions, respectively, and predict an automatic shut down of the positive feedback loop once the early phase of LTD induction is complete. We also explain the dual role of nitric oxide in LTP and LTD, as well as the observation that deletion of β-CaMKII results in the switching of LTD to LTP under LTD induction conditions [9]. 1 Wang, Y. T. & Linden, D. J. *Neuron* **25**, 635-647, doi:10.1016/s0896-6273(00)81066-1 (2000). 2 Kakegawa, W. & Yuzaki, M. *PNAS* **102**, 17846-17851, doi:10.1073/pnas.0508910102 (2005). 3 Tanaka, K. & Augustine, G. J. *Neuron* **59**, 608-620, doi:10.1016/j.neuron.2008.06.026 (2008). 4 Lev-Ram, V., Wong, S. T., Storm, D. R. & Tsien, R. Y. *PNAS* **99**, 8389-8393, doi:10.1073/pnas.122206399 (2002). 5 Wang, D. J. *et al. J. Neurosci.* **34**, 2355-2364, doi:10.1523/jneurosci.4064-13.2014 (2014). 6 Bouvier, G. *et al. Cell Rep.* **15**, 104-116, doi:10.1016/j.celrep.2016.03.004 (2016). 7 Huang, Y.

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Disclosures: A.R. Gallimore: None. E. De Schutter: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.13/N16

Topic: B.08. Synaptic Plasticity

Support: BBSRC EastBio

Title: JAK/STAT signalling underlies leptin-induced LTD at temporoammonic-CA1 synapses in adult hippocampus

Authors: *G. MCGREGOR, J. HARVEY;
Div. of Neurosci., Univ. of Dundee, Dundee, United Kingdom

Abstract: Increasing evidence indicates that the adipocyte-derived hormone leptin has the ability to regulate excitatory synaptic transmission within the hippocampus as well as regulating satiety as a means to maintain energy expenditure (Irving and Harvey, 2014). Recent investigations indicate that leptin can modulate synaptic plasticity in an age-dependent manner at hippocampal schaffer collateral (SC)-CA1 synapses (Moult and Harvey, 2011). However, leptin also regulates the anatomically distinct temporoammonic (TA) input to CA1 synapses, as leptin can induce a novel form of long term potentiation (LTP) at juvenile hippocampal TA-CA1 synapses (Luo et al. 2015). However the effects of leptin on excitatory synaptic transmission at adult hippocampal TA-CA1 synapses is unknown. Here, we used standard extracellular recordings to investigate the effects of leptin on excitatory synaptic transmission in male adult (12-24 week) rats. Selective inhibition of synaptic transmission by dopamine (100 μM; 5 min) confirmed TA stimulation (Luo et al. 2015; Otmakhova & Lisman, 1998). Addition of leptin (25 nM; 15 min) induced a persistent depression of synaptic transmission (long term depression; LTD) at TA-CA1 synapses (to 76 ± 5% of baseline; $n = 4$; $P < 0.001$). This effect was NMDA receptor-dependent as blockade of NMDA receptors with 50 μM D-AP5 inhibited leptin-induced LTD ($n = 5$). The signalling pathways underlying leptin-induced LTD were also examined and were found to involve JAK2/STAT3 signalling as inhibitors of JAK/STAT signalling such as AG490 (95 ± 9% of baseline; $n = 5$; $P > 0.05$) and stattic (104 ± 5% of baseline; $n = 5$; $P > 0.05$) blocked leptin-induced LTD. In parallel immunocytochemistry studies, leptin-induced LTD was also examined

in hippocampal cultures. In the presence of low Mg^{2+}, leptin resulted in a reduction in GluA1 surface expression (to 79 ± 5% of control; $n = 3$; $P < 0.01$) suggesting that leptin increased the internalisation of AMPA receptors. Moreover the ability of leptin to reduce GluA1 surface expression was blocked by inhibitors of JAK/STAT signalling (e.g. 97 ± 4% and 92 ± 4% of control in the presence of AG490 and stattic respectively; $n = 3$; $P > 0.05$ for each). Accumulating evidence suggests that the JAK/STAT pathway is involved in neuroprotection and Alzheimer's disease (AD; Chiba et al. 2009). Moreover leptin prevents the detrimental actions of amyloid beta at hippocampal synapses (Doherty et al, 2013). Thus the ability of leptin to regulate excitatory synaptic strength at TA-CA1 synapses is likely to have important implications for the role of the leptin system in health and CNS-driven disease.

Disclosures: G. McGregor: None. J. Harvey: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.14/N17

Topic: B.08. Synaptic Plasticity

Support: JSPS Grants 15J02047, 15H04259, 15K18338

MEXT Grants 25110717, 15H01556

Takeda Science Foundation

the Naito and the Uehara Memorial Foundations

Title: Analyses of cell-surface amount, individual endo- and exocytosis of AMPA receptors, revealed suppression of exocytosis is important in hippocampal LTD

Authors: *S. FUJII, H. TANAKA, T. HIRANO;
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Abstract: Synaptic plasticity is critical in learning, memory, psychiatric and neurological disorders. At excitatory synapse, AMPA-type glutamate receptors (AMPARs) dynamically move into and out of synapses, and the increase or decrease in number of cell-surface AMPARs by exocytosis and endocytosis has been regarded as a main cellular mechanism for long-term potentiation (LTP) or depression (LTD). Here, we clarified AMPAR trafficking during LTD in a hippocampal culture preparation with total internal reflection fluorescence microscopy (TIR-FM). First, we attempted to estimate the change of amount of AMPARs on the cell-surface using

super-ecliptic pHluorin (SEP; pH-sensitive GFP variant). However, a recent study showed that significant SEP-AMPAR signals come from inside of a neuron, and that the intensity of such signal changes with intracellular acidification caused by neuronal activities. Therefore, we applied rapid pH exchange methods using U-tube and estimated accurately the amount of AMPARs by subtracting the signal intensity at pH 6.0 corresponding to the intracellular signal from that at pH 7.4. By this way, we revealed that the amount of AMPARs on both post- and extrasynaptic membrane gradually decreased during 5 min NMDA application. Next, we attempted to clarify the frequencies of endo- and exocytosis during LTD by observing individual events. Discrete endocytosis events were visualized by changing extracellular pH intermittently to an acidic condition. At pH 6.0, cell-surface AMPAR-SEP signals were quenched, and only signals derived from endocytosis vesicles were detected. NMDA application transiently (within 1 min) increased the frequency of clathrin/dynamin-dependent AMPAR endocytosis. On the other hand, the frequency of exocytosis showed gradual decrease during 5 min NMDA application. Considering the time courses of cell-surface receptor amount, and frequencies of exocytosis and endocytosis, we suggest that LTD is expressed not by the increase in endocytosis frequency but by the decrease in exocytosis frequency of AMPAR.

Disclosures: S. Fujii: None. H. Tanaka: None. T. Hirano: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.15/N18

Topic: B.08. Synaptic Plasticity

Support: Boston Children’s Hospital Translational Research Program

Department of Defense W81XWH-13-1-0118

Repository Core for Neurological Disorders, Department of Neurology, Boston Children’s Hospital

IDDRC (NIH P30HD018655)

Title: Uniform electric field stimulation induces region-specific long-term modulation of cortical excitability in mouse primary motor cortex *In vitro*

Authors: *Y. SUN, S. C. DHAMNE, A. ROTENBERG;
Dept. of Neurol. and the F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA

Abstract: Background: Transcranial direct current stimulation (tDCS) is a method for focal noninvasive in vivo brain stimulation, which leads to lasting changes in cortical excitability, resembling long-term potentiation or long-term depression (LTP/LTD). Although widely used in human studies, the anatomic tDCS effects at cortical layer resolution are incompletely understood. We applied direct current stimulation (DCS) in vitro to test whether DC field stimulation modulates cortical excitability uniformly throughout the cortical thickness. **Methods:** DCS was delivered to isolated mouse primary motor cortical (M1) slices through Ag/AgCl electrodes over a range of DC field orientations relative to the neuronal projections. Cortical excitability was monitored by field excitatory postsynaptic potentials (fEPSPs) evoked by stimulating layer V of the M1 slice using an 8X8 microelectrode array which spanned across all cortical layers. The %change in fEPSP slope throughout the M1 slice, 1 hour after DCS, was plotted as an interpolated two-dimensional color map. **Results:** With the cathode positioned above the pial surface, an LTD-like effect (DCS-LTD) on excitatory synaptic strength was reliably present in superficial cortical layers, while an LTP-like effect (DCS-LTP) was observed in layers V/VI below the stimulating site. Similarly, DCS-LTP was nearly uniform, except a small region of LTD in layer V/VI after DCS with the anode positioned over the pial surface. These modulatory patterns of the synaptic strength were specific to DCS, which was not found in either chemically or conventional pulsatile electrical protocols for LTP or LTD induction in M1 slices. Further, we identified an mGluR5-dependency of DCS-LTD induced by cathodal DCS. Bath application of mGluR5 negative allosteric modulator 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP, 10 μM) selectively blocked DCS-LTD while DCS-LTP was preserved. **Conclusion:** DCS effects on cortical excitability are not uniform throughout the cortex. Rather, DCS induces pathway-specific and layer-specific changes in excitatory synaptic strength that are reliant on distinct molecular mechanisms.

Disclosures: Y. Sun: None. S.C. Dhamne: None. A. Rotenberg: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.16/O1

Topic: B.08. Synaptic Plasticity

Support: MRC Grant

Title: Roles of SUMOylation in hippocampal synaptic plasticity

Authors: *E. BRAKSATOR[1], K. A. WILKINSON[2], Z. I. BASHIR[3], J. M. HENLEY[4]; [1]Sch. of Physiology, Pharmacol. & Neurosci., [2]Biochem., [3]Physiology, Pharmacol. and Neurosci., [4]Univ. of Bristol, Bristol, United Kingdom

Abstract: SUMOylation is a lysine-targeted posttranslational modification in which members of the small ubiquitin-like modifier (SUMO) family are covalently bound to target proteins to alter their function. SUMOylation is involved in multiple neuronal signalling cascades and is strongly implicated in many neurological and neurodegenerative diseases. Fast excitatory synaptic transmission in the CNS is mediated by AMPARs and their tightly regulated trafficking underlies the cellular basis of learning and memory. Long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy are mediated by the activity-dependent increase or decrease in surface expressed postsynaptic AMPARs respectively. We have shown recently that SUMOylation is required for AMPAR trafficking in synaptic scaling and LTP. In this study we investigated whether SUMOylation is also required for mGluR- and/or NMDAR- dependent-LTD of AMPAR transmission in the CA1 area of the hippocampus. AMPAR excitatory post-synaptic currents (EPSCs) were recorded from CA1 pyramidal neurons using whole-cell patch-clamp electrophysiology in hippocampal slices obtained from postnatal day 13-15 male Wistar rats. mGluR-LTD was induced by the mGluR agonist DHPG (100μM) as well as by a paired-pulse low frequency stimulus protocol (PP-LFS). NMDAR-LTD was induced by low frequency stimulation (LFS). We found that reducing protein SUMOylation either by infusing a constitutively active form of SENP or a dominant negative form of the essential SUMO conjugating enzyme Ubc9 via the patch pipette, significantly reduced DHPG-induced LTD but not NMDAR-LTD. Interestingly, PP-LFS induced mGluR-LTD was not significantly changed when protein SUMOylation was reduced. These data show that SUMOylation regulates DHPG-induced LTD but not NMDAR-LTD in the CA1 area of the hippocampus. Future experiments will investigate the mechanisms by which SUMOylation regulates mGluR-LTD and whether the aberrant LTD observed in Alzheimer's disease models can be altered by SUMO modification.

Disclosures: E. Braksator: None. K.A. Wilkinson: None. Z.I. Bashir: None. J.M. Henley: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.17/O2

Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust

BBSRC

Berrow Foundation

Title: Synaptic plasticity during cortical slow oscillations *In vitro*

Authors: *M. KAHN, J. BARTRAM, S. TUOHY, E. MANN;
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Abstract: It has been proposed that a key function of slow-wave sleep is to provide a period of net synaptic weakening. While there is evidence in support of this idea, it remains unclear what mechanisms could mediate such a function. Here we explore this question by means of a slice model of the slow oscillation in mouse entorhinal cortex. We find that the depolarised phase of the slow oscillation (UP state) depresses subthreshold inputs unless they are immediately followed by a postsynaptic spike. Analysis of the coefficient of variation suggests this weakening may be predominantly expressed presynaptically. However, its initiation is sensitive to inhibition of postsynaptic NMDA receptors. We are currently further characterising the molecular requirements for this process. The UP state dependent depression we describe here could be an important mechanism contributing to synaptic plasticity during slow-wave sleep. Therefore, identifying its molecular requirements may enable us to test the functional importance of synaptic weakening during sleep.

Disclosures: M. Kahn: None. J. Bartram: None. S. Tuohy: None. E. Mann: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.18/O3

Topic: B.08. Synaptic Plasticity

Support: AHA Grant 11POST7580006

NIH Grant R01NS081248

NIH Grant R01NS040701

Title: CaMKII regulates the depalmitoylation and synaptic removal of AKAP79/150 to mediate structural LTD

Authors: *K. WOOLFREY, H. CABALLES, D. GOODELL, M. DELL'ACQUA, U. BAYER;
Pharmacol., Univ. of Colorado Denver, Aurora, CO

Abstract: Long term depression (LTD) is a process essential for regulating synaptic strength, but its underlying mechanisms have not been fully elucidated. Intriguingly, there is substantial overlap in the molecular players that mediate both LTD and long term potentiation (LTP), which is an opposing form of synaptic plasticity. A recently described example of this pathway commonality is Ca2+/calmodulin (CaM)-dependent protein kinase II (CaMKII). Here we explore the role of CaMKII in the cellular processes that support LTD. We identify the synaptic scaffold AKAP79/150 as a direct substrate of CaMKII and find that phosphorylation disrupts AKAP79/150 association with F-actin and facilitates its removal from spines. We further explore the importance of AKAP79/150 depalmitoylation and removal from spines in support of LTD and establish CaMKII as a critical regulator of AKAP79/150 palmitoylation state. Together, these findings provide mechanistic insight into how CaMKII mediates LTD and provide the first direct evidence for a function of the well-described LTD-induced trafficking of AKAP79/150.

Disclosures: K. Woolfrey: None. H. Caballes: None. D. Goodell: None. M. Dell'Acqua: None. U. Bayer: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.19/O4

Topic: B.08. Synaptic Plasticity

Support: NSERC #249853

CIHR #125888

Title: Ethanol has age- and region-dependent effects on synaptic plasticity in the hippocampus.

Authors: *S. SAWCHUK, J. D. SHIN, B. R. CHRISTIE;
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Abstract: The role of particular NMDA subunits in long-term depression (LTD) remains a controversial subject. NMDA receptors are composed of four distinct subunits, two obligatory GluN1 subunits, and two GLuN2 (A,B,C,D) or GluN3(A,B) subunits. Variation within these latter two subunits direct many of the ionotropic properties for the NMDA receptor. Previous studies have indicated that ethanol and the GluN2B subunit antagonist ifenprodil enhances LTD in the CA1 subfield (Hendricson et al., 2002), while other studies conclude that both ethanol and ifenprodil inhibit the induction of LTD (Izumi *et al,* 2005). Furthermore, studies investigating the role of specifically blocking GluN2B subunit containing NMDA receptors with ifenprodil found

no evidence to support any particular role for specific NMDA subunits in directing synaptic plasticity (Morishita et al., 2007). Factors contributing to the confounding results may stem from the wide ranges of ages studied, as well as variable concentrations of ethanol used during the experiments. We hypothesized that differential expression of GluN2 subunits in the hippocampus during such a wide age range might be an influencing factor in these results. To examine this issue we obtained field recordings from transverse hippocampal slices (350 micron) from animals at three distinct post-natal day time points (PND 14, 21 or 28). We found that ethanol (either 50 or 100 mM) did not significantly influence the induction of LTD in the Dentate Gyrus (DG) of animals at any age. Conversely, LTD induction in the CA1 subfield was significantly reduced in slices at PND14 and 28, but not PND21, and exposure to 3uM ifenprodil had no influence on the induction of LTD in the CA1 at PND28. These results indicate that ethanol can have region- and age-specific effects on long-term depression in the hippocampus.

Disclosures: S. Sawchuk: None. J.D. Shin: None. B.R. Christie: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.20/O5

Topic: B.08. Synaptic Plasticity

Support: NIAAA Grant K22 AA021414

Title: Mechanism of ethanol-induced disinhibition of nucleus accumbens core

Authors: *M. H. PATTON, B. M. ROBERTS, T. AKINTOLA, B. N. MATHUR;
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Abstract: Alcoholism is characterized by an enhancement of incentive salience attributed to alcohol related cues. This is likely due to alterations in signaling within the nucleus accumbens (NAc), an integration center for motivational circuitry. The NAc receives glutamatergic and GABAergic inputs from various brain regions and the balance between excitation and inhibition onto NAc principal medium spiny neurons (MSNs) is critical in shaping the output of this structure. While ethanol depresses inhibition onto MSNs in the nearby dorsal striatum, whether ethanol affects GABAergic signaling in the ventral striatum (NAc) that may induce hyper-motivated states remains unexplored. Here, we test the effects of ethanol on inhibitory synaptic strength onto NAc MSNs using whole-cell patch-clamp electrophysiology in acute mouse slices. We find a postsynaptically expressed, concentration-dependent ethanol-induced depression of inhibitory inputs onto MSNs. Further, MSN disinhibition is occluded following *in vivo* ethanol

exposure, suggesting a role for inhibitory synapses in the NAc during acute intoxication. These data represent a novel mechanism through which ethanol modulates NAc output, which could contribute to the rewarding properties of alcohol.

Disclosures: M.H. Patton: None. B.M. Roberts: None. T. Akintola: None. B.N. Mathur: None.

Poster

223. Long-Term Depression

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 223.21/O6

Topic: B.08. Synaptic Plasticity

Support: NIH Z01 ES100221

Title: Function consequences of structural heterogeneity in hippocampal CA2 pyramidal neurons.

Authors: *T. D. HELTON, M. ZHAO, S. M. DUDEK;
Neurosci. Lab., Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

Abstract: The hippocampus is critical for many cognitive functions, including certain forms of learning and memory, and recent evidence shows that CA2 pyramidal neurons play a role in social recognition memory (Hitti, 2014; Smith 2016). Interestingly, anatomical studies in guinea pig (Bartesaghi, 1999) and *in vivo* recordings in rat (Kay, 2016) suggest that CA2 pyramidal neurons are heterogeneous in their dendritic branching patterns and firing properties, respectively. In addition we reported that induction of long-term depression (LTD) is similarly heterogeneous (Zhao, 2007). We therefore sought to determine whether the dendritic branching patterns in CA2 confer functional properties that underlie differences in synaptic plasticity as well as the relative effectiveness of different synaptic inputs. Long-term potentiation (LTP) cannot readily be induced at excitatory synapses in CA2 stratum radiatum (SR) in response to pairing or high-frequency synaptic stimulation (HFS, 100 Hz) and LTD is expressed in only some of CA2 neurons in response to a low-frequency stimulation (LFS, 2Hz). However, we can more accurately describe the synaptic plasticity as falling into a richer array of 'plasticity phenotypes': 1) responding to LFS with LTD; 2) unresponsive to LFS, but expressing LTD in response to HFS; 3) unresponsive to LFS or HFS; or less frequently, 4) potentiating in response to either type of stimulation. In this study we have confirmed the presence of four CA2 pyramidal neuron morphologies in rat and have attempted to correlate the structural subtypes to the plasticity phenotypes. Although we observed some differences in the distributions of plasticity for each cell morphology type, they were not informative into the nature of the

relationship between morphology and synaptic plasticity. We noted however that one of the morphological cell types (Mb) had a significantly greater number secondary of branches within the SR. We therefore tested whether synaptic inputs in the SR (largely from CA3 neurons) were relatively more effective than synapses stimulated in the stratum lacunosum-moleculare (SLM; largely from entorhinal cortex) in these neurons compared with the other morphological types. Indeed, when recording from Mb cells, we found that EPSCs evoked with SR stimulation were roughly 3 fold larger than those evoked in SLM. In contrast, EPSCs recorded from Ma, Mc, and B cells were roughly similar when either the SR or SLM was stimulated. These results indicate that the relative effectiveness of different synaptic inputs in the SR and SLM can vary in the four morphological cell types and perhaps underlie the diverse firing properties of CA2 neurons in vivo.

Disclosures: T.D. Helton: None. M. Zhao: None. S.M. Dudek: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.01/O7

Topic: B.08. Synaptic Plasticity

Support: NRF Grant/2015R1C1A1A02036674

Title: Simultaneous observation of transcription of Arc mRNA and Ca^{2+} spikes in live hippocampal neurons

Authors: *H. MOON[1], R. H. SINGER[2,3,4], H. PARK[1];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Transcription Imaging Consortium, Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; [3]Dept. of Anat. and Structural Biol., [4]Gruss-Lipper Biophotonics Ctr., Albert Einstein Col. of Med., Bronx, NY

Abstract: The pattern of gene expression in neuronal network is believed to be the essential element for memory formation. Especially, immediate early gene Arc is known to be deeply involved in modulation of synaptic plasticity. The expression of Arc occurs in a highly activity-dependent manner through various signaling pathways such as PKA, CamK and MAPK/ERK. Arc PBS KI mouse is a mouse line developed for single Arc mRNA imaging by knocking in 24 tandem arrays of PP7 binding site (PBS) in 3′ untranslated region (3′ UTR) of Arc gene. In this study, we observed the transcription of Arc in live hippocampal primary cultures. By simultaneously imaging relative Ca^{2+} concentrations and Arc mRNA transcription in various conditions induced by electrical stimulation and pharmacological reagents such as TTX and

bicuculline, we attempt to reveal the relationship between the activity of neurons and expression of Arc with single mRNA resolution. This study may elucidate new perspectives about the mechanism of synaptic plasticity in transcriptional level.

Disclosures: H. Moon: None. R.H. Singer: None. H. Park: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.02/O8

Topic: B.08. Synaptic Plasticity

Support: Human Frontiers Science Program (T.L.)

NIH grant R01MH080047 (R.Y.)

NIH grant 1DP1NS096787 (R.Y.)

Max Planck Florida Institute

Title: *In vivo* 2 photon Fluorescence lifetime imaging (2pFLIM) measurements of protein activity in the brain

Authors: *T. LAVIV[1], B. KIM[2], J. CHU[2], A. LAM[2], M. Z. LIN[2], R. YASUDA[1];
[1]Max Planck Florida Institue For Neurosci., Jupiter, FL; [2]Stanford Univ., Stanford, CA

Abstract: A major quest in neuroscience is to understand the remarkable dynamics of the structure and function of the brain in response to sensory experience. Detailed investigation of synaptic plasticity *in vitro* have yielded exquisite molecular mechanisms, while new *in vivo* imaging techniques have enabled high resolution exploration at the functional level of small neuronal circuits. However, it is still unknown what molecular mechanisms the brain utilizes to encode experience-dependent plasticity. In order to address this question, we have established an imaging based approach to enable *in vivo* 2 photon Fluorescence lifetime imaging (2pFLIM) measurements of protein activity in the mouse cerebral cortex. In order to measure molecular dynamics at the level of single cells, we have established and characterized a FLIM sensor for CREB, a well-known transcription factor which plays a vital role in long term memory and synaptic plasticity. This system allows us to perform chronic 2pFLIM based measurements of CREB activity levels in single cell resolution in cortical layer 2/3 cells. Using this technique we were able to demonstrate a dynamic increase in CREB activity during enriched sensory environment. Furthermore, we have established a novel dual-color FLIM imaging method using

CyRFP, a red shifted RFP well suited for FLIM. Using EGFP/CyRFP dual imaging in hippocampal spines, we show differential RhoA and CaMKII activity during structural LTP in a single spine. We are now using this method to establish FLIM based measurements of single spine molecular dynamics *in vivo*. This approach may help to uncover the molecular mechanisms underlying experience dependent plasticity in the living brain.

Disclosures: T. Laviv: None. B. Kim: None. J. Chu: None. A. Lam: None. M.Z. Lin: None. R. Yasuda: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.03/O9

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS083085

Title: Translation dynamics of single mrnas in live neurons

Authors: *B. WU, C. ELISCOVICH, Y. J. YOON, R. H. SINGER;
Albert Einstein Col. of Med., Bronx, NY

Abstract: Translation is the fundamental biological process that converts the mRNA information into proteins. Local production of protein is essential for axon guidance, synaptic plasticity as well as learning and memory. Single molecule imaging in live cells has illuminated the dynamics of RNA transcription, however, it is not yet applicable to translation. We report here the development of Single molecule Imaging of NAscent PeptideS (SINAPS) to assess translation in live neurons. As with transcription assays, the approach provides direct readout of the initiation frequency, the elongation rate and the location of translation sites within the cell. Single molecule fluorescence recovery after photobleaching provides direct measurement of elongation rates. We applied SINAPS in cultured primary hippocampal neurons. The results indicate that mRNAs are translated in proximal dendrites, but are repressed in distal dendrites with occasional "bursting" translation. Quantitative fluctuation analysis reveals the dynamics of these bursts. This technology enables the quantitative spatial and temporal analysis of translation of single mRNAs in living neuron and provides a new tool for addressing the mechanism of local translation in dendrites and axons.

Disclosures: B. Wu: None. C. Eliscovich: None. Y.J. Yoon: None. R.H. Singer: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.04/O10

Topic: B.08. Synaptic Plasticity

Support: NS83085

Title: Visualization of transcription dynamics of immediate early gene Arc in hippocampal neurons

Authors: *S. DAS[1], B. WU[1], Y. YOON[1], H. PARK[2], R. SINGER[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Dept. of Physics & Astronomy, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Neuronal activity triggers the transcription of many Immediate Early (IE) genes. Among them, Arc (activity-regulated cytoskeletal-associated), also known as Arg 3.1, has garnered particular interest due to its crucial role in long-lasting synaptic plasticity and memory consolidation. The primary regulation of Arc is at the level of its transcription and targeting of its mRNA to potentiated synapses. While most *in vivo* studies provide insights about the spatial regulation of Arc mRNA, however, the dynamics of endogenous Arc mRNA in response to neuronal activity or LTP induction has not been characterized with high temporal resolution. We have generated a transgenic mouse by knocking in 24 tandem arrays of bacteriophage-derived PP7-binding sites (PBS) into the 3'-untranslated region (3'-UTR) of the Arc gene. These PBS stem loops are recognized by their cognate binding PP7 coat protein (PCP), tagged with a fluorescent molecule, therefore allowing for dynamic visualization of active Arc loci as well as individual transcripts. Using single molecule fluorescent *in situ* hybridization (smFISH) performed on hippocampal neurons from this mouse, we can detect and quantify individual mRNAs after triggering neuronal activity. Furthermore, real-time measurements of transcription and dendritic mRNAs reveal the kinetic profile of Arc transcription and the localization of these mRNAs. Overall, our results provide interesting insights about the induction of Arc transcription from ensemble level to single cell and finally to single genetic locus level. This approach allows for detailed examination of neuronal activity-regulated gene expression in response to different stimulation paradigms.

Disclosures: S. Das: None. B. Wu: None. Y. Yoon: None. H. Park: None. R. Singer: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.05/O11

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS82085

Title: Localization of newly synthesized proteins within dendritic spine microdomains

Authors: *Y. J. YOON[1], B. WU[2], R. H. SINGER[2];
[1]Anat. and Structural Biol., [2]Albert Einstein Col. of Med., Bronx, NY

Abstract: Long-lasting synaptic plasticity in neurons requires protein synthesis. However it is not clear where and how newly translated proteins can effect changes in synaptic compartments. To test whether newly synthesized synaptic proteins exhibit discrete localization within spine heads, we performed Halo-labeling on a variety of synaptic proteins to determine differences in subspine localization of new and preexisting proteins. We observed newly synthesized β-actin proteins discretely positioned at the periphery of dendritic spines following glutamate uncaging stimulation. Sorting of new actin to a microdomain within spine heads demonstrated that stimulation-dependent synthesis of actin could mediate long-lasting structural modification of spines. Our results indicate that the location of newly translated proteins may indicate local function of new proteins and provide a link to the essential role of protein translation during synaptic plasticity and spine remodeling.

Disclosures: Y.J. Yoon: None. B. Wu: None. R.H. Singer: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.06/O12

Topic: B.08. Synaptic Plasticity

Support: JSPS kAKENHI 15K06771

Title: Novel splice variants of gtf2i expressed in the neuronal dendrites of rat brain.

Authors: Y. SHIRAI[1], *T. SUZUKI[1,2];
[1]Shinshu Univ. Grad. Sch. Med., Matsumoto, Japan; [2]Biol. Sci. for Intractable Neurol. Dis., Inst. Biomed. Sci, Shinshu Univ., Matsumoto, Japan

Abstract: We have reported that a variety of mRNAs are attached to post-synaptic density (PSD) purified from cerebral cortex of rat brain (Suzuki et al., Neurosci.Res. 57, 61-85). More than one hundred among thousands of PSD-mRNAs code not only synaptic proteins but also nuclear proteins. General transcription factor II-I (Gtf2i) is one of such PSD-mRNAs coding nuclear proteins. Gtf2i is a transcription factor and one of the genes implicated in Willams-Beuren syndrome, an autism spectrum disorder. We are studying molecular mechanisms of dendritic localization and local-translation of Gtf2i mRNA, and nuclear translocation of Gtf2i protein and its function in long-term synaptic plasticity. In this study, we investigated splice variants of the Gtf2i gene in both the 5'untranslated region (5'UTR) and the coding region. To search for novel 5'UTRs of Gtf2i, we utilized the cap analysis gene expression database of the mouse. We identified seven novel Gtf2i transcripts with alternatively spliced 5'UTRs in the rat brain. We also identified four novel splice variants in the coding sequence of Gtf2i. Furthermore, we identified a selective usage of certain types of 5'UTR by coding variants. In situ hybridization demonstrated a differential pattern of expression of Gtf2i mRNAs with alternatively spliced 5'UTRs among neuronal cells, and the localization of one of the variants in neuronal dendrites in the rat brain. Immunohistochemistry also demonstrated a distribution of Gtf2i-immunoreactivity in the dendrites. These results suggest multiple pathways of expression of Gtf2i gene in the brain. The expression patterns may be under the control of alternative promoters coupled to the alternative splicing in the coding region. Differential localization of mRNA to neuronal dendrites suggests spatiotemporal specific translation at the post-synaptic sites that is involved in transfer of synaptic activity to expression of specific sets of genes in the nucleus.

Disclosures: Y. Shirai: None. T. Suzuki: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.07/O13

Topic: B.08. Synaptic Plasticity

Support: NIMH Grant 5F31MH107210-02

Title: Synaptically generated changes in CRTC1 and its role in activity-dependent transcription

Authors: *S. L. BONANNO[1], M. DESALVO[2], C. CHRONIS[2], T. J. O'DELL[3], K. PLATH[2], K. C. MARTIN[2];
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Abstract: CRTC1 is a potent co-activator of gene expression and has been show to be necessary for the maintenance of long-term potentiation in the hippocampus. CRTC1 acts as a retrograde signaling molecule that travels from synapse to nucleus, where it modulates CREB-mediated gene expression. Previous work revealed that CRTC1 undergoes dramatic and complex post-translational modifications following NMDA receptor activation, which correlate with its nuclear transport. Present work shows that distinct plasticity-inducing stimuli generate distinct profiles of CRTC1 post-translational modification with distinct levels of nuclear accumulation in cultured hippocampal neurons. Current work is aimed at correlating specific patterns of CRTC1 modification with different programs of gene expression using RNA sequencing and chromatin immunoprecipitation sequencing (ChIP-seq).

Disclosures: S.L. Bonanno: None. M. DeSalvo: None. C. Chronis: None. T.J. O'Dell: None. K. Plath: None. K.C. Martin: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.08/O14

Topic: B.08. Synaptic Plasticity

Support: NIH-NS12333

F31NS083349A

Title: Activity-driven phosphorylation of ribosomal protein S6 is differentially regulated in dendrites vs. cell bodies

Authors: *P. SALGADO[1], O. STEWARD[2];
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Abstract: Induction of perforant path long-term potentiation triggers phosphorylation of ribosomal protein S6 (rpS6) in the region of activated synapses (middle molecular layer) and also in cell bodies of dentate granule cells. Our previous studies demonstrate that activation of rpS6

phosphorylation in both dendrites and cell bodies is blocked by NMDA receptor antagonists. Also, phosphorylation of rpS6 within cell bodies is selectively blocked by wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3-K), suggesting involvement of the PI3-K/AKT/mTOR pathway. The signaling pathways that regulate rpS6 phosphorylation within dendrites are poorly understood, but one candidate is the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway, which is strongly activated by perforant path stimulation. Here, we assess the respective roles of MAPK/ERK vs. AKT/mTOR in regulating phosphorylation of rpS6 at synapses vs. neuronal cell bodies. As in previous studies, phosphorylation of rpS6 was induced by repeated delivery of high frequency stimulation to the perforant path in anesthetized rats. During stimulation, selective pharmacological inhibitors were locally infused via a glass microelectrode placed in the granule cell layer of the dentate gyrus, which also served to record responses generated by perforant path stimulation. Phosphorylation of rpS6 was assessed by immunohistochemistry using phospho-specific antibodies that recognize subsets of serine residues, ser235/236 and ser240/244. Local infusion of the MEK inhibitor, U0126, attenuated rpS6 phosphorylation in the dendritic laminae but did not affect rpS6 phosphorylation in granule cell bodies. Local infusion of the mTOR inhibitor, rapamycin, attenuated rpS6 phosphorylation in both the dendritic lamina and within granule cell bodies. These results support the concept that induction of rpS6 phosphorylation at activated synapses is regulated predominately via MAPK/ERK signaling, whereas activation of rpS6 phosphorylation in granule cell bodies is regulated predominately by PI3-K/AKT signaling. This offers a mechanism by which local protein synthesis may be selectively regulated in dendrites following synaptic stimulation to accomplish specific molecular pathway-dependent functions.

Disclosures: P. Salgado: None. **O. Steward:** Other; co-founder of the company called "Axonis.".

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.09/O15

Topic: B.08. Synaptic Plasticity

Title: Npas4 as a marker of recent neuronal activity

Authors: *D. G. WHEELER[1], Y. BEAVER[1], J. LAPIRA[1], P. M. ARDESTANI[2], K. MARUYAMA[1], F. SERNEO[1], D. ELOW[1], K. BAUMGAERTEL[1], R. SCOTT[1], M.

SHAMLOO[2], M. PETERS[1];
[1]Dart NeuroScience, San Diego, CA; [2]Stanford Univ., Palo Alto, CA

Abstract: Memory formation engages a specific set of neurons activated by environmental stimuli to initiate a cascade of activity-dependent signaling that alters gene expression. Identification and isolation of those neurons is critical to the study of memory and excitation-transcription coupling in the nervous system. This has traditionally been achieved through investigation of a subset of immediate-early genes (IEGs) such as cFos, Arc and Egr1 (Zif268) using techniques such as *in situ* hybridization, immunostaining or transgenic reporter animals. While proving to be useful as tools for marking activated neurons, the expression of these genes may not be entirely tuned to neuronal activity alone. IEG activation in neurons is linked to Ca^{2+} entry and synaptic activity, but it is also regulated by growth factor signaling, neurotrophins and neuromodulators. In contrast, Npas4 is expressed in a neuron-specific manner and is induced selectively by neuronal activity *in vitro*, suggesting that it may be a better marker of neuronal activation *in vivo*. In RNA-Seq experiments on primary cortical cultures, we found that *Npas4* mRNA levels increase dramatically in response to synaptic activity, but not in response to cAMP elevation by forskolin. This was paralleled *in vivo* by Npas4 induction in the hippocampus and retrospenial cortex in response to contextual learning, and with faster kinetics than observed for other IEGs. Interestingly, non-specific stressors such as restraint and vehicle injection failed to induce Npas4, while cFos, Arc and Egr1 expression were all elevated. Systemic administration of the NMDA receptor antagonist MK-801 blocked memory formation and completely prevented training-induced Npas4 expression, but it only partially inhibited induction of other IEGs. Thus, Npas4 couples to neuronal activity and memory formation but lacks the sensitivity of other IEGs to non-specific stressors. To mark recently active neurons we developed a rabbit monoclonal antibody against Npas4. Npas4 immunoreactivity in unstimulated cultured neurons was found to be low, and it was strongly induced by depolarization but not cAMP elevation. *In vivo*, immunoreactivity was observed in a sub-set of neurons after contextual fear conditioning. In addition, we observed strong induction of Npas4 immunoreactivity following ischemia in mice. Critically, we validated the specificity of our Npas4 antibody using Npas4 knock-out animals and found no cross-reactivity with other targets. Our data demonstrate that Npas4 is uniquely tuned to respond to neuronal activity and that our newly developed monoclonal antibody can be used to identify recently activated neurons *in vivo*.

Disclosures: **D.G. Wheeler:** A. Employment/Salary (full or part-time): Dart NeuroScience LLC. **Y. Beaver:** A. Employment/Salary (full or part-time): Dart NeuroScience. **J. Lapira:** A. Employment/Salary (full or part-time): Dart NeuroScience. **P.M. Ardestani:** None. **K. Maruyama:** A. Employment/Salary (full or part-time): Dart NeuroScience. **F. Serneo:** A. Employment/Salary (full or part-time): Dart NeuroScience. **D. Elow:** A. Employment/Salary (full or part-time): Dart NeuroScience. **K. Baumgaertel:** A. Employment/Salary (full or part-time): Dart NeuroScience. **R. Scott:** A. Employment/Salary (full or part-time): Dart NeuroScience. **M. Shamloo:** None. **M. Peters:** A. Employment/Salary (full or part-time): Dart NeuroScience.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.10/O16

Topic: B.08. Synaptic Plasticity

Support: NSF GRFP

NIH R01 MH101528-01

Title: Transient elevations in neuronal activity induce a subset of the neuronal activity-regulated gene program defined by MAPK/ERK-dependent enhancers.

Authors: *K. TYSSOWSKI, J. M. GRAY;
Genet., Harvard Med. Sch., Boston, MA

Abstract: Brief neuronal activation can strengthen synapses, whereas sustained neuronal activation weakens them. Yet in each case synaptic plasticity is dependent on activity-regulated gene expression. We therefore asked whether neuronal gene expression differs in response to transient versus sustained activity. Using RNA-Seq, we found that transient neuronal activity induces expression of only a subset of the activity-regulated genes that are induced by sustained activity. This subset includes true immediate early genes (IEGs) but not the more slowly induced delayed primary response genes (dPRGs) or late response genes (LRGs). Two distinguishing features of genes induced by transient activity are a requirement for MAPK/ERK signaling and the presence of nearby enhancers whose enhancer RNA induction is also MAPK/ERK-dependent. We also used genome-wide experiments to investigate the mechanism of enhancer activation by MAPK/ERK. We found that enhancer RNA induction but not histone acetylation at IEG enhancers requires MAPK/ERK, indicating that MAPK/ERK functions downstream of histone acetylation to activate neuronal enhancers. Our data reveal a mechanism by which distinct patterns of neuronal activity may encode distinct biological outcomes.

Disclosures: K. Tyssowski: None. J.M. Gray: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.11/O17

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

Support: NIH Grant DA041878

Title: Single cell analysis of neuronal activity-dependent gene transcription shows role for chromatin regulation at distal enhancers in transcriptional inducibility

Authors: *A. E. WEST[1], L.-F. CHEN[2], M. G. YANG[2], A. ZHOU[2];
[1]Neurobiol, Duke Univ. Hosp., Durham, NC; [2]Neurobiol, Duke Univ., Durham, NC

Abstract: Although the mechanisms of neuronal activity-dependent gene transcription have been well characterized by population based biochemical assays, very little is known about how individual neurons behave in response to neuronal activity. Understanding transcription at the single neuron level is important because many neuronal activity-regulated genes encode proteins that modulate the strength and function of neuronal connections; thus the degree of cell-to-cell variation in activity-dependent gene transcription has direct consequences for neuronal network plasticity. We have performed single-molecule RNA fluorescence in situ to quantify mRNA transcript numbers and active gene loci of two neuronal activity regulated genes (*Fos* and *Npas4*) in cultured embryonic mouse hippocampal neurons following a uniform membrane depolarization stimulus. Even among neurons that experience the same upstream activation we find substantial gene to gene and allele to allele variation in transcriptional induction, suggesting a gene local mechanism of variability in the transcriptional response. We hypothesized that variation in the activation state of distal enhancers could underlie these differences in the inducibility of activity-dependent genes. To test this hypothesis, we recruited a dCas9-p300 fusion protein to distal enhancers of the *Fos* gene to locally induce histone acetylation. Enhancer recruitment of dCas9-p300 was sufficient to both activate *Fos* transcription in unstimulated neurons and increase the induction of *Fos* expression in activated neurons in a manner that was dependent on the histone acetyltransferase activity of p300. Taken together, these data establish a causal relationship between enhancer chromatin state and neuronal activity-inducible gene transcription at the level of single neurons.

Disclosures: A.E. West: None. L. Chen: None. M.G. Yang: None. A. Zhou: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.12/O18

Topic: B.08. Synaptic Plasticity

Support: National Institute on Drug Abuse DA034681 & DA039650

Startup funds from University of Alabama at Birmingham

Evelyn F. McKnight Brain Research Foundation

Title: Functional modulation of activity-dependent gene expression by non-coding enhancer RNAs

Authors: *R. C. SIMON, N. V. N. GALLUS, K. E. SAVELL, F. A. SULTAN, J. J. DAY; Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Enhancers are DNA regulatory elements that contribute to establishment of cell identity during development by modulation of gene expression both *in cis* and *in trans*. Arising from enhancers are recently-discovered noncoding RNAs, coined enhancer RNAs (eRNAs). While evidence suggests eRNAs are involved in transcriptional pausing, their potential role in activity-dependent neuronal function and development remains unclear. Here, to investigate eRNA function *in vitro*, we selected an eRNA site upstream of *Fos*, an immediate early gene that codes for a transcription factor implicated in neuronal plasticity and cognitive processes. Consistent with previous studies, we show that neuronal activation leads to recruitment of RNA Polymerase II (PolII) to the *Fos* enhancer, resulting in bidirectional RNA PolII-dependent eRNA synthesis. In addition, neuronal silencing with tetrodotoxin decreases *Fos* eRNA expression and blocks the ability of glutamate receptor agonists to upregulate *Fos* eRNA. To determine whether eRNAs have a functional role in expression of the associated protein-coding *Fos* mRNA, we employed a selective eRNA knockdown approach using stable anti-sense oligonucleotides. Remarkably, whereas *Fos* mRNA knockdown had no effect on eRNA levels, eRNA knockdown resulted in a concomitant downregulation of protein-coding mRNA, suggesting a functional role for this non-coding transcript. Moreover, single-molecule RNA FISH revealed that *Fos* eRNAs are restricted to the nuclei of neurons. Finally, we employed mobility shift assays to show that *Fos* eRNAs bind directly to epigenetic modifiers conventionally believed to bind DNA. These findings indicate that eRNAs directly modulate gene expression and suggest that activity-driven induction of eRNAs could be an important regulatory mechanism in the central nervous system.

Disclosures: R.C. Simon: None. N.V.N. Gallus: None. K.E. Savell: None. F.A. Sultan: None. J.J. Day: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.13/P1

Topic: B.08. Synaptic Plasticity

Support: DIR NICHD

Title: Gene regulatory networks activated by pattern-specific generation of action potentials in dorsal root ganglia neurons

Authors: *P. R. LEE[1], J. E. COHEN[2], D. A. IACOBAS[3], S. IACOBAS[3], R. D. FIELDS[1]; [1]NIH/NICHD, Bethesda, MD; [2]Centerfor Biologics Evaluation and Res., FDA, Silver Spring, MD; [3]Dept. of Pathology, New York Med. Col. Sch. of Med., Valhalla, NY

Abstract: Development and plasticity in the nervous system are regulated by specific patterns of neural impulse activity. Patterned activity orchestrates spatial and temporal changes in the expression of networks of genes and their translated products. These gene regulatory networks underlie the long-term changes in cell specification, growth of synaptic connections, and adaptation that occur throughout neonatal and postnatal life. Here we show that the temporal nature of action potentials and downstream signaling differentially regulates the expression of hundreds of neuronal genes through distinct gene regulatory networks. Analysis of upstream regulatory regions showed enrichment in transcription factor binding sites for NF-Kappa B that was action potential pattern specific. Gene-set ontology enrichment of specific pathways for growth and development of neurons is also shown to be action potential pattern dependent. Beyond widely used bioinformatics approaches to understanding transcriptomic data we have used prominent gene analysis and pair wise topology measurements to quantify transcription networks which are sensitive to the timing of action potential bursting. Our new findings demonstrate spike-frequency decoding by action potentials at the transcriptional level by pattern-specific activation of transcription factors and associated gene-regulatory networks that operate to adjust the modalities of sensory neurons.

Disclosures: P.R. Lee: None. J.E. Cohen: None. D.A. Iacobas: None. S. Iacobas: None. R.D. Fields: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.14/P2

Topic: B.08. Synaptic Plasticity

Support: Howard Hughes Medical Institute

NIH NS094637

The Jackson Laboratory Postdoctoral Fellowship

Title: The role of a brain specific tRNA isodecoder in neuronal function

Authors: *M. KAPUR[1,2], G. NAGY[3], S. ADAMSON[4], J. H. CHUANG[4], C. ROSE[5], S. GYGI[5], S. L. ACKERMAN[1,2];
[1]Cell. and Mol. Med., UCSD, San Diego, CA; [2]Howard Hughes Med. Inst., Chevy Chase, MD; [3]The Jackson Lab. for Mammalian Genet., Bar Harbor, ME; [4]The Jackson Lab. for Genomic Med., Farmington, CT; [5]Dept. of Cell Biol., Harvard Med. Sch., Boston, MA

Abstract: The genomes of higher eukaryotes contain hundreds of transfer RNA (tRNA) genes. Surprisingly, while numerous genetic disorders have been linked to mutations in enzymes that process or modify tRNAs, a disease-linked mutation in a nuclear-encoded tRNA gene has not been reported. Until recently, the prevailing view was that isodecoder tRNAs, that is, tRNAs that share the same anticodon and differ only in their body sequence, were functionally redundant, and thus could compensate for a mutated tRNA. However, growing evidence indicates that isodecoder tRNAs may have different functional efficiencies.
Our lab recently identified the first tissue-specific mammalian tRNA gene, *n-Tr20*. *n-Tr20* is one of 5 isodecoders in the nuclear encoded $tRNA^{Arg}_{UCU}$ family, and in contrast to the other members of this family, is specifically expressed in the nervous system. Loss of *n-Tr20* in mice dramatically reduced the $tRNA^{Arg}_{UCU}$ pool in the nervous system, resulting in ribosome stalling on the cognate AGA codons. This stalling was severely exacerbated in the absence of the ribosome recycling factor GTPBP2, leading to widespread neurodegeneration.
Here, we find that expression of this single tRNA gene modulates complex phenotypes such as seizure susceptibility, and surprisingly, that loss of this tRNA results in widespread transcriptional reprogramming. This study sheds light on the role of individual tRNA isodecoder genes, and their possible role in human disease.

Disclosures: M. Kapur: None. G. Nagy: None. S. Adamson: None. J.H. Chuang: None. C. Rose: None. S. Gygi: None. S.L. Ackerman: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.15/P3

Topic: B.08. Synaptic Plasticity

Title: Synaptic vesicles contain small ribonucleic acids (sRNAs) including transfer RNA fragments (trfRNAs) and micro-RNAs (miRNAs)

Authors: *M. L. HARLOW, H. LI, C. WU, R. ARAMAYO, M. S. SACHS;
Biol., Texas A&M Univ., College Station, TX

Abstract: Protein synthesis is necessary for both the consolidation of new memories and the reconsolidation of existent memories. In neurons, much of the protein synthesis occurs away from the cell body at specialized regions within the dendritic arbor and postsynaptic boutons, and is referred to as local protein synthesis. Local protein synthesis at the synapse is regulated by the presynaptic activity of synaptic vesicles (SVs), and requires a host of translation factors, ribosomes, mRNAs, and small non-coding RNAs (sRNAs; 20-50 nucleotides [nt] in length). We have demonstrated that, in addition to neurotransmitters, cholinergic SVs isolated from the peripheral nervous system (PNS) of the model organism *Torpedo californica* (Pacific electric ray) and heterogeneous SVs isolated from the central nervous system (CNS) of Mus musculus (mouse) also contain sRNAs - primarily the 5' fragments of transfer RNAs (tRNAs) termed tRNA fragments (trfRNAs). trfRNAs, once thought to be degradation products, have recently been implicated in the paternal influence on offspring metabolism, the post-transcriptional regulation of protein synthesis, and the suppression of cancer cell metastasis. In addition to trfRNAs, SVs contain micro RNAs (miRNAs) known to be involved in transcriptional and translational regulation. The discovery that SVs contain sRNAs suggests that, in addition to inducing changes in local dendritic excitability through the release of neurotransmitters, SVs may, through the release of specific trfRNAs and miRNAs, directly regulate local protein synthesis. We believe these findings have broad implications for the study of chemical synaptic transmission.
Li, H., Cheng, W., Aramayo, R., Sachs, M.S. & M.L. Harlow (2015) Synaptic vesicles contain small ribonucleic acids (sRNAs) including transfer RNA fragments (trfRNA) and microRNAs (miRNA). *Sci. Rep.* 5, 14918; doi: 10.1038/srep14918.

Disclosures: M.L. Harlow: None. H. Li: None. C. Wu: None. R. Aramayo: None. M.S. Sachs: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.16/P4

Topic: B.08. Synaptic Plasticity

Support: Estonian Research Council (IUT19-18)

Estonian Research Council (Grant 8844)

by the European Union through the European Regional Development Fund (Project No. 2014-2020.4.01.15-0012)

Title: The effect of sumoylation on the transactivation capacities of different human NFAT isoforms in neurons

Authors: *H. VIHMA, T. TIMMUSK;
Tallinn Univ. of Technol., Tallinn, Estonia

Abstract: The nuclear factor of activated T-cells (NFAT) family of transcription factors encompasses four closely related calcium-regulated members, NFATc1, NFATc2, NFATc3, and NFATc4, that upon activation by the calcium-dependent phosphatase calcineurin translocate from cytosol to the nucleus and regulate their target genes. In the nervous system, NFAT-dependent gene regulation has been shown to be involved in axonal growth and guidance, synaptic plasticity, and neuronal survival. We have previously shown that there are a number of alternative splice variants of NFAT genes expressed in the brain and that the transcriptional activity of different human NFAT proteins in neurons in response to membrane depolarization is isoform specific. For example, NFATc3 and NFATc4 are the strongest transcriptional activators in neurons, and NFATc1 and NFATc2 display isoform-specific transcription activation capacities. Here, in an attempt to identify the motifs responsible for isoform-specific transactivation of different human NFAT proteins, we looked at the effect of sumoylation as a potential regulator of transcription. Protein sumoylation is a post-translational modification in which a member of the small ubiquitin-like modifier (SUMO) family of proteins is covalently coupled to lysine residues in target proteins and sumoylation has been shown to regulate many cellular processes including repression and activation of transcription. For that, we mutated lysine residues to arginine of all predicted sumoylation sites on different human NFAT isoforms and studied the transactivation capacities between the potentially SUMO-modified wild-type and unmodified mutant forms of NFAT isoforms in rat primary cortical or hippocampal neurons in response to membrane depolarization and compared the induced transactivation levels in neurons to those obtained from HEK293FT and BHK21 cells in response to calcium signaling. Our results showed that the effect of sumoylation on the transactivation capacities of NFAT isoforms

was dependent on the amount of sumoylation sites on specific isoform and varied between cell types.

Disclosures: H. Vihma: None. T. Timmusk: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.17/P5

Topic: B.08. Synaptic Plasticity

Support: NIH Grant EY018441

Title: Transcriptional regulation of BDNF exon IX: Role of Nuclear respiratory factor 2 (NRF-2)

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

Abstract: Brain-derived neurotrophic factor (BDNF) is considered as the most abundant and active neurotrophin expressed throughout the nervous system. BDNF plays an important role in synaptic transmission, plasticity, neuronal proliferation, differentiation, survival, and death. A distinct feature of the BDNF gene is the existence of multiple non-coding exons and a single coding exon (exon IX). Although the regulation of BDNF expression by neuronal activity has received a great deal of attention, little is known about the transcriptional regulation of the BDNF gene itself, nor about transcription factor candidates that may play such a role. The goal of the present study was to begin an in-depth investigation of the key transcription factor/s that regulate/s the single coding exon IX of the rat BDNF gene. Our initial in silico analysis revealed tandem binding sites for nuclear respiratory factor 2 (NRF-2) on the promoter of exon IX. NRF-2 is of special significance because we have previously found it to co-regulate the transcriptional expressions of mediators of energy metabolism (cytochrome c oxidase) and mediators of neuronal activity (glutamatergic receptors). To test our hypothesis that NRF-2 also regulates the BDNF gene, we performed electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP), promoter cloning and site-directed mutagenesis, real-time quantitative PCR, and western blotting. Results indicate that NRF-2 functionally regulates exon IX of the rat BDNF gene. The binding sites of NRF-2 are conserved between rats and mice. Over-expressing NRF-2 up-regulated the expression of BDNF exon IX, whereas knocking down NRF-2 down-regulated such expression. These findings are consistent with our hypothesis that NRF-2, in addition to regulating the coupling between neuronal activity and energy metabolism,

also regulates the expression of BDNF, which is intimately associated with energy-demanding neuronal activity.

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

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.18/P6

Topic: B.08. Synaptic Plasticity

Support: NIH

NSF

Title: Differential genomic expression analysis of neurons mediating the *Aplysia* californica siphon/gill-withdrawal reflex.

Authors: *C. BOSTWICK[1], Q. YANG[2], A. B. KOHN[3], R. D. HAWKINS[2], L. L. MOROZ[4]; [1]Neurosci., Univ. of Florida Whitney Lab. for Marine Biosci., Saint Augustine, FL; [2]Neurosci., Columbia Univ., New York, NY; [3]Whitney Lab., [4]Neurosci., Univ. of Florida, Saint Augustine, FL

Abstract: A major goal of modern neuroscience is to gain a comprehensive understanding of the molecular processes necessary for the formation and maintenance of long-term memories using identified neural circuits known to mediate behaviors. We sampled individual neurons comprising the sensory, motor, and interneuronal components of the defensive siphon/gill-withdrawal reflex from the abdominal ganglion of the sea hare *Aplysia californica* to determine each neuron's unique transcriptome.

We identified transcripts that are differentially expressed between the different neuron subtypes of the reflex circuit as well as differential expression between the motor neurons controlling siphon and gill withdrawal, respectively. Hundreds of transcripts are differentially expressed when comparing the sensory LE cells to their downstream motor or interneurons. We found nearly 300 molecules to be differentially expressed between sensory neurons and the L29 interneurons of the reflex. Some of these transcripts encode structural proteins, enzymes, transcription factors, and regulatory kinases and phosphatases. Many of these differentially expressed transcripts are "uncharacterized", meaning their function or cellular role is currently unknown. These transcripts may serve a variety of roles, including regulating gene expression, cellular processes, or modifying RNA after it is transcribed. Additionally, these transcripts could

potentially be neuronal-specific noncoding RNAs that mediate other unknown regulatory roles within the nervous system. Our study of the molecular processes and players involved in the formation and potential maintenance of long-term memory in *Aplysia californica* is vital to understanding the fundamentals of memory formation and learning in other organisms.

Disclosures: C. Bostwick: None. Q. Yang: None. A.B. Kohn: None. R.D. Hawkins: None. L.L. Moroz: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.19/P7

Topic: B.08. Synaptic Plasticity

Title: Estrogen prevents input-deprivation related down-regulation of gene expression in the proestrus female rat barrel cortex: RNA-Seq

Authors: *J. J. ORCZYK[1], A. GORE[1], R. BATKA[1], P. E. GARRAGHTY[1,2];
[1]Psychological and Brain Sci., [2]Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Our laboratory studies the central effects of peripheral denervation in rats and previously in squirrel monkeys. Previous results have led us to investigate the role of gonadal hormones in denervation-associated sensory plasticity. Prior work has demonstrated the primary somatosensory cortex undergoes topographic reorganization in response to the loss of input from a specific peripheral location, such as in the case of limb amputation or denervation. We hypothesized that reorganization is driven by a mixture of homeostatic and use-dependent plasticity mechanisms. Interestingly, estrogen activates the same cAMP-response element binding proteins (CREB) as neural activity. Thus, we investigated the effects of unilateral infraorbital nerve (ION) transection on gene expression in the female rat barrel cortex using RNA seq in both proestrus and gonadectomized female rats (N = 7 in each group) after a 24-h survival duration. In gonadectomized females, 109 genes are down-regulated and 3 are up-regulated when comparing gene expression in the input-deprived barrel cortex with input-intact cortex. In contrast, 5 genes are down-regulated and 23 are up-regulated in the proestrus female rat barrel cortex after input-deprivation. Genes down-regulated in the gonadectomized female cortex include GABA receptor subunits, neurotrophins, and dopamine release and reuptake proteins. Genes up-regulated in the proestrus rat female barrel cortex include prolactin, NHLH1, and C1QL2. Despite the differences in gene expression, the functional outcome is predicted to be similar in both proestrus and gonadectomized females. In both cases, a reduction in both excitatory and inhibitory neurotransmission, and in the number of synapses is expected to occur

although by disparate mechanisms. In the gonadectomized females, neuronal atrophy is expected to be the primary driver of a reduction in the number of synapses. In the proestrus females, changes in gene expression favor the activation of microglia that consume inactive synapses, perhaps recognizing them through a tagging mechanism mediated by C1QL2. Other researchers have implicated a microglia response in sensory-deprivation associated plasticity. Further research is required to determine the extent to which C1QL2 may be involved in mediating adult synaptic plasticity. A consequence of our results is the necessity to take hormonal status into account as a dependent variable when performing animal research. A further corollary is that sex absolutely must be taken into consideration when designing animal research as differing hormonal environments between the sexes almost surely influence gene expression.

Disclosures: **J.J. Orczyk:** A. Employment/Salary (full or part-time): Indiana University Bloomington. **A. Gore:** None. **R. Batka:** None. **P.E. Garraghty:** A. Employment/Salary (full or part-time): Indiana University Bloomington.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.20/P8

Topic: B.08. Synaptic Plasticity

Title: Haloperidol modulates glutamate system functional connectivity: results from a translational immediate early gene based network study.

Authors: *E. F. BUONAGURO, G. LATTE, C. TOMASETTI, F. MARMO, C. AVAGLIANO, L. VELLUCCI, F. IASEVOLI, A. DE BARTOLOMEIS;
Univ. Federico II, Napoli, Italy

Abstract: Schizophrenia has been conceptualized both as a synaptic plasticity and a functional connectivity disorder. Antipsychotic drugs, such as haloperidol, are mainstays of schizophrenia pharmacotherapy. Although extensively studied, the effects of haloperidol are not completely understood at a network level, as well as its role in synaptic plasticity and functional connectivity. Homer1a (*H1a*) is an Immediate Early Gene (IEG) expressed in an activity-dependent manner, coding for a protein involved in the activity-induced reorganization of glutamatergic synapses. Its expression may be induced by acute antipsychotic administration, and may be affected by dopaminergic or glutamatergic psychotomimetic compounds. We tested the hypotesis that acute haloperidol would modulate functional connectivity in the glutamate system among brain regions relevant to schizophrenia pathophysiology. We used *H1a* expression as a molecular tool to assess putative changes in brain regional interactivity after acute treatment

with haloperidol. Sprague-Dawley rats were randomly assigned to two treatment groups (n=23), receiving vehicle (NaCl 0.9%; VEH) or haloperidol 0.8 mg/kg (HAL) i.p. injection. *H1a* induction was evaluated by means of *in situ* hybridization. Signal intensity analysis was performed in 11 Regions of Interest (ROIs) in the cortex, in the caudate-putamen and in the nucleus accumbens. Student's t-test was used to detect treatment effects. A signal correlation analysis was performed, computing all possible pairwise Pearson correlations among ROIs separately in the two groups. Using significant correlations, two networks were created for HAL and VEH group, and their network, node and edge properties were assessed. Consistent with previous observations, haloperidol induced *H1a* in all striatal ROIs. The VEH and the HAL network showed differences in network parameters (connected components, diameter, radius, characteristic path length), node attributes (degree, eccentricity, closeness, radiality, betweenness, stress, centroid, eigenvector, bridging centralities) and edge attributes (edgebetweenness). The HAL network showed enhanced interactivity between cortical and striatal regions, and reduced interactivity between caudate-putamen and nucleus accumbens. The main result of this work is the characterization of two distinct large-scale cortico-subcortical brain networks after VEH or HAL acute administration. These functional connectivity changes are potentially related to neural activity and synaptic plasticity within the glutamate system, and may play a role in haloperidol therapeutic and side effects.

Disclosures: E.F. Buonaguro: None. G. Latte: None. C. Tomasetti: None. F. Marmo: None. C. Avagliano: None. L. Vellucci: None. F. Iasevoli: None. A. de Bartolomeis: None.

Poster

224. Transcription and Translation: Mechanisms and Dynamics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 224.21/P9

Topic: B.08. Synaptic Plasticity

Support: NIH Grant 2-R01-NS036715-18

NIH Grant 4-P50-MH100024-04

Title: Comprehensive proteomic analysis of synaptosome protein turnover during environmental enrichment

Authors: *S. HEO[1,3], G. H. DIERING[1,3], R. S. NIRUJOGI[4,5], J. L. BACHMAN[1], A. PANDEY[4,2], R. L. HUGANIR[1,3];
[1]Neurosci., [2]Biol. Chemistry, Pathology and Oncology, Johns Hopkins Univ. Sch. of Med.,

Baltimore, MD; [3]Kavli Neurosci. Discovery Inst., Baltimore, MD; [4]McKusick-Nathans Inst. of Genet. Med., Baltimore, MD; [5]Inst. of Bioinformatics, Bangalore, India

Abstract: Communication between cells in the brain occurs at specialized sites of contact called synapses. The specific formation, removal, and efficacy of synaptic connections are dynamically regulated and modified by experience. Each neuron is made up of collections of proteins (called the proteome) that harbor characteristic turnover profiles of synthesis and degradation. The ability to alter a neuronal proteome, for example through changes to protein stability, is a fundamental factor affecting overall brain function, learning and memory. Recent advances in techniques to manipulate or monitor brain function have enabled scientists to study cellular and molecular mechanisms underlying learning and memory. However, cell biological mechanisms of proteostasis such as synthesis, degradation, modification, trafficking (or delivery) and stability of proteins during learning and memory or global neuronal activity changes remain poorly understood. While large differences in protein stability can be observed in the brain, the precise role of protein stability modification in the long-term maintenance of brain function is unclear. Here we explored dynamics of a rodent model system synaptic proteome and their modifications using sub-cellular fractionation, biochemistry and quantitative proteomics. We provide a system-wide identification of proteins in mouse forebrain synaptosomes and their half-lives during environmental enrichment using the Stable Isotope Labeling in Mammals (SILAM) technique and high-resolution mass spectrometry. We also observed that synaptic proteins underwent stabilization or destabilization dynamically during exposure to enriched environment. These findings will provide critical evidence to elucidate basic molecular mechanisms that regulate synaptic plasticity, receptor trafficking and long-term memory formation in terms of protein turnover and protein modifications such as phosphorylation. Furthermore this study can help to find targets that have significant relevance for many neurological disorders resulting from abnormal synaptic transmission and synaptic plasticity.

Disclosures: S. Heo: None. G.H. Diering: None. R.S. Nirujogi: None. J.L. Bachman: None. A. Pandey: None. R.L. Huganir: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.01/P10

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

Support: NIH P30AG035982

NIH P20GM103418

AA IIRG-10-172459

KU Funds

Title: Human ApoE ε2 promotes regulatory mechanisms of bioenergetic and synaptic function in female brain: a focus on V-type H^+-ATPase

Authors: *L. ZHAO[1], S. WOODY[1], H. ZHOU[2], S. IBRAHIMI[1], Y. ZHANG[2];
[1]Sch. of Pharm., Univ. of Kansas, Lawrence, KS; [2]Sch. of Med., Univ. of Kansas, Kansas City, KS

Abstract: Humans possess three major isoforms of the apolipoprotein E (ApoE) gene encoded by three alleles: ApoE ε2 (ApoE2), ApoE ε3 (ApoE3), and ApoE ε4 (ApoE4). It is established that the three ApoE isoforms confer differential susceptibility to Alzheimer's disease (AD); however, an in-depth molecular understanding of the underlying mechanisms is currently unavailable. In this study, we examined the cortical proteome differences among the three ApoE isoforms using 6-month-old female, human ApoE2, ApoE3, and ApoE4 gene-targeted replacement mice and two-dimensional proteomic analyses. The results reveal that the three ApoE brains differ primarily in two areas: cellular bioenergetics and synaptic transmission. Of particular significance, we show for the first time that the three ApoE brains differentially express a key component of the catalytic domain of the V-type H^+-ATPase (Atp6v), a proton pump that mediates the concentration of neurotransmitters into synaptic vesicles and thus is crucial in synaptic transmission. Specifically, our data demonstrate that ApoE2 brain exhibits significantly higher levels of the beta subunit of Atp6v (Atp6v1B2) when compared to both ApoE3 and ApoE4 brains, with ApoE4 brain exhibiting the lowest expression. Our additional analyses show that Atp6v1B2 is significantly impacted by aging and AD pathology and the data suggest that Atp6v1B2 deficiency could play a role in the progressive loss of synaptic integrity during early development of AD. Collectively, our findings indicate that human ApoE isoforms differentially modulate regulatory mechanisms of bioenergetic and synaptic function in female brain. A more efficient and robust status in both areas could serve as a potential mechanism contributing to the neuroprotective and cognition-favoring properties associated with the ApoE2 genotype.

Disclosures: L. Zhao: None. S. Woody: None. H. Zhou: None. S. Ibrahimi: None. Y. Zhang: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.02/P11

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

Support: NIH P30AG035982

NIH P20GM103418

AA IIRG-10-172459

KU Funds

Title: Human ApoE isoforms differentially modulate brain bioenergetic metabolism

Authors: *L. WU[1], L. ZHAO[1,2];
[1]Pharmacol. & Toxicology, [2]Neurosci. Grad. Program, Univ. of Kansas, Lawrence, KS

Abstract: Alzheimer's disease (AD) is an irreversible neurodegenerative disorder characterized clinically by progressive loss of memory and cognitive decline. There are two types of AD: early-onset and late-onset (LOAD); LOAD account for over 95% of all AD cases. Humans possess three genetic isoforms of apolipoprotein E (ApoE)—ApoE2, ApoE3 and ApoE4—that confer differential risk for LOAD; however, the underlying mechanisms are poorly understood. This study was sought to investigate the impact of human ApoE isoforms on brain energy metabolism, an area significantly perturbed in preclinical AD. A Taqman custom array was performed to examine the expression of a total of 43 genes involved in glucose and ketone body uptake, transport and metabolism in human ApoE gene-targeted replacement mice (hAPOE-TR). Consistent with our previous findings, ApoE2-bearing brains exhibited the most robust while ApoE4 brains were associated with the most deficient profile on both the uptake and metabolism of glucose, the primary fuel for the brain. In particular, the three ApoE genotypes significantly differed in the expression of facilitated glucose transporters, which mediate the entry of glucose into neurons, and hexokinases, which act as the "gateway enzyme" in glucose metabolism by converting glucose to glucose-6-phosphate, the initial step in the glycolysis, glycogen synthesis and pentose phosphate pathway. Interestingly, on the uptake and metabolism of ketone bodies, the secondary energy source for the brain, ApoE2 and ApoE4 brains exhibited a similar level of robustness, while ApoE3 brains presented a relatively deficient profile. Moreover, ingenuity pathway analysis (IPA) indicated that PPAR-γ/PGC-1α signaling pathway could be activated in the ApoE2 brain and inhibited in the ApoE4 brain. Taken together, our data provide additional evidence that human ApoE isoforms differentially modulate brain bioenergetic metabolism, which could serve as a potential underlying mechanism contributing to their discrete risk impact in AD. A therapeutic approach that promotes glucose uptake and glycolytic metabolism may hold the promise to reduce the AD risk in ApoE4 carriers; however, a ketogenic strategy may provide a more meaningful benefit in ApoE3 than in ApoE4 carriers.

Disclosures: L. Wu: None. **L. Zhao:** None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.03/P12

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

Support: NIH P30AG035982

NIH P20GM103418

AFPE Predoctoral Fellowship

KU Funds

Title: Redistribution of brain clusterin (CLU) isoforms in neurodegeneration

Authors: *S. K. WOODY, L. ZHAO;
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Abstract: Clusterin (CLU), also known as apolipoprotein J (APOJ), has recently been identified as the third most significant genetic risk factor for the development of late-onset Alzheimer's disease (LOAD). As approximately 36% of the population carries two copies of the CLU AD-risk variant (rs11136000 C allele; CLU-C), this gene is of great significance to public health. However, despite the clinical importance, an extensive gap exists in the literature pertaining to the basic properties of CLU in the brain. In this study, we demonstrate that CLU, which is predominantly expressed in the brain, exists as two isoforms: mature CLU (mCLU) and nuclear CLU (nCLU). Of particular significance, our data indicate the localization of nCLU in brain and neuronal mitochondria; a novel finding in the CLU research field. Additionally, our analyses demonstrate a rapid rearrangement of neuronal CLU isoforms in response to neurotoxic insult, including a significant removal of cytosolic mCLU and an accumulation of nCLU in the cytosol that appears to result from declining nCLU levels in the mitochondria. These initial findings suggest that: 1) Contrary to the long-held notion that nCLU is a stress-inducible and solely pro-apoptotic protein, nCLU may play an important regulatory role in neuronal homeostasis; 2) Neurodegenerative challenge induces redistribution of neuronal CLU isoforms; this redistribution results in intracellular CLU dyshomeostasis and mitochondrial instability which may account for the pro-apoptotic properties attributed to nCLU following cellular stress.

Disclosures: S.K. Woody: None. L. Zhao: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.04/Q1

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

Support: Ekam Imaging Inc

Horizon Discovery

Title: APOE epsilon-4 knock-in rat model of Alzheimer's disease - gender differences in cognitive behavior and gray matter microarchitecture: Insights on the neuropathology of disease progression using MRI.

Authors: *P. P. KULKARNI[1], J. HONEYCUTT[2], M. S. TRIVEDI[3], H. BRENHOUSE[2], M. NEDELMAN[4], K. GAMBER[5], C. F. FERRIS[1];
[1]Psychology, Northeastern Univ. Dept. of Psychology, Boston, MA; [2]Psychology, Northeastern Univ., Boston, MA; [3]Pharmaceut. Sci., Nova Southeastern Univ., Fort Lauderdale, FL; [4]Ekam Imaging Inc, Boston, MA; [5]Horizon Discovery, St Louis, MT

Abstract: The most prevalent genetic risk for sporadic Alzheimer's disease is the allele Ɛ4 of the apolipoprotein E4 (APOE Ɛ4). The many neuropathological findings that define AD are associated with APOE Ɛ4 carriers together with general cerebrovascular impairment and neuroinflammation. There are reports from human imaging studies that suggest the apoE4 protein could affect neurodevelopment many years before the onset of AD. Indeed, brain structure alterations may precede overt cognitive impairment in AD by several decades. Early detection of these alterations holds inherent value for the development and evaluation of preventive treatment therapies.In collaboration with Horizon Discovery (St Louis) we characterized the brain and cognitive development of male and female APOE Ɛ4 knock-in (KI) rats, a preclinical model of Alzheimer's disease. Testing memory in the novel preference task showed no difference between WT and KI females at seven months of age, but a significant loss of memory in KI males as compared to WT males. Using a newly developed non-invasive technology combining quantitative anisotropy and computational analysis of 174 brain areas of the rat we identified subtle changes in gray matter microarchitecture between genotypes. There were only 6 areas with significant differences in indices of anisotropy (IA) values, e.g. fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity, between female genotypes. In contrast, there were 33 brain areas showing significant changes in FA values between male genotypes. 17 of these areas included cerebral cortex, 8 the cerebellar complex and 5 the primary olfactory system. The same pattern was reflected in the RD values - very few differences in females with 55 differences between male genotypes strongly favoring the cerebellum, cortex and olfactory system. Areas that showed little change were the thalamus, amygdala, hypothalamus, basal

ganglia and to our surprise hippocampal complex. The precise molecular and biochemical processes contributing to these putative microarchitectural changes in gray matter are being studied in postmortem brain samples collected from this study. In summary, male but not female APOE Ɛ4 rats showed deficits in memory. Putative changes in gray matter microarchitecture across 174 brain areas were both gender and genotype specific. Male but not female rats, expressing the APOE Ɛ4 protein showed significant differences in cerebellum, cerebral cortex and primary olfactory system, but not the hippocampal complex.

Disclosures: **P.P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc. **J. Honeycutt:** None. **M.S. Trivedi:** None. **H. Brenhouse:** None. **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc. **K. Gamber:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.05/Q2

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

Support: Ekam Imaging Inc.

Horizon Discovery

Title: Using quantitative anisotropy and computational analysis across 174 brain areas to identify changes in gray matter microarchitecture in the apoe knock-out rat: evidence of gender differences and alterations in cortico/thalamic and cerebellar connectivity

Authors: ***J. QIAO**[1], C. GHARAGOUZLOO[2], P. KULKARNI[3], L. TIMMS[4], K. GAMBER[5], M. NEDELMAN[6], A. VEN[4], S. SRIDHAR[4], C. F. FERRIS[3];
[1]MIE, [2]Bioengineering, [3]Psychology, [4]Physics, Northeastern Univ., Boston, MA; [5]Horizon Discovery, St. Louis, MT; [6]Ekam Imaging Inc., Boston, MA

Abstract: In the central nervous system, apolipoprotein E (APOE) is produced in glia and functions in the transport of cholesterol to neurons via APOE receptors. The absence or dysregulation of this lipoprotein increases risk for vascular disorders and Alzheimer's disease (AD). The recent development of transgenic homozygous APOE -/- knock out (KO) rats

provided an opportunity to apply a new imaging methodology "in vivo neuropathology" to non-invasively study subtle changes in gray matter microarchitecture in WT and KO female and male rats using quantitative anisotropy across 174 brain regions. In addition, T1 and T2 relaxivity measures were collected across these brain regions and comparisons made between gender and the APOE genotype. All studies were done on a Bruker 7T MR scanner while rats were anesthetized with 2% isoflurane. Both males and female APOE KO rats showed over 40 brain areas with altered T1 relaxivity as compared to gender matched WT controls. The entire cerebellum and deep cerebellar nuclei were affected as were many of their afferent and efferent connections in brainstem and pons. There was little difference in T1 measures between genders. Changes in T2 relaxivity between APOE genotypes were less pronounced and numbered only 10 in females and18 in males. Again areas of the cerebellum and their afferent and efferent connections were involved. Quantitative anisotropy showed clear male/female differences. In the males this imaging technique complemented the relaxivity measures as the cerebellum again showed differences between APOE genotypes for fractional anisotropy and radial diffusivity. In addition, many thalamic areas and their cortical connections were altered. In females the changes were fewer and localized primarily to hypothalamus and amygdala. In this study multiple non-invasive MRI protocols were used to identify putative changes in gray matter microarchitecture across 174 brain areas in a rat model of AD. The changes were both gender and APOE specific. Rats, male and female, lacking apolipoprotein E showed significant differences in cerebellum and its afferent and efferent connections for measures of T1. This profile was consistent for males for T2 and indices of anisotropy but different for females. There was little evidence of any changes in the hippocampal complex between gender and APOE genotype. Brains were harvested and are undergoing immunhistochemical analysis for glial fibrillary associated protein and activated microglia to assess neuroinflammation in areas identified with these imaging protocols.

Disclosures: **J. Qiao:** None. **C. Gharagouzloo:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc.. **L. Timms:** None. **K. Gamber:** None. **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc.. **A. Ven:** None. **S. Sridhar:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging Inc..

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.06/Q3

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

Support: Ekam Imaging

Lundbeck

Title: Mapping brain neural circuitry in response to pro-cognitive therapeutics: a pharmacological MRI study in the awake rat

Authors: *C. F. FERRIS[1], P. KULKARNI[2], M. NEDELMAN[3], I. E.M DE JONG[4];
[1]Psychology, [2]Northeastern University, Ctr. for Translational NeuroImaging, Boston, MA;
[3]Ekam Imaging, Boston, MA; [4]Lundbeck, Valby, Denmark

Abstract: Combination therapies are being developed to improve memory in cases of mild cognitive impairment and early stage Alzheimer's. To this end, we followed changes in brain activity caused by a serotonin receptor antagonist given alone or in combination with an acetylcholinesterase inhibitor using functional magnetic resonance imaging (fMRI) in the awake rat. Studies were performed on a Bruker 7T MRI scanner using a restraining system and RF coil design customized for awake rat imaging. Rats were acclimated to restraining system prior to imaging. During the scanning session the compounds were given intravenous and BOLD (Blood Oxygen Level Dependent) fMRI used to study activation in 171 predefined regions of the brain. The serotonergic antagonist alone had only a modest effect on brain activity affecting 8 brain regions primarily localized to the ventral forebrain. The acetylcholinesterase inhibitor activated 19 brain areas when given alone affecting the serotonergic raphe nucleus, areas of cholinergic neurotransmission and areas associated with the hippocampal complex. When both drugs were given together, there was a robust activation pattern of 36 brain regions. The pattern of significantly activated brain regions could be reconstructed into three integrated neural networks - Extended Amygdala, Striato-Pallidum and Septo-Hippocampus, and interestingly much of the primary olfactory system. This example of a potential combination therapy shows a clear synergistic effect of both compounds that extended beyond the effects of each alone. The combination treatment recruits a constellation of integrated neural circuits associated with cognition, emotion and motivation as well as extroceptive (olfaction) and introceptive cues (brainstem). These may collectively contribute to cognition, by enriching learning and memory processes with motivational salience and the context of the extroceptive and introceptive worlds. Studies are presently underway to test this combination therapy in the APOE epsilon 4 knock-in rat model of Alzheimer's that presents with deficits in learning and memory.

Disclosures: **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research, Ekam Imaging. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **I. E.M de Jong:** None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.07/Q4

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

Title: First capillary blood volume full-brain atlas and absolute functional CBV measurements using QUTE-CE MRI in sprague dawley rats: Preliminary results in the APOE Epsilon 4 knock-in rat model of Alzheimer's disease.

Authors: *C. A. GHARAGOUZLOO[1], L. TIMMS[2], J. QIAO[3], Z. FANG[2], J. NNEJI[4], A. V. VEN[2], P. KULKARNI[5], C. F. FERRIS[5], K. GAMBER[6], S. SRIDHAR[2];
[1]BioEngineering, Northeastern Univ., Medford, MA; [2]Physics, [3]Mechanical and Industrial Engin., [4]BioEngineering, [5]Psychology, Northeastern Univ., Boston, MA; [6]Horizon Discovery, St. Louis, MT

Abstract: Cerebral blood volume (CBV) is an important indicator of tissue health and function. Dynamic susceptibility contrast (DSC) MRI is commonly used for measuring CBV values but requires accurate determination of the arterial input function (AIF), or GBCA concentration versus time curve which is typically 15-30% inaccurate. Furthermore a fast acquisition protocol (such as echo-planar imaging [EPI]) must be employed, which inherently limits both the spatial resolution and the signal-to-noise ratio (SNR). It has been shown that CBV measurements with DSC-MRI are even more inaccurate in ischemic tissue because of late, unpredictable arterial arrival of CA. Other techniques for measuring the CBV, such as steady-state susceptibility contrast mapping (SSGRE), steady state CBV (SS_CBV), and ΔR2 all utilize T2 and T2* effects, which are susceptible to intra- and extra-voxular dephasing as well as flow artifacts. We have developed a quantitative blood volume atlas of the rat brain using the **Q**uantitative **U**ltra-short **TE** **C**ontrast **E**nhancement (QUTE-CE) MRI technique based on an absolutely quantitative signal and partial voluming. Unlike usual techniques that render only semi-quantitative raw MR signal[9], QUTE-CE is insensitive to extravoxular susceptibility and motion/flow artifacts. We

employ superparamagnetic iron-oxide nanoparticles (SPIONs) for intra-vascular contrast enhancement, and our technique provides clearly delineated positive contrast of the vascular compartment compared to the commonly employed T2-weighted techniques which produce negative contrast. We develop the Atlas both in terms of absolute CBV and capillary blood volume, and demonstrate that the technique can be utilized for quantitative steady-state functional imaging by measuring changes in CBV in Sprague Dawley rats induced by a 5% CO2-challenge and anesthesia by 3% isoflurane. A reference 3D MRI atlas with 174 segmented and annotated regions was utilized to construct the vascular atlas. In collaboration with Horizon Discovery we ran preliminary studies on capillary density measures in male and female APOE Ɛ4 knock-in (KI) rats and found gender differences in the hippocampus and cortex. At 7 months of age male KI, but not female KI, showed deficits in memory as compared to gender-matched, wild-type controls. Female KI showed significantly higher capillary densities in the hippocampus (CA1, CA3 and dentate gyrus) and cortex as compared to KI males but no difference from WT females. These preliminary findings may reflect the positive influence of estrogen on angiogenesis, aging and early disease progression.

Disclosures: C.A. Gharagouzloo: None. L. Timms: None. J. Qiao: None. Z. Fang: None. J. Nneji: None. A.V. Ven: None. P. Kulkarni: None. C.F. Ferris: None. K. Gamber: None. S. Sridhar: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.08/Q5

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

Support: R21 AG-040683

R21 AG-040753

Title: Particulate air pollutants accelerate neurodegeneration in EFAD mice

Authors: *M. CACCIOTTOLO[1], A. SAFFARI[2], C. SIOUTAS, 90089-0191[2], M. LADU[3], T. E. MORGAN[1], C. E. FINCH[1];
[1]USC Davis Sch. of Gerontology, Los Angeles, CA; [2]USC Viterbi Sch. of Engin., Los Angeles, CA; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Particulate air pollutants are increasingly recognized for their neurotoxic impact in human populations and in experimental rodent studies but very little is known about the

mechanisms involved and interaction with the apoE ε4 risk factor for Alzheimer disease (AD). To evaluate the mechanism behind air pollutant neurotoxicity, we exposed 20 female EFAD transgenic mice (5xFAD+/-/human APOE ε3 or ε4 +/+) to nanosized urban airborne particulate material (nPM) for 225h (5h/day, 3d/wk, 15wks, at a dose of 340ug/m3 nPM) and analyzed brain tissues for hallmarks of AD pathology. nPM exposure induced 2.8-fold greater increment in cortical plaque load in E4FAD mice than in E3FAD mice. However, both APOE alleles responded with Aβ oligomer increases in soluble extracts of cerebral cortex. Silver staining showed selective neuronal atrophy in response to nPM in the hippocampus, restricted to CA1 neurons, without significant changes in the contiguous CA2/3 neurons, or in the dentate gyrus (DG). The selective morphological changes in CA1 neurons with nPM exposure paralleled their vulnerability in AD pathogenesis. At the protein level, nPM exposure induced a robust decrease of GluR1 protein levels in both genotypes. No changes were detected in other glutamatergic receptor subunits (GluR2, NR2A and NR2B) or in other common synaptic proteins (pre-synaptic: synaptophysin; post-synaptic: PSD95). These findings support the hypothesis that particulate air pollutants accelerate pathological brain aging, with potentially greater impact in APOE ε4 carriers. We propose that the underlying mechanisms of neurotoxicity involve increased cerebral Aβ production and glutamatergic remodeling.

Disclosures: M. Cacciottolo: None. A. Saffari: None. C. Sioutas: None. M. LaDu: None. T.E. Morgan: None. C.E. Finch: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.09/Q6

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

Support: UIC Start-up Fund

Title: APOE modulated cerebrovascular dysfunction in Alzheimer’s disease

Authors: *L. M. TAI[1], F. M. MAROTTOL[1], K. P. KOSTER[1], A. MORRIS[1], R. THOMAS[2]; [1]Anat. and Cell Biol., [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Cerebrovascular (CV) dysfunction is emerging as a critical component of Alzheimer disease (AD). Although CV damage is well documented in AD, the extent is variable and this variability may be related to the ability of known AD risk factors to affect CV function. Our research is focused on the role of known AD risk factors in CV dysfunction: *APOE* and female sex. *APOE4* is the greatest genetic risk for sporadic AD, with a greater effect in females.

Therefore, the role of *APOE* and female sex in CV dysfunction was assessed in novel EFAD transgenic mice, which express human *APOE3* or *APOE4* and overproduce human Aβ. Our data demonstrate that *APOE4* and Aβ induce cognitive impairments and increase CV leakiness in female mice (E4FAD mice) at 8 months of age.In female E4FAD mice, cognitive defects correlated with vessel degeneration. Angiogenic signaling in brain endothelial cells (BECs) is key for controlling vessel degeneration and the complex metabolic, transport and signaling functions of the CV. Therefore, angiogenic growth factors (GFs) may ameliorate CV dysfunction in AD. Our comparison of the main angiogenic GFs demonstrate that EGF protects against Aβ-induced disruption of vessel formation and vessel degeneration in single BEC cultures. EGF also protects against Aβ42-induced vessel degeneration in triple cultures (astrocytes, pericytes and BECs) designed to mimic the blood-brain barrier (BBB). Additionally, EGF receptor ligands induced functional effects in all cell types of the BBB for Aβ-induced activation. Based on the *in vitro* findings, we treated female E4FAD mice with EGF (300µg/kg/week) from 6-8 months in a prevention paradigm. EGF prevented the age-dependent cognitive decline as assessed by spontaneous alternation (Y-maze), novel object recognition and platform latency (Morris water maze) and prevented CV leakiness and vessel degeneration. Collectively these data support that *APOE4 and Aβ predispose the CV to damage with an "accelerating hit", which can be ameliorated by EGF.*

Disclosures: L.M. Tai: None. F.M. Marottol: None. K.P. Koster: None. A. Morris: None. R. Thomas: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.10/Q7

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

Support: NIA AG12311

Windgate Foundation

STOPAlzheimer's

Title: Apoe affects autophagy through TFEB and transcriptional regulation in Alzheimer's and epilepsy.

Authors: *P. A. PARCON[1], M. BALASUBRAMANIAM[3,4], R. A. JONES[2,6], L. LIU[5], R. E. MRAK[7], S. T. GRIFFIN[2,6];

[1]RIOA, [2]Geriatrics, UAMS, Little Rock, AR; [3]Bioinformatics, Univ. of Arkansas, Little Rock, Little Rock, AR; [4]Geriatrics, [5]Univ. of Arkansas for Med. Sci., Little Rock, AR; [6]Geriatric Res. Educ. and Clin. Ctr., Central Arkansas Veterans HealthCare Syst., Little Rock, AR; [7]Pathology, Univ. of Toledo Hlth. Sci. Campus, Toledo, OH

Abstract: Autophagy is a major protein and organelle degradation pathway that is often preferentially affected in neurodegenerative diseases. Autophagy is regulated to a great degree by the transcription factor TFEB, which in turn is regulated at the lysosome by its phosphorylation state: This determines if TFEB can be bound to 14-3-3 and remain in the cytoplasm, or be transported to the nucleus for transcriptional upregulation of autophagy genes. The APOE ε4 allele is the single greatest genetic risk factor for development of Alzheimer's disease, with no single mechanism yet agreed upon, while the APOE ε3 allele is considered normal risk. Using RT-PCR on human hippocampal tissue, we have found that autophagy genes LC3B, LAMP2, and SQSTM1/p62 transcripts are significantly higher in the hippocampus of those with Alzheimer's disease with two copies of APOE ε3 compared to those homozygous for APOE ε4. Based on confocal immunofluorescence and *in situ* proximity ligation assay in human hippocampus, both AD groups had elevated nuclear levels of TFEB compared to age-matched controls, along with lower levels of the cytoplasmic 14-3-3 and TFEB complex, but were not significantly different from each other. We found further evidence in western blot of epilepsy brain tissue, as we noted that those patients with at least one copy of APOE ε4 have a great amount of nuclear TFEB without a corresponding increase in autophagy mRNAs. This implies a dysfunction in transcriptional upregulation of autophagy at a point between nuclear translocation and transcription. We have found that *in silico* simulations of ApoE4 protein have a stronger binding to the conserved nucleotide sequence for TFEB promotor regions, and therefore hypothesize that ApoE4 increases risk for disease, at least, in part by inhibiting compensatory upregulation of autophagy transcription. Supported in part by NIA AG12411, the Windgate Foundation, and STOP Alzheimer's.

Disclosures: P.A. Parcon: None. M. Balasubramaniam: None. R.A. Jones: None. L. Liu: None. R.E. Mrak: None. S.T. Griffin: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.11/Q8

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

Support: NIH Grant AG037481

NIH Grant AG037919

NIH Grant AG044490

NIH Grant ES024233

NIH Grant ES021243

DOD Grant W81XWH-13-1-0384

Title: Integrated systems approach identifies gene networks in APOE3 & APOE4 expressing mice in response to HFD

Authors: *K. NAM[1], A. MOUNIER[1], C. M. WOLFE[1], N. F. FITZ[1], J. SCHUG[2], I. LEFTEROV[1], R. KOLDAMOVA[1];
[1]Envrn. and Occup. Heath, Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Genet., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Alzheimer's disease (AD) is a complex multifactorial disease with a significantly increased incidence of its late onset form in ε4 carriers, in individuals exposed to various risk factors, including diet, and higher prevalence in females. It has been demonstrated that high fat diet (HFD) exacerbates memory deficits and amyloid pathology also in APP transgenic mice. To get further insight in the effect of ApoE on the response to HFD, young APP mice expressing human ApoE3 or ApoE4 isoforms were fed HFD for 3 months and Morris Water Maze (MWM) task performed to assess spatial learning and memory retention. Following the behavior testing, brain tissue was collected for histological analysis and RNA-seq. To determine how ApoE isoforms and diet affect amyloid deposition, we measured compact and diffuse plaques by X-34 and 6E10 staining in hippocampus and cortex. To assess the effect of diet and ApoE isoform on the transcriptome, in APP mice genome-wide, we applied RNA-seq followed by Weighted Gene Co-expression Network Analysis (WGCNA). WGCNA organizes genes in functional groups (*modules*) based on topological overlap. We identified 15 modules associated with diet, APOE genotype and gender and further characterized 3 of them, representing immune response (GO:0006955), protein localization (GO:0015031) and regulation of transcription (GO: 0016481). We determined that immune response positively correlated to diet and APOE4 genotype in female mice. In contrast regulation of transcription was positively associated to diet in male mice. Highly connected hub genes, identified in each module (Trem2, Tyrobp, B2m, Cx3cr1 – immune response; Tgfb1, Cst, Nr2f6 – regulation of transcription; Appbp2, Med13, Ube3a – protein localization) were used to generate high connectivity gene networks. Selected genes within the hub gene networks were validated by RT-QPCR and western blot. The results clearly demonstrate that APOE isoform and gender affect the response to diet on transcriptional level.

Disclosures: K. Nam: None. A. Mounier: None. C.M. Wolfe: None. N.F. Fitz: None. J. Schug: None. I. Lefterov: None. R. Koldamova: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.12/Q9

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

Support: grants-in-aid for scientific research from JSPS

Title: Dietary cholesterol increases neurodegenerative pathology through the blood-cerebrospinal fluid barrier in rabbits

Authors: *F. OBATA, R. MINAI, C. WANG, B. NING, M. NIIMI, J. FAN;
Univ. Yamanashi, Dept. Mol Pathol, Yamanashi, Japan

Abstract: Rabbit has been used as cholesterol-sensitive Alzheimer's disease (AD) model. We fed Japanese white rabbits with 0.3 % cholesterol diet for 16 weeks, which is a condition to induce hypercholesterolemia as well as atherosclerosis in aorta, but less severe condition than reported 1-2 % cholesterol diet AD models. And we used Watanabe Hereditary Hyperlipidemic (WHHL) rabbits as a high serum cholesterol control as they are hypercholesterolemic from very beginning of their lives. Bielschowsky's silver stain was performed to rabbit brains and neurodegenerative changes were recognized in cerebral cortex and hippocampus of WHHL rabbits in high degree but less in 0.3 % cholesterol diet group. In the choroid plexus, lipid-laden foam cells and cholesterol crystal lesions were apparent in WHHL, whereas small lesions of foam cells were recognized in 0.3 % cholesterol diet group. Normal chow diet group (0 % cholesterol control) had no foam cells in the choroid plexus. Rabbit macrophage-specific marker RAM11 immunohistochemisty (IHC) revealed that these foam cells are macrophagic origin. Apoliporotein CIII (ApoCIII) IHC showed positivity in the foam cells, choroid plexus epithelial cells and cilia of ependymal cells in 0.3 % cholesterol group and WHHL rabbits in which intensity of the stain was higher in WHHL. As ApoCIII is a component of very low-density lipoprotein (VLDL), which increases in hypercholesterolemia, and VLDL receptor is known to be expressed at the choroid plexus, the result suggests accumulation and transportation of cholesterol through the blood-cerebrospinal fluid barrier (BCSFB). On the other hand, Apoliporotein E (ApoE) IHC signals were increased in neuronal and glial cytoplasm but not in the choroid plexus and ependymal cells in hyperlipidemic rabbits. Upregulation of intrabrain ApoE under high serum cholesterol suggests that brain parenchyma senses increased cholesterol signal. Current study suggests that the BCSFB may serve as a novel route for cholesterol to enter brain parenchyma.

Disclosures: F. Obata: None. R. Minai: None. C. Wang: None. B. Ning: None. M. Niimi: None. J. Fan: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.13/Q10

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

Title: Impaired cholesterol homeostasis contributes to the increased secretion of β-amyloid peptide in Familial Alzheimer's disease-associated presenilin mutant

Authors: *Y. CHO, O.-H. KWON, H. OH, S. CHUNG;
Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Cerebral deposition of amyloid β-peptide (Aβ) is crucial in the pathogenesis of Alzheimer's disease (AD). The highly amyloidogenic 42-residue Aβ (Aβ42) is the first species to be deposited in both sporadic and familial AD (FAD). Mutations in FAD-associated presenilin1 (PS1) and 2 (PS2) increase the ratio of Aβ42 over 40-residue (Aβ40). A PS mutant reportedly elevates the level of cholesterol due to the increased expression of CYP51, which is critical for cholesterol biosynthesis. Since elevated cholesterol is a well-known risk factor for AD, it may contribute to the increased Aβ production in PS1 mutant cells. However, this possibility has never been directly examined. In this study, we confirmed that the expression of CYP51 and cholesterol level were elevated in CHO cells transfected with PS1 ΔE9 mutant, compared to cells transfected with PS1 wild type (WT). The elevated cholesterol level in PS1 ΔE9 cells was decreased to a level comparable to that in PS1 WT cells by incubating cells with CYP51 specific inhibitor, tebuconazole. In PS1 ΔE9 cells, tebuconazole significantly decreased secreted level of Aβ42, but not that of Aβ40, confirming that the increase cholesterol level contributes to the increased Aβ42 from PS1 ΔE9 cells . In PS1 WT cells, however, levels of Aβ42 and Aβ40 were not affected by tebuconazole. When cholesterol was depleted by incubating cells in lipid depleted serum, the amount of decreased Aβ42 was larger than of Aβ40, suggesting that cholesterol level and Aβ42 level is closely linked. These results suggest that the impaired cholesterol homeostasis in FAD PS mutants contributes to increased Aβ42 production.

Disclosures: Y. Cho: None. O. Kwon: None. H. Oh: None. S. Chung: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.14/Q11

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

Support: NIH R01 AG043522

Title: Bexarotene discontinuation maintains behavioral improvement independent of changes in amyloid pathology

Authors: *B. CASALI, G. LANDRETH;
Neurosci., Case Western Reserve Univ., Cleveland, OH

Abstract: Treatment of Alzheimer's disease (AD), a neurodegenerative disorder characterized by pathological hallmarks of beta-amyloid (Aβ) plaque deposits, inflammation, and cognitive decline, remains a clinical obstacle in part due to the disease's lack of successful targeted therapies. Our lab established that short-term treatment with the RXR agonist bexarotene ameliorates amyloid pathology and reverses cognitive deficits in murine models of AD, but long-term treatment resulted in enhanced cognition despite no changes in amyloid plaque burden. This suggests that improved cognition may not strictly correlate with decreases in the pathological hallmarks of AD. Using the APP/PS1 murine model of AD, we find that not only does bexarotene improve short-term memory deficits after two weeks of treatment, but that this cognitive improvement is maintained despite a two-week discontinuation of bexarotene. Paradoxically, cognitive improvement was independent of changes in plaque burden and insoluble Aβ species, despite engagement of RXR target genes with bexarotene in both the two-week bexarotene treated and the bexarotene discontinued animals. *In vitro* studies modeling bexarotene discontinuation in primary astrocytes resulted in enhanced LXR target genes, confirming our *in vivo* findings. These data suggest that bexarotene may exert cognitive benefits in AD mouse models through different mechanisms from those classically observed previously. These findings may inform future clinical studies involving RXR agonists.

Disclosures: **B. Casali:** A. Employment/Salary (full or part-time): Brad Casali, Gary Landreth. Other; Gary Landreth. **G. Landreth:** A. Employment/Salary (full or part-time): Gary Landreth. Other; Gary Landreth.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.15/Q12

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

Support: NIH Grant R01AG035355

NIH Grant P01AG030128

Mayo Clinic Alzheimer’s Disease Research Center pilot grant to N. Z.

Title: Apolipoprotein E4 inhibits cerebral insulin signaling and insulin-regulated glucose metabolism

Authors: *N. ZHAO, C.-C. LIU, C. M. LINARES, M. M. PAINTER, G. BU;
Mayo Clin. Jacksonville, Jacksonville, FL

Abstract: Diabetes and impaired insulin signaling in the brain have been linked to the pathogenesis of Alzheimer’s disease (AD). Epidemiological studies show that diabetic patients are at higher risk for AD. The ε4 allele of the apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for late-onset AD. Interestingly, the association between diabetes and amyloid pathology is stronger among *APOE4* carriers. In addition, recent clinical trials revealed that intranasal insulin administration significantly improves the cognitive function of AD patients in a manner that depends on *APOE* genotype. *APOE4* carriers, either as healthy adults or with dementia, have lower cerebral glucose metabolism in FDG-PET studies. Despite these observations in humans, the mechanism by which apoE isoforms differentially regulate brain insulin signaling and glucose metabolism remain unclear. Here we report apoE isoform-dependent glucose metabolism and insulin signaling in human apoE-targeted replacement (TR) mouse models. We found that cerebral blood flow was reduced in aged apoE4-TR mice compared with apoE3-TR mice. Aged apoE4-TR mice also had impaired insulin signaling and reduced capacity to metabolize cerebral glucose upon glucose challenge when examined by *in vivo* microdialysis. These effects were further exacerbated when apoE4-TR mice were fed with high-fat diet (HFD). At the cellular level, we found that apoE4 inhibited insulin signaling and insulin signaling-regulated mitochondrial respiration and glycolysis in primary neurons, likely by reducing the amount of insulin receptor at the cell surface. Together, our studies provide novel insights into the pathogenic mechanisms of apoE4 and insulin resistance in AD and have implications in
designing apoE-targeted therapy to improve cerebral insulin signaling and glucose metabolism to treat AD.

Disclosures: N. Zhao: None. C. Liu: None. C.M. Linares: None. M.M. Painter: None. G. Bu: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.16/Q13

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

Title: Effect of apoe e4 variant on progression from mild cognitive impairment to alzheimer's disease

Authors: *B. P. TAYLOR[1], S. RISACHER[3], J. KRANTZ[2];
[2]Psychology, [1]Hanover Col., Hanover, IN; [3]IUPUI Neurosci. Ctr., Indianapolis, IN

Abstract: Alzheimer's disease is a very prevalent and fatal disorder in older adults, and Mild Cognitive Impairment is often seen as a precursor to Alzheimer's disease. Alzheimer's disease is a form of Dementia that is characterized by loss of cognitive abilities while aging. It is ultimately fatal. Mild Cognitive Impairment is more of an intermediate stage between normal mental decline with aging and the more serious decline of dementia. The ApoE E4 gene has been shown to be highly correlated with a greater likelihood of acquiring late-onset Alzheimer's disease. This study looked to see the effect that the ApoE E4 gene has on the rate of brain volume loss (specifically in the hippocampus, entorhinal cortex, and cerebral cortex) in patients who transition from Mild Cognitive Impairment to Alzheimer's disease. This was done by comparing people, gathered from the Alzheimer's Disease Neuroimaging Initiative, with Mild Cognitive Impairment who test positive for the E4 allele vs. those with Mild Cognitive Impairment who test negative for the E4 allele, to see which group has a faster loss of brain volume to Alzheimer's disease. My study found that testing positive for the E4 allele led to faster neurodegeneration in both people with Mild Cognitive Impairment who converted to Alzheimer's and even those who did not convert to Alzheimer's. This study is beneficial because it could provide further insight into the potential genetic cause of Alzheimer's disease, especially if the study is replicable. It is unique from other studies of this nature because it involves a new cohort.

Disclosures: **B.P. Taylor:** A. Employment/Salary (full or part-time): I am a Teacher Assistant at San Diego State University. **S. Risacher:** A. Employment/Salary (full or part-time): Researcher at IUPUI Neuroscience Center. **J. Krantz:** A. Employment/Salary (full or part-time): Professor of Psychology at Hanover College.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.17/Q14

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

Support: NIH T32 DA007097

Center on Aging, College of Pharmacy, Academic Health Center of the University of Minnesota

Title: An HDL-mimetic peptide increases secretion and lipidation of human apoE

Authors: *D. S. CHERNICK[1], G. W. REBECK[3], L. LI[1,2];
[1]Pharmacol., [2]Exptl. and Clin. Pharmacol., Univ. of Minnesota, Minneapolis, MN; [3]Neurosci., Georgetown Univ., Washington, DC

Abstract: The Apolipoprotein E (apoE) ε4 allele is the primary genetic risk factor for late-onset Alzheimer's disease (AD). ApoE in the brain is produced primarily by astrocytes and microglia. Once secreted, apoE binds lipids and forms high-density lipoprotein (HDL)-like particles in the interstitial and cerebrospinal fluid. ApoE interacts with Aβ, and the level and lipidation state of apoE affects Aβ aggregation and clearance pathways. ApoE may also play a role in AD via effects on cholesterol/lipid metabolism, synaptic plasticity, cell signaling, and inflammation. Apolipoprotein A-I (apoA-I) and HDL levels are inversely correlated with AD risk and severity, and overexpression of human apoA-I attenuated memory deficits in a mouse model of AD. ApoA-I has been shown to increase apoE secretion in both peripheral macrophages and primary mixed glia. We have previously shown that 4F, an apoA-I mimetic peptide, increases both secretion and lipidation of apoE in primary murine glial cells. The current study aims to determine the ability of 4F to recapitulate this effect on human apoE. In order to perform these studies we have used immortalized human targeted-replacement apoE3 and apoE4 mouse astrocytes. Preliminary experiments show that 4F increases secretion and lipidation of both apoE3 and apoE4 in these astrocytes. Future studies aim to elucidate whether the effects of 4F on apoE3 and apoE4 are differential, and whether the increase in apoE lipidation occurs *in vivo*. ApoE as a therapeutic target for AD remains controversial due to the detrimental potential of increasing apoE4 levels. However, by improving the lipidation status of apoE4, many of its deleterious effects may be ameliorated. Finally, we will determine whether 4F is capable of reducing pathology and memory deficit in mouse models of AD.

Disclosures: D.S. Chernick: None. G.W. Rebeck: None. L. Li: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.18/R1

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

Support: Cure Alzheimer's Fund

American River Nutrition, Inc

Title: Tocotrienols for Alzheimer's disease: vitamin E beyond tocopherols

Authors: *W. XIA[1,2], C.-L. SHEN[3], H. MO[4];
[1]Dept. of Pharmacol. & Exptl. Therapeut., Boston Univ. Sch. of Med., Boston, MA; [2]Grecc, ENR Mem. Veterans Hosp., Bedford, MA; [3]Pathology, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [4]Nutr., Georgia State Univ., Atlanta, GA

Abstract: Neuropathological hallmarks in brains of Alzheimer's disease (AD) patients include amyloid β protein containing neuritic plaques and Tau containing neurofibrillary tangles. Multiple therapeutics have been tested to reduce the pathological burden and to maintain cognitive function. However, currently there is no cure for AD. One promising therapeutic direction is based on the observation that lower incidents of AD and mild cognitive impairment (MCI) have been reported in populations exposing to higher levels of vitamin E, particularly tocotrienols, but not tocopherols. In this study, we compared tocotrienols to statins, which target 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and decrease downstream levels of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), intermediate products of cholesterol metabolism. In murine 3T3-F442A adipocytes, tocotrienols induced a dose-dependent reduction of the membrane HMG-CoA reductase. As a consequence of tocotrienol-mediated depletion of FPP and GGPP, prenylation of small GTPase is impaired, rendering unprenylated Ras prone to degradation; in human melanoma cells, we found that tocotrienols induced dose-dependent reduction of Ras protein. We analyzed levels of Aβ and Tau after chronic dosing with tocotrienols in a high-fat-diet induced obese mice for 3 months. Tocotrienols exhibited efficacy similar to statins that reduce Aβ in cells and animals. Our studies will provide results from cellular and animal studies to explore whether tocotrienols exert protection against AD without the dose-limiting toxicities of statins.

Disclosures: **W. Xia:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; grant support by American River Nutrition. **C. Shen:** 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; American River Nutrition. **H. Mo:** 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; American River Nutrition.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.19/R2

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

Title: Molecular mechanism and functional effect of apoE-TREM2 interaction in microglia

Authors: *Y. ATAGI, M. M. PAINTER, C.-C. LIU, J. D. FRYER, G. BU;
Neurosci., Mayo Clin. Florida, Jacksonville, FL

Abstract: Mutations in the triggering receptor expressed on myeloid cells 2 (TREM2) have been identified as risk factors for late-onset Alzheimer’s disease (LOAD). In the central nervous system (CNS), TREM2 is expressed primarily in microglia, and shown to play important roles in controlling neuroinflammatory events including phagocytosis and production of pro-inflammatory cytokines. Recently, we have identified apolipoprotein E (apoE), a lipid carrier and the strongest genetic risk factor for LOAD, as a novel, endogenous ligand of TREM2. Interestingly, AD risk-associated TREM2 variants have reduced binding to apoE, which promotes phagocytic uptake of apoptotic neurons by microglia in a TREM2-dependent manner. In this study, we have further characterized the molecular mechanism and functional importance of apoE-TREM2 interaction. Using biochemical approaches, we have defined TREM2-binding domain on apoE. As phospholipids have also been shown to bind to TREM2, we further investigated the biochemical relevance and functional significance of apoE-TREM2 interaction using reconstituted liposomes containing defined amounts of phospholipid, cholesterol, and apoE. Our studies provide molecular insights into the apoE-TREM2 interaction and have implications in designing strategies targeting apoE-TREM2 interaction to treat AD.

Disclosures: Y. Atagi: None. M.M. Painter: None. C. Liu: None. J.D. Fryer: None. G. Bu: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.20/R3

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

Support: K01AG044490

R01ES024233

R01AG037481

R01AG037919

Title: Effects of Liver X receptor agonist treatment on Alzheimer's disease-like phenotype and neuronal regulatory networks in human APOE3 and APOE4-targeted replacement mice

Authors: *N. F. FITZ[1], A. MOUNIER[1], C. W. WOLFE[1], A. Y. CARTER[1], K. NAM[1], J. SCHUG[2], I. LEFTEROV[1], R. KOLDAMOVA[1];
[1]Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA; [2]Functional Genomics Core, Dept. of Genet., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Inheritance of ε4 allele of APOE is the major genetic risk factor for late-onset Alzheimer disease (AD), however, the true biochemical mechanisms underlying this association remain elusive. Epidemiological and clinical studies have suggested a link between cholesterol metabolism and AD pathology. In the CNS, cholesterol and lipoprotein metabolism is important for normal neuronal function as well as for Aβ aggregation and clearance from the brain. The liver X receptors (LXRs) and Retinoid X Receptors (RXR) are transcription factors that control the expression of genes involved in cholesterol and lipoprotein metabolism. One of the major LXR/RXR target genes is ATP binding transporter A1 (ABCA1) that regulates cholesterol efflux and is essential mediator of HDL level in plasma and brain. We have shown that the lack of ABCA1 in APP transgenic mice results in almost complete absence of HDL, diminished ApoE and correlated increase in parenchymal amyloid plaques and oligomeric Aβ. We previously confirmed that RXR agonist treatment resulta in reversal of memory deficits in APP/PS1ΔE9 mice expressing human APOE3 or APOE4 to the levels of their nontransgenic controls and significant increase in Aβ clearance, but no effects on amyloid deposition. Furthermore, we were the first to show RXR agonist treatment affected signaling pathways associated with neurogenesis and neuron projection development and can ameliorated the damaged dendrite complexity and loss of neurites caused by $A\beta_{42}$. Here we wanted to examine the therapeutic potential of a LXR ligand to ameliorate AD phenotype, in APP/PS1ΔE9 mice expressing human APOE3 and APOE4. Furthermore using Abca1 deficient mice expressing APOE3/4 determine if

the therapeutic potential of LXR ligands is mediated through Abca1. All mice were treated for 1 month with T0901317 (20mg/kg mouse; T0) in the normal rodent chow starting at 5 months of age. The mice were compared for behavioral, amyloid deposition, and transcriptional profiles in the brain. Our data demonstrate that T0 is able to reverse behavioral deficits in both APOE isoforms compared to mice fed control diet. There was no significant change in the levels of amyloid deposition. The results of this study will reveal transcriptional changes initiated by LXR activation and how these modifications impact AD pathology. The ability to associate changes at gene expression level, or transcript enrichment within gene networks and clusters, to pathogenic phenotypes of AD will have a significant impact on defining the therapeutic mechanisms of LXR ligands in AD.

Disclosures: N.F. fitz: None. A. Mounier: None. C.W. Wolfe: None. A.Y. Carter: None. K. Nam: None. J. Schug: None. I. Lefterov: None. R. Koldamova: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.21/R4

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

Support: HDH Wills 1965 Charitable Trust 1117747

UK Medical Research Council: G1001354

Oxford Biobank funded by the Oxford BRC

Gordon Edward Small's Charitable Trust Scottish Charity Register SC008962

University of Oxford Clarendon Scholarship

ARUK studentship English Charity Register 1077089

Title: Apolipoprotein E and ageing: How is genetic risk and resilience for Alzheimer's disease represented within the resting brain?

Authors: *S. SURI[1], N. FILIPPINI[1], A. TRACHTENBERG[1], V. HEISE[1], E. ZSOLDOS[1], A. MAHMOOD[1], A. SINGH-MANOUX[2], M. KIVIMAKI[2], K. EBMEIER[1], C. MACKAY[1]; [1]Univ. of Oxford/Department of Psychiatry, Oxford, United Kingdom; [2]Dept. of Epidemiology & Publ. Hlth., Univ. Col. London, London, United Kingdom

Abstract: The apolipoprotein E (*APOE*) gene has three alleles (ε2, ε3, ε4) that differently influence lifetime risk for developing late-onset Alzheimer's disease (AD). *APOE* ε4 is the best-established genetic risk factor for sporadic AD, whereas the ε2 allele may be protective. We (and others) have previously shown that ε4 influences brain function decades before potential cognitive decline, but that its effects on cognition and brain activity may vary with age. Paradoxically, the ε4 allele appears to confer a cognitive advantage in younger ages, and exert its deleterious effects in later life. However, little is known about the equivalent for ε2, which remains largely under-investigated. Studying ε2 would not only complement our current understanding of the complex effects of ε4, but could also lend valuable insights into why ε2 carriers lead relatively long and healthy lives.

Here, we present the first comprehensive analysis of functional connectivity (FC) of the brain in carriers of all three *APOE* alleles. AD patients exhibit disruptions in FC, measured as correlations in spontaneous activity between remote regions within the resting brain. Accordingly, altered resting-state FC, particularly within a network called the "default mode network", has gained attention as a potential biomarker of the neurodegenerative process. We therefore asked whether genetic risk and protection for AD were differently represented within the resting brain across the lifespan, with a particular focus on "default mode" areas.

Fifty-four cognitively healthy younger (20-40 years, 17ε2/19 ε3/18ε4 carriers), 84 middle-aged (40-55 years; 25ε2/24ε3/35ε4 carriers) and 291 older individuals (60-85 years; 36ε2/191ε3/64ε4 carriers) received a 3T resting-state functional MRI scan. *APOE*-related differences were studied using FSL tools. We performed a detailed examination of FC using (1) a seed in the posterior cingulate cortex, which is a key hub of the default mode network, (2) large-scale resting-state networks, and (3) recently developed network modeling techniques that allow for a richer analysis of network connections (FSLNets). We find that like ε4, the effects of ε2 on FC appear to be age-specific. Thus while younger ε2 carriers show significantly lower FC of the posterior cingulate cortex relative to ε3 homozygotes and ε4 carriers, the opposite effect is observed in older individuals ($P<0.05$, corrected for voxel-wise multiple comparisons). We suggest a critical "transition" phase (40-60 years) during which the effects of ε2 and ε4 on FC appear to cross over. This may be a particularly useful age-range for participants of clinical trials targeting preventative treatments.

Disclosures: S. Suri: None. N. Filippini: None. A. Trachtenberg: None. V. Heise: None. E. Zsoldos: None. A. Mahmood: None. A. Singh-Manoux: None. M. Kivimaki: None. K. Ebmeier: None. C. Mackay: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.22/R5

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

Support: NMRC Centre Grant NMRC/CG/013/2013 and NMRC/CG/NUHS/2010

Biomedical Research Council, Singapore BMRC 04/1/36/372

National Medical Research Council, Singapore /CIRG/1390/2014

Duke-NUS Graduate Medical School Signature Research Program funded by Ministry of Health, Singapore

Title: Differential white matter extracellular water increase in APOE e4 carriers and non-carriers among healthy normal, mild cognitive impairment, and Alzheimer's disease

Authors: *F. JI[1], O. PASTERNAK[2], Y. LOKE[1], S. LIU[1], S. HILAL[3], B. TAN[4], N. VENKETASUBRAMANIAN[5], C.-H. CHEN[3], J. ZHOU[1];
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Abstract: Introduction: Alzheimer's disease (AD) is associated with white matter (WM) microstructure abnormalities revealed by diffusion tensor imaging (DTI) method. The apolipoprotein-E epsilon4 (APOE-ε4) allele is a major genetic risk factor for AD, which may also impact WM microstructure such as demyelination and axonal damage. However, the current DTI model suffers from the partial volume effect, therefore preventing accurate quantification of WM abnormalities in AD patients. Free-water (FW) imaging can solve this problem by differentiating the water compartment in the extracellular space from the tissue compartment in a voxel-based manner using DTI data. Here, using FW techniques in subjects with no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD, we hypothesized that APOE-ε4 carriers and non-carriers would have differential trajectories of WM damage along the AD progression.
Methods: 167 subjects (13 NCI carriers, 50 non-carriers; 22 MCI carriers, 35 non-carriers; and 18 AD carriers, 29 non-carriers) underwent DTI scans (Siemens Tim Trio, 3T, 61 diffusion directions at b=1150 s/mm^2 and 7 b0 maps). The DTI data were preprocessed using FSL. FW method was applied to derive the individual FW and tissue compartment maps. To access the group difference in FW and tissue compartments (carriers vs. non-carriers in NCI, MCI and AD),

we carried out voxel-wise statistics on the FW and tissue compartments images using two-sample t-tests. All analyses were controlled for age, gender, handedness, and ethnicity. **Results:** There was no difference in FW and tissue compartments between carriers and non-carriers in AD and NCI group. In MCI group, carriers had higher WM FW mostly at posterior temporal/parietal and occipital regions compared to non-carriers. Tissue compartment did not differ. Moreover, we extracted the mean FW values of these significant regions identified from the MCI group and compared the carriers and non-carriers across the three groups. Differential trajectories of WM FW in carriers and non-carriers across the three groups were observed: in carriers, there was WM FW increase in MCI compared to the NCI; in contrast, in non-carriers, there was WM FW increase in AD compared to MCI stage but not MCI compared to NCI. **Conclusion:** Our findings suggest that APOE genotype influences the FW concentration of WM in MCI. APOE-ε4 carriers may feature an early increase in neuroinflammation, making them more vulnerable to Alzheimer's disease.

Disclosures: F. Ji: None. O. Pasternak: None. Y. Loke: None. S. Liu: None. S. Hilal: None. B. Tan: None. N. Venketasubramanian: None. C. Chen: None. J. Zhou: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.23/R6

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

Support: R01AG051386-01

Title: A03 reverses ApoE4-induced reductions in SirT1 *In vitro*

Authors: J. CAMPAGNA[1], P. SPILMAN[1], D. BAI[1], H. ROLLINS[2], R. DAMOISEAUX[3], J. PHAM[4], T. BILOUSOVA[1], B. JAGODZINSKA[1], M. JUNG[4], D. E. BREDESEN[1], *V. JOHN[5]; [1]Neurol., [3]MSSR, [4]Chem., [2]UCLA, Los Angeles, CA; [5]Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Expression of apolipoprotein ε4 (ApoE4) is the major genetic risk factor for sporadic, late-onset Alzheimer's disease (LOAD). The recent studies of Theendakara et al. (2013, 2015) have shown that one of the contributing factors to increased AD risk is likely reduction expression of the major longevity determinant sirtuin 1 (SirT1) in ApoE4-positive individuals. As patients suffering from AD have lower SirT1 (Kumar et al. 2013), lower prodromal levels may put individuals closer to a threshold for disease development. Thus, therapeutics that can increase SirT1 in the presence of ApoE4 my confer protection against disease development or

may be of therapeutic benefit after Mild Cognitive Impairment (MCI) or AD onset. To identify such a compound, we screened a small clinical compound library using human neuroblastoma A172 cells transiently transfected with ApoE4 and found selective serotonin-reuptake inhibitor alaproclate ("A03") increased SirT1 as determined by AlphaLISA (Perkin-Elmer). A03 was validated using stably ApoE4-transfected N2a cells. In addition, A03 increase trophic amyloid precursor protein (APP) fragment soluble APP alpha (sAPPα). A03 showed good brain-penetance and a good brain:plasma ratio in *in vivo* pharmacokinetics studies comprising oral and subcutaneous (SQ) injection of adult wildtype mice with A03 at 10 mg/kg and euthanasia of mice at 1, 2, 4, 6, and 8 hours post-dosing. A03 levels in brain and plasma were measured at Integrated Analytical Solutions (IAS), and Pk parameters were calculated using PK Solutions software (Summit PK). Pilot *in vivo* efficacy studies were run using ApoE4 targeted replacement (ApoE4 TR) and ApoE4 TR x 5XFAD (APP with 3 mutations, presenilin 1 with two mutations) mice, with dosing by SQ injection at 10 mg/kg/day for 28 days. Levels of SirT1 were determined in frontal and parietal cortex of study mice as it has been reported that SirT1 is lower in frontal cortex of ApoE4 TR mice (Lattanzio et al. 2014). There was a trend toward SirT1 and sAPPα increases in ApoE4 TR mice and no observable side-effects or toxicity. A03 is the first compound reported to increase SirT1 and sAPPα *in vitro* in presence of ApoE4 expression. The trends towards SirT1 and sAPPα enhancement *in vivo* were promising as well as the absence of any apparent toxicity. Future studies will be directed toward identification of additional SirT1 enhancers, as well as to A03 analog development to identify a lead compound with greater *in vivo* efficacy.

Disclosures: J. Campagna: None. P. Spilman: None. D. Bai: None. H. Rollins: None. R. Damoiseaux: None. J. Pham: None. T. Bilousova: None. B. Jagodzinska: None. M. Jung: None. D.E. Bredesen: None. V. John: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.24/R7

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

Support: CIHR Operating Grant MOP-37754

Title: APOE genotype effects on cortical folding of Alzheimer's Disease patients, MCI and healthy people

Authors: *A. FANG[1], R. TORO[2], J. LEWIS[1], A. EVANS[1];
[1]Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]Inst. Pasteur, Paris, France

Abstract: Objectives To examine the relationship between APOE genotype and cortical folding degree. **Methods** Data from the Alzheimer's disease Neuroimaging Initiative (ADNI) was used. A fully automated structural MRI image processing pipeline (CIVET 2.0) was utilized for data analysis of totally 510 AD, late mild cognitive impairment (LMCI), early mild cognitive impairment (EMCI), and age-matched healthy controls (NC) from the longitudinal Alzheimer's Disease Neuroimaging Initiative (ADNI), of whom 198 were homozygous for the APOE ε3 allele, 166 were homozygous for the APOE ε4 allele, while 146 were heterozygous for the APOE ε3 and ε4 alleles. Cortical folding degree analysis was done with software package "Surface Ratio". Statistical analysis was performed with MATLAB software. **Results** In all four groups, the cortical folding degrees of the people with different genotypes decreased with the development of the disease. In NC and EMCI groups, the cortical folding degrees of both APOE ε3 homozygous carriers and APOE ε3ε4 heterozygous carriers were significant larger than APOE ε4 homozygous carriers (All $p<0.01$, Table 1&2). In LMCI and AD groups, the cortical folding degrees of and APOE ε3ε4 heterozygous carriers were significant larger than APOE ε4 homozygous carriers (LMCI: $p<0.01$; AD $p<0.05$, Table 1&2).

	ε3 homozygous	**ε3 ε4 heterozygous**	**ε4 homozygous**
AD	3.86 ± 0.02	3.76 ± 0.06	3.75 ± 0.01
LMCI	3.90 ± 0.02	3.84 ± 0.02	3.81 ± 0.02
EMCI	3.94 ± 0.02	3.92± 0.02	3.91 ± 0.02
NC	3.96 ± 0.02	3.92 ± 0.02	3.85 ± 0.02

Table 1. Cortical folding degrees of different group/genotype

AD ε3 ε3 vs. ε3 ε4	**AD ε3 ε3 vs. ε4 ε4**	**AD ε3 ε4 vs. ε4 ε4**
P=0.05	P=0.75	P<0.05
LMCI ε3 ε3 vs. ε3 ε4	**LMCI ε3 ε3 vs. ε4 ε4**	**LMCI ε3 ε4 vs. ε4 ε4**
P=0.14	P=0.50	P<0.01
EMCI ε3 ε3 vs. ε3 ε4	**EMCI ε3 ε3 vs. ε4 ε4**	**EMCI ε3 ε4 vs. ε4 ε4**
P=0.27	P<0.01	P<0.01
NC ε3 ε3 vs. ε3 ε4	**NC ε3 ε3 vs. ε4 ε4**	**NC ε3 ε4 vs. ε4 ε4**
P=0.12	P<0.01	P<0.01

Table 2. p-values between different group/genotype **Conclusions** The preliminary results suggest an effect of the APOE ε4 allele on cortical folding degrees. APOE genotype may play a critical role in human gyrification indices. APOE ε4 carriers might have less folding in the brain from very early stages of AD, even there is no disease onset. Investigation of the relation between APOE genotype and cortical folding degrees may lead us to predictive biomarkers and drug targets of dementia.

Disclosures: A. Fang: None. R. Toro: None. J. Lewis: None. A. Evans: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.25/R8

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

Title: Sex and ApoE e4 gene dose effects on lateralized hippocampal volumes in older adults with normal cognition, mild cognitive impairment, and Alzheimer's disease

Authors: *Z. HOBEL, B. SHEN, J. PA;
Mark and Mary Stevens Neuroimaging and Informatics, Los Angeles, CA

Abstract: The apolipoprotein E epsilon 4 allele (APOE4) is the major genetic risk factor for late onset Alzheimer's dementia (AD) and is associated with smaller hippocampal volume. A previous study suggests that, in individuals with mild cognitive impairment (MCI), reductions in hippocampal volume may be related to sex and APOE4 gene dose. However, the effect of sex and APOE4 gene dose on lateralized hippocampal volumes in a large cohort has not been examined. We investigated the relationship between sex, APOE4 genotype, and both left and right hippocampal volumes in older adults with normal cognition (NC), MCI, and AD.
T1 MRI data and demographics for NC, MCI and AD subjects were acquired from 4 large, publicly available cohorts (ADNI, AIBL, NACC, and PPMI; n=2464). Hippocampal segmentation and volume measurements were completed using FreeSurfer and visually inspected for accuracy. Hippocampal volumes (HCV) were adjusted for age and total intracranial volume. ANOVA and posthoc t-tests were performed to compare HCV across APOE4 genotypes within each diagnosis group and sex.
Our main finding is that NC subjects showed a lateralized, APOE4-related reduction in HVA which was dose-dependent in women. For NC women, the left HCV was significantly smaller with increasing APOE4 alleles, while the right HCV was only smaller with 2 alleles. NC Men had significantly smaller right HCV only with both 1 and 2 APOE4 alleles when compared to

non-carriers, and did not show a APOE4 dose effect. In subjects with MCI, the APOE4 dose effect was observed in women in both the left and right HCV, while men showed an APOE4 dose effect in only the left HCV. A trend for a dose effect was observed in the left HCV of both male and female AD patients. Regardless of diagnosis or sex, APOE4 carriers had smaller left and right HCV compared to non-carriers. This trend was true for all subjects, although it did not reach significance for the left HCV of NC men.
In the absence of cognitive symptoms, the APOE4 allele appears to have a differential effect on male and female HCV, associated with smaller left hippocampus in women and right in men. In terms of an APOE4 dose effect, NC and MCI women showed this effect in both hippocampi, while NC men showed no dose effect and MCI men only had a dose effect in the left HCV. AD men and women both exhibited the dose effect in left HCV but not right. Taken together, our results suggest that hippocampi of NC and MCI women are more negatively affected by the presence of 2 APOE4 alleles than men. Longitudinal studies of these cohorts are needed to examine the differential impact of sex, APOE4 alleles, and baseline left and right hippocampal volumes on future cognitive function and conversion to dementia.

Disclosures: Z. Hobel: None. B. Shen: None. J. Pa: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.26/R9

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

Support: P01AG03012801

R21AG048498

R21 AG051233-01

NIH R21 AG044682-01A1

Title: Mechanism for APOE4-induced Alzheimer’s disease risk predicts therapeutic targets

Authors: *N. C. COLLINS[1], J. GEORGE[1], S. GHURA[1], A. VALENCIA[1], L. VAN ELDIK[2], M. LADU[1];
[1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Sanders-Brown Ctr. on Aging, Univ. Of Kentucky, Lexington, KY

Abstract: Alzheimer's disease (AD) is the most common form of dementia, representing a significant social and financial burden. Soluble forms of the amyloid-β (Aβ) peptide, including oligomeric Aβ (oAβ) and Aβ42, are likely the proximal neurotoxins in AD. The greatest genetic risk factor for AD is the *APOE4* allele of apolipoprotein E (apoE), which increases risk up to 15-fold compared to the more common *APOE3* allele. Aβ levels are higher with *APOE4* than *APOE3*, evidence that apoE isoforms differentially modulate the aggregation and clearance of Aβ. Our overall hypothesis is that apoE4 is less lipidated than apoE3, leading to lower apoE4/Aβ complex levels and higher soluble Aβ levels, resulting in synaptic loss and eventual dementia. In EFAD mice (transgenic mice that overexpress human Aβ42 and express one of the human apoE isoforms), apoE4 is less lipoprotein-associated than apoE3, based on the reduced levels of apoE4 extracted in the lipid-soluble extraction fraction. In addition, apoE/Aβ complex levels are lower and soluble Aβ levels are higher in E4FAD mice compared to E3FAD mice. Using human brain samples from our collaboration with the University of KY, the goal of this study is to validate these observations in control and AD samples with genotypes *APOE3/3, APOE3/4,* or *APOE4/4.* Analysis demonstrated that lipoprotein-associated apoE is significantly lower in AD patients with the *APOE4/4* genotype vs. *APOE3/3* AD patients and all genotypes in the control cohort. Soluble Aβ (oAβ and Aβ42) levels are higher in the AD cohort compared to the control cohort, and are highest in AD patients with an *APOE4/4* genotype. Finally, apoE/Aβ complex levels are higher in both control and AD subjects with *APOE3/3* than complex levels in both control and AD subjects with *APOE4/4*. These results are consistent with the hypothesis that *APOE4* causes a reduction in apoE lipidation and apoE/Aβ complex, resulting in inefficient clearance of soluble Aβ, synaptic loss, and dementia. Together, these results suggest apoE lipidation as a potential therapeutic target for *APOE4*-induced AD risk.

Disclosures: N.C. Collins: None. J. George: None. S. Ghura: None. A. Valencia: None. L. Van Eldik: None. M. LaDu: None.

Poster

225. Alzheimer's Disease: Apolipoproteins

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 225.27/R10

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

Support: ADRC

Title: Apolipoprotein E4 drives amyloid pathology in an inducible mouse model - Effects at different stages of amyloid pathology

Authors: *C.-C. LIU, N. ZHAO, N. WANG, J. ROGER, C. LINARES, C.-W. TSAI, G. BU; Neurosci. Res. Dept., Mayo Clin., Jacksonville, FL

Abstract: Accumulation of amyloid-β (Aβ) peptide in the brain is a critical and perhaps initiating event in the pathogenesis of Alzheimer's disease (AD). Aβ accumulation leads to the formation of Aβ aggregates which disrupt synaptic functions and may lead to eventual neurodegeneration. Among the three polymorphic alleles, the ε4 allele of the apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for sporadic AD. In addition to increasing the prevalence of AD, the presence of *APOE4* also lowers the age of onset. Although there is strong evidence from both humans and animal models that apoE4 enhances amyloid pathology, the critical period when apoE4 has the strongest effects on amyloid pathology is not clear. We have developed cell type-specific, inducible mouse models in which the expression of apoE isoforms can be turned on or off in a cell type-specific manner. Using these models, we found that overexpression of apoE4 in astrocytes during the initial seeding stage as oppose to the rapid growth period is critical for its effect on accelerating amyloid and promoting astrogliosis and microgliosis in the background of $APP_{SWE}/PS1_{\Delta E9}$ amyloid model mice. By measuring Aβ half-life using *in vivo* microdialysis, we found that apoE4 inhibits Aβ clearance. Overexpression of apoE3 did not affect the amyloid plaque load or Aβ clearance, but did enhanced synaptic plasticity measured by long-term potentiation. Together, our results define a critical role of apoE4 in driving amyloid pathology during the initial seeding period, likely by inhibiting Aβ clearance and promoting Aβ aggregation. Our findings provide novel mechanistic insights into differential functions of apoE isoforms in amyloid pathogenesis and have implications for designing mechanism-based, apoE-targeted AD therapy.

Disclosures: C. Liu: None. N. Zhao: None. N. Wang: None. J. Roger: None. C. Linares: None. C. Tsai: None. G. Bu: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.01/R11

Topic: C.04. Movement Disorders

Title: Alterations in the density of neurotransmitter receptors in the brain of the zQ175KI mouse model of Huntington's disease

Authors: T. HUHTALA[1], J. RYTKÖNEN[1], *T. PARKKARI[1], P. J. SWEENEY[1], O. KONTKANEN[1], L. C. PARK[2], L. MRZLJAK[2];
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Abstract: Huntington's disease (HD) is a neurodegenerative genetic disorder that affects brain functions, muscle coordination and leads to cognitive decline and psychiatric problems. In HD patients, changes in expression of receptors for neurotransmitters, such as glutamate, dopamine, GABA, acetylcholine and adenosine, have been reported. In order to determine if similar changes can be also detected in the zQ175KI knock-in mouse model of HD, we applied autoradiography to compare receptor densities of dopamine 1 (D1R), dopamine 2 (D2R), cannabinoid 1 (CB1) and gamma-aminobutyric acid (GABA) receptors in the brain of wild-type (WT) and heterozygous (HET) mutant male mice at the age of 6, 9, 12 and 15 months. For receptor density analysis, coronal brain sections were prepared and Bmax values of corresponding tritiated ligands to D2R (^{3}H-raclopride), D1R (^{3}H-SCH-23390), CB1 (^{3}H-MePPEP) and $GABA_A$ (^{3}H-flumazenil) receptors were measured. Specific binding of each ligand in the globus pallidus, substantia nigra, striatum, somatomotor cortex and cingulate cortex was calculated as the difference between total and non-specific binding. Bmax values were determined using non-linear regression analysis. We observed significantly ($P < 0.05$) lower densities of D2R and D1R in the striatum of HET mice compared to values detected in WT. Furthermore, the density of CB1 receptors in the globus pallidus, substantia nigra and striatum was also lower in HET mice. In contrast, a significant increase in the $GABA_A$ receptor density was noted in the globus pallidus, substantia nigra, striatum and cingulate cortex of HET mice. We conclude that alterations in the density of several neurotransmitter receptors in the brain of the zQ175KI mouse HD model largely replicate clinical findings in humans.

Disclosures: T. Huhtala: None. J. Rytkönen: None. T. Parkkari: None. P.J. Sweeney: None. O. Kontkanen: None. L.C. Park: None. L. Mrzljak: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.02/R12

Topic: C.04. Movement Disorders

Title: Changes in cerebral blood flow in conscious zQ175KI Huntington’s disease mouse model mice

Authors: *T. HUHTALA[1], J. RYTKÖNEN[1], L. TOLPPANEN[1], P. J. SWEENEY[1], T. PARKKARI[1], O. KONTKANEN[1], L. C. PARK[2], L. MRZLJAK[2];
[1]Charles River Discovery, Kuopio, Finland; [2]CHDI Management/CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease (HD) is a neurodegenerative genetic disorder that affects brain functions, muscle coordination and leads to cognitive decline and psychiatric problems. Glucose

metabolism and cerebral blood flow (CBF) are useful correlates of brain functions, as changes in these parameters are often associated with clinical symptoms in neurodegenerative disorders. In HD patients, cerebral artery flow is significantly lower than in control subjects. In order to determine if similar changes can be also detected in a mouse HD model, we used autoradiography to compare brain perfusion in conscious zQ17KI heterozygote (HET) and wild-type (WT) male mice at the age of 9 and 12 months. One of the major challenges to study neurological function in rodents is the necessity to use restraint, anesthesia or paralyzing agents, all of which impact neuronal function. Delivery of a radiotracer that reaches cerebral equilibrium within a short time frame may allow imaging of an immediate neuronal effect with great temporal resolution. One of the most applicable tracers in this regard is ^{14}C-iodoantipyrine, whose concentration is strictly linearly dependent on tissue radioactivity and CBF when data are captured within a brief interval (~10 s) after the tracer injection. Therefore, in this study, we applied ^{14}C-iodoantipyrine as a radiotracer to study CBF in conscious WT and HET mice. To be able to capture CBF of conscious animals, mice were cannulated into the jugular vein. After the mice had recovered from the surgery, they were connected to a tether, and the radiotracer was injected followed by immediate dosing of the terminal solution. Brains were collected, sectioned in the coronal plane at a 100-µm interval and radioactivity was quantified by autoradiography. We observed that in the 9-month-old zQ175KI HET mouse brains, a lower amount (by 3-25 %) of the perfusion tracer was detected in all studied regions (cortex, striatum, hippocampus and cerebellum), compared to the levels determined in WT mice. In the subsequent study, a similar comparison will be carried out between the brains of 12-month-old zQ175KI HET and WT mice to understand how disease progression affects CSF in this animal model.

Disclosures: T. Huhtala: None. J. Rytkönen: None. L. Tolppanen: None. P.J. Sweeney: None. T. Parkkari: None. O. Kontkanen: None. L.C. Park: None. L. Mrzljak: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.03/R13

Topic: C.04. Movement Disorders

Title: The branched DNA methodology and analysis of dysregulated expression of Huntington's disease related genes of interest.

Authors: M. VIHMA[1], S. MIETTINEN[1], *J. KURKIPURO[1], T. PARKKARI[1], M. CERRADA-GIMENEZ[1], A. NURMI[1], D. LAVERY[2], L. C. PARK[2];
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Abstract: Huntington's disease (HD) is a fatal neurodegenerative genetic disorder characterized by movement and psychiatric impairment as well as cognitive decline. The genetic background of HD contains the abnormal expansion of the glutamine repeats in the huntingtin gene (HTT) in addition to dysregulated expression profiles of several other key genes, such as dopamine receptors, cannabinoid receptor, dopamine and cAMP-regulated phosphoprotein and phosphodiesterase 10A (PDE10A). Branched DNA (bDNA) signal amplification technology enables the multiplex measurement of 3-80 target genes directly from tissue homogenates or from RNA samples with unparalleled accuracy and precision. In this study, the bDNA methodology was used to analyze the cortex and striatum homogenate samples from two separate animal models of HD; 6 and 12 weeks old wild-type and transgenic R6/2 mice as well as 3, 6, 9, 11 and 16 months old wild-type and heterozygote zQ175 knock-in (HET zQ175 KI) mice. The expression levels of several typical target genes related to HD were analyzed and compared to standard qPCR methodology in terms of robustness and data variability. The use of bDNA technology showed statistically significant ($p < 0.05$) changes in the expression levels of several HD key genes in the HET zQ175KI male mice when compared to the WT mice. For example, PDE10a, Drd2 and Cnr1 expression levels in 9 mo HET zQ175 KI mouse brains are expressed at 61 %, 67 % and 32 %, respectively, compared to those in aged-matched WT. Furthermore, the data from bDNA methodology was similar to data from qPCR but showed less variation even when using small number of samples. The current study demonstrates the value of the bDNA methodology as a useful tool for the assessment of gene expression changes in genetically modified animals.

Disclosures: M. Vihma: None. S. Miettinen: None. J. Kurkipuro: None. T. Parkkari: None. M. Cerrada-Gimenez: None. A. Nurmi: None. D. Lavery: None. L.C. Park: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.04/R14

Topic: C.04. Movement Disorders

Support: The 2014 Princess Royal Tenovus Scotland Medical Research Scholarship

Title: Episodic memory and cognitive flexibility in the Hdh^{Q111} mouse model of Huntington's disease

Authors: *A. MARIANO[1], O. MONTEIRO[1], E. MOSS[1], C. BRAZAITIS[1], V. BROWN[2], J. LAMBERT[1], R. LANGSTON[1];

[1]Div. of Neurosci., Univ. of Dundee, Dundee, United Kingdom; [2]Sch. of Psychology and Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Huntington's disease (HD) is a progressive, fatal neurodegenerative disorder caused by an unstable trinucleotide (CAG) repeat expansion within the Huntingtin gene, which initiates a chain of downstream toxic events affecting brain function. Although HD is mainly known as a motor disease and is usually associated with movement dysfunctions, recent finding have provided evidence that cognitive deficits can appear up to 15 years before the motor symptoms, during the prodromal phase of the disease. Our mouse model of HD, the heterozygous Hdh^{Q111} mouse ($Hdh^{Q111+/-}$), accurately recapitulates the protracted prodromal disease phase and provides a window of time during which the cognitive phenotype of this mouse can be characterized. Using a battery of memory tests that rely upon spontaneous novelty detection in mice, we have shown substantive deficits in hippocampus-dependent episodic-like associative memory by 10-12 weeks of age in the $Hdh^{Q111+/-}$ mouse. However, using the Morris water maze paradigm, we do not observe any impairment in hippocampal-dependent spatial learning or long-term memory at the same age (3 months) or even by 10 months of age. When presented with a serial reversal learning task, 10-month old $Hdh^{Q111+/-}$ mice showed mild deficits in memory flexibility compared to wild type littermates. *When trained to criterion, in fact,* $Hdh^{Q111+/-}$ mice take significantly longer to learn a new platform location. It has previously been shown that water maze training is associated with a mild stress reaction and increased corticosterone levels that might affect learning performance of the mice. Therefore, detection of corticosterone levels in blood samples is underway to determine whether a difference in the stress response between tasks and/or between genotypes could be the reason for the apparent discrepancy in hippocampal learning and memory performance in these different behavioural conditions.

Disclosures: A. Mariano: None. O. Monteiro: None. E. Moss: None. C. Brazaitis: None. V. Brown: None. J. Lambert: None. R. Langston: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.05/R15

Topic: C.04. Movement Disorders

Support: CIHR FDN 143210

Title: Adaptation of a home-cage motor learning task to assess behaviour in the YAC128 model of Huntington's disease

Authors: C. L. WOODARD, F. BOLAÑOS, J. D. BOYD, G. SILASI, T. H. MURPHY, *L. A. RAYMOND;
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Abstract: Behavioural testing of disease models in rodents can be useful in assessing the extent of cognitive and physical impairment, as well as evaluating the efficacy of potential interventions. However, many traditional behavioural paradigms involve testing the animal for short periods in stressful or unfamiliar environments, often during times of the day when they would typically be sleeping, and this can introduce significant variability to the data. To address this problem, we have adapted a new paradigm to identify behavioural phenotypes in models of chronic, progressive neurodegenerative disease. This paradigm involves the incorporation of a lever pull task into the mouse home-cage, allowing continuous access to the task in a naturalistic environment. Task difficulty can be changed dynamically based on performance by requiring the mouse to hold the lever in a more narrow range, or for a longer period, to receive a water reward. Additionally, kinematic analysis of lever movement can be used to assess motor control and persistence during task performance. Video and lever pulling data is collected automatically, allowing for minimal human interaction and continuous assessment of multiple animals over long periods. We have used this paradigm to investigate behavioural phenotypes in the YAC128 mouse model of Huntington's disease at 2 months (presymptomatic), 4 months (early symptomatic) and 6 months (symptomatic) of age. Specifically, we have obtained measures of motor learning and control, as well as cognitive flexibility and memory, in order to further define the deficits seen in these animals. We hope that this will prove to be an effective tool, more generally, for increasing the sensitivity and automation of cognitive and motor assessments in models of neurodegenerative disease. *Funded by the Canadian Institutes for Health Research.*

Disclosures: C.L. Woodard: None. F. Bolaños: None. J.D. Boyd: None. G. Silasi: None. T.H. Murphy: None. L.A. Raymond: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.06/R16

Topic: C.04. Movement Disorders

Title: Altered dopamine receptor 2 signaling in the zQ175 mouse model of Huntington's disease.

Authors: *H. B. JANSSENS[1], A. RASSOULPOUR[2], I. MUNOZ-SANJUAN[3], L. MRZLJAK[3], R. CACHOPE[3];

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Abstract: Huntington's disease (HD) is a genetically inherited neurodegenerative disorder characterized by severe motor dysfunction, cognitive decline and psychiatric disturbances, associated with profound striatal and cortical loss. Altered dopaminergic transmission is thought to play a key role in HD pathophysiology, and has been reported in HD patients as well as in rapidly-progressing rodent models of HD. To enhance our understanding of such differences, we aimed to characterize dopamine dynamics in the slower-progressing knock-in zQ175 mouse model of HD.
Electrically-evoked striatal dopamine release from anesthetized 7 month old heterozygous zQ175 and WT mice was detected by fast-scan cyclic voltammetry. Oxidative currents were measured in the ventral striatum while increasing intensities of electrical stimulation were delivered to the VTA/SNc after vehicle and after raclopride (D2 antagonist; 1 mg/kg) i.p. injection. Dopamine levels detected from WT and Q175 mice after vehicle treatment were not significantly different. However, raclopride-evoked enhancement of phasic dopamine release was attenuated in Q175 animals as compared with WT. There was no difference in the rate of reuptake between genotypes as measured by Tau75. These results indicate presynaptic D2 receptor dysfunction in Q175 mice, and suggest that similar functional alterations may exist at the postsynaptic level, impairing striato-pallidal indirect pathway transmission.

Disclosures: H.B. Janssens: None. A. Rassoulpour: None. I. Munoz-Sanjuan: None. L. Mrzljak: None. R. Cachope: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.07/R17

Topic: C.04. Movement Disorders

Support: Huntington Society of Canada

Research Center on Aging

Canada Research Chair

Title: Early olfactory behavior deficits associated with olfactory bulb atrophy and caspase-8 activation in HD rodent models

Authors: *R. K. GRAHAM[1], M. LAROCHE[1], M. LESSARD-BEAUDOIN[1], M. GARCIA-MIRALLES[2], C. KREIDY[2], L. YU-TAGER[3], M. R. HAYDEN[4], H. NGUYEN[3], M. A. POULADI[2];
[1]Dept. de Pharmacologie et Physiologie, Univ. of Sherbrooke, Sherbrooke, QC, Canada; [2]Med., Natl. Univ. of Singapore, Singapore, Singapore; [3]Med. Genet., Univ. of Tuegingen, Tuegingen, Germany; [4]Med. Genet., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Olfactory dysfunction and altered neurogenesis are observed in several neurodegenerative disorders including Huntington disease (HD). These deficits are an early symptom and correlate with decline in cognitive performance, depression and degeneration of olfactory regions in the brain. Despite clear evidence demonstrating olfactory dysfunction in HD patients, only limited details are available in murine models and the underlying mechanisms are unknown. We have previously shown decreased olfactory cortical volume in the YAC128 HD model. We now examined odor investigation behaviors and show that at 1 and 3 months YAC128 mice do not habituate to the odor as quickly in trial 2 ($p<0.05$), and at 5 months the YAC128 mice investigate trial 1 odors longer than WT littermates ($p<0.05$). Initial data also show an increase in GFAP and Iba-1 in the olfactory bulb (OB) in post-symptomatic YAC128 compared to WT. Furthermore, an increase in the active form of caspase-6 and caspase-8 are observed at 3 months in YAC128 OB vs WT. We also assessed the rat BACHD model. In the BACHD model, early increases in NeuN and atrophy of the OB (ANOVA $p=0.02$) are observed. In addition, an increase in caspase-8 (ANOVA $p=0.02$) expression at 6 months is observed vs. WT. Supporting this, we detect an increase in casp8 activation in the OB at 3 months of age. Identification of early markers for HD will help inform therapeutic approaches and will clarify the utility of olfactory function tests in at risk HD individuals.

Disclosures: R.K. Graham: None. M. Laroche: None. M. Lessard-Beaudoin: None. M. Garcia-Miralles: None. C. Kreidy: None. L. Yu-Tager: None. M.R. Hayden: None. H. Nguyen: None. M.A. Pouladi: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.08/S1

Topic: C.04. Movement Disorders

Support: CHDI Grant A-7601

Title: Longitudinal assessment of cortico-striatal projections in the zQ175 mouse model of Huntington's Disease

Authors: *N. FOSTER, H. HINTIRYAN, M. BECERRA, I. BOWMAN, K. COTTER, M. BAY, M. ZHU, M. S. BIENKOWSKI, M. Y. SONG, L. GOU, S. YAMASHITA, H. DONG; USC, Los Angeles, CA

Abstract: The zQ175 mouse is a recently developed knock-in model of the neurodegenerative, genetically based Huntington's Disease (HD). The heterozygous zQ175 mouse closely mimics the mutant genotype in most human patients with HD and thus has good genetic validity. In humans with HD and other mouse models of the disease, both cortex and striatum are severely affected. One possible mechanism underlying the dysfunction seen in HD is degeneration of axonal projections between brain regions, or connectopathy. We endeavored to assess whether connectopathic changes were occurring in the cortico-striatal axonal connections in these mice. Injections of anterograde axonal tracers were made into the primary motor cortex, secondary motor cortex, and medial prefrontal cortex in groups of zQ175 and their wild type littermates. Axonal terminations that were labeled by these injections were quantified in the dorsal striatum and compared between groups. Additionally, groups of zQ175 and wild type mice at 2-, 6-, and 12-months of age were used to observe the development of any connectopathic changes occurring during progression of the Huntington's phenotype.

Disclosures: N. Foster: None. H. Hintiryan: None. M. Becerra: None. I. Bowman: None. K. Cotter: None. M. Bay: None. M. Zhu: None. M.S. Bienkowski: None. M.Y. Song: None. L. Gou: None. S. Yamashita: None. H. Dong: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.09/S2

Topic: C.04. Movement Disorders

Support: CHDIF

The Methodist Hospitals Endowed Professorship in Neuroscience

Title: Basal ganglia pathology in heterozygous 18 month-old Q175 knock-in Huntington's disease mice

Authors: *Y. DENG, H. WANG, M. JONI, A. REINER;
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Progressive basal ganglia neurodegeneration is a well-known feature of Huntington's disease (HD). The Q175 knock-in mouse is a slowly progressing HD model, but its basal ganglia

pathology is not well characterized. Using stereology, quantitative immunohistochemistry, and in situ hybridization histochemistry, we examined striatal projection neuron, striatal interneuron, and striatal projection system pathology, as well as pallidal, nigral and subthalamic nucleus pathology in 18 month-old heterozygous Q175 mice. We found that although total striatal neuron numbers and striatal volumes revealed by stereology were not significantly changed in 18 month-old Q175 mice, DARPP32-positive striatal projection neurons were significantly reduced in abundance by 40%. Enkephalin (ENK) message in indirect pathway striatal projection neurons (iSPNs) was also significantly decreased, but substance P (SP) message in direct pathway striatal projection neurons (dSPNs) was unchanged, suggesting the DARPP32 perikaryal reduction was preferential for ENK iSPNs. Consistent with this, we found reduction in DARPP32 in the striatal terminals in globus pallidus externus (GPe) but not in striatal terminals in globus pallidus internus (GPi) or substantia nigra pars reticulata (SNr). Despite the reduced ENK message and unaltered SP message in striatum, ENK was increased in striato-GPe terminals and SP was increased in striatal terminals in GPi and SN, likely reflecting striatal neuron dysfunction and decreased neurotransmitter release in striatal target areas. Of note, the DARPP32 reductions in striatal neuron abundance and striato-GPe fibers, and ENK and SP retention in terminals in striatal target areas were all correlated with motor abnormalities detected by rotarod testing and open field testing. The abundance of striatal parvalbuminergic (PARV), cholinergic and NOS-containing interneurons were not changed in 18 month-old Q175 mice, nor were PARV neuron numbers in GPe, GPi and SNr. Neuron abundance in the subthalamic nucleus and dopaminergic neuron abundance in substantia nigra pars compacta (SNc) were also unaltered. Our results suggested that Q175 knock-in mice resembles a very early stage of adult onset human HD and could be useful for testing HD therapeutics.

Disclosures: Y. Deng: None. H. Wang: None. M. Joni: None. A. Reiner: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.10/S3

Topic: C.04. Movement Disorders

Support: NIH Grant DA026430

NIH Grant MH104450

Title: HdhQ200/200 mouse model displays rapid Huntington’s Disease-like behavior and pathology

Authors: *J. K. CAO, K. SWINNEY, L. ZWEIFEL, N. STELLA;
Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Huntington's Disease (HD) is a devastating inherited autosomal dominant neurodegenerative disease with no known cure and few palliative treatment options available. Characterized by progressive deterioration of motor and cognitive functions, the average survival time is 15-25 years after disease onset and end-stage patients are profoundly demented and debilitated. The HdhQ mouse model was developed by inserting pathogenic-sized CAG repeats into the *Hdh* gene, the mouse homolog of the HD gene. An allelic series of the HdhQ mouse model has been generated with 200 CAG repeats in length that recapitulate critical components of HD pathogenesis. Here we characterized in further detail several behavioral and pathological impairments that are reliably measured in the homozygous HdhQ200/200 model. Longitudinal studies of behavioral phenotypes and histology revealed disease onset as early as 6 months of age and end stage at 12 months. Female and male HdhQ200/200 animals showed deficits in weight, grip strength, motor coordination, and circadian activity by 8 months of age. Analysis of pathology by semi-quantitative immunohistochemistry at 12 months of age revealed reduced DARPP-32 and CB_1R expression. There was also indication of increased mutant huntingtin aggregates in the striatum of these mutants. Using *in vivo* calcium imaging with GCaMP calcium indicator technologies, we found pronounced alterations in activity-dependent calcium dynamics in medium spiny neurons of 10-month-old HdhQ200/200 mice. Previous work suggests that chronic cannabinoid treatments may alleviate behavioral and pathological deficits seen in other HD models. Our next set of experiments is designed to target the endocannabinoid signaling system and test its potential therapeutic effect in this model. Our study will disclose detailed behavioral and pathological changes in the HdhQ200/200 mouse line and whether cannabinoid treatments alleviate these changes. Supported by NIH (DA026430 to NS and MH104450 to LZ).

Disclosures: J.K. Cao: None. K. Swinney: None. L. Zweifel: None. N. Stella: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.11/S4

Topic: C.04. Movement Disorders

Support: CHDI grant to GM

Title: Axonal injury is protein kinase JNK3 dependent in a murine model of Huntington's disease.

Authors: *R. G. GATTO[1], M. KANG[1], Y. CHU[2], H. FRIEDECK[1], B. KIM[1], J. H. KORDOWER[2], S. T. BRADY[1], G. A. MORFINI[1];
[1]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Dept. of Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Huntington's disease (HD) is an autosomal dominant disease resulting from expansion of a polyglutamine tract in mutant huntingtin (*mhtt*). Our previous work showed that mhtt promotes deficits in axonal transport, a cellular process critical for the appropriate maintenance of axonal connectivity, further demonstrating a role of the protein kinase JNK3 and its toxic effect. These findings prompted us to assess the contribution of JNK3 to mHtt-induced pathology in-vivo by analyzing R6/2 mice featuring genetic ablation of JNK3. Collectively, results from these studies demonstrate a major role of JNK3 on the axonal pathology induced by *mhtt in vivo*.

Disclosures: R.G. Gatto: None. M. Kang: None. Y. Chu: None. H. Friedeck: None. B. Kim: None. J.H. Kordower: None. S.T. Brady: None. G.A. Morfini: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.12/S5

Topic: C.04. Movement Disorders

Title: Investigation of corticostriatal synaptic transmission and cerebellar Purkinje neuron firing in the R6/2 mouse model of Huntington's disease by using multi-electrode arrays

Authors: *M. KOPANITSA, O. KONTKANEN, A. NURMI, P. SWEENEY;
Charles River Discovery, Kuopio, Finland

Abstract: Many clinical manifestations of Huntington's disease are likely caused by degeneration of striatal medium-sized spiny neurons (MSNs). It has been suggested that certain behavioral disturbances in individuals with Huntington's disease (HD) may arise due to derangement of synaptic transmission in the striatum that occurs prior to pronounced neuronal loss. For this reason, corticostriatal synaptic transmission in genetic mouse models of HD has been a subject of intense investigation. Changes such as increased membrane input resistance and a reduction in membrane capacitance of MSNs as well as presynaptic phenotypes have been noted in several HD mouse models. We sought to determine if electrophysiological phenotypes in the striatum can be also detected by measuring field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of corticostriatal pathways with multi-electrode arrays (MEAs) in horizontal brain slices of 8-week old R6/2 male mice. We found that while input-output

relationships and LTP induced by theta-burst stimulation were similar in slices from R6/2 and WT mice, paired-pulse facilitation (PPF) at an interpulse interval of 100 ms was higher in mutants (127.5 ± 4.5) than in WTs (112.8 ± 2.6; $P = 0.005$, Mann-Whitney test). This observation is in line with previous reports of increased PPF in genetically altered mice bearing several HD-related mutations (Milnerwood and Raymond, 2007). There have been some disagreement as to whether motor symptoms in HD may be also caused by dysfunction of the cerebellum. Some evidence of impaired Purkinje neuron functions in R6/2 mice have been reported (Dougherty et al., 2012). Using MEA-based recordings, we examined tonic spontaneous discharges of individual Purkinje neurons in parasagittal slices from 8-week old R6/2 and WT mice. We found that Purkinje neurons from mutant mice exhibited a trend toward a lower firing rate and had a significantly higher coefficient of variation of the interspike interval (R6/2: 0.31 ± 0.02; WT: 0.18 ± 0.01; $P = 0.016$, 3-level nested ANOVA). We conclude that MEA-based approach provides a convenient, easy-to-multiplex setting for detecting relevant electrophysiological phenotypes in brain slices from mouse models of HD. References: Milnerwood and Raymond (2007) J Physiol 585(Pt 3): 817-31. Dougherty et al. (2012) Exp Neurol 236(1): 171-178.

Disclosures: M. Kopanitsa: None. O. Kontkanen: None. A. Nurmi: None. P. Sweeney: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.13/S6

Topic: C.04. Movement Disorders

Support: NIH Grant NS41574

Title: Direct and indirect striatal output pathways differentially affect their targets in mouse models of Huntington’s Disease

Authors: *J. BARRY, C. CEPEDA, M. S. LEVINE;
IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington’s disease (HD) is a hereditary neurological disorder characterized by chorea, cognitive deficits and psychiatric symptoms. As HD progresses there is a massive loss of medium-sized spiny neurons (MSNs) in the human striatum. MSNs expressing dopamine (DA) D1 receptors project to the substantia nigra (SN) (direct pathway), whereas MSNs expressing DA D2 receptors project to the external segment of the globus pallidus (GPe) (indirect pathway). It is believed that MSNs of the indirect pathway are affected earlier than MSNs of the direct

pathway. To examine how these two pathways are altered in HD, channelrhodopsin (ChR2) was injected into the striatum of symptomatic R6/2 (2-3 month) and symptomatic (12 month) YAC128 mice or their wildtype (WT) littermates, which were crossed to either D1- or D2-Cre expressing mice. These mice then express ChR2 in either the direct (D1-Cre) or indirect (D2-Cre) striatal output pathways. Recording in brain slices, we examined spontaneous excitatory (sEPSC) and inhibitory (sIPSC) postsynaptic currents, and IPSCs evoked by optogenetic stimulation of direct or indirect pathway terminals in either the SN or the GPe to determine alterations in the neurons of output structures. sIPSC frequency was reduced in the SNr of both R6/2 and 12 month-old YAC128 D1-Cre mice, while sIPSC amplitude was significantly decreased in R6/2 mice only. Optogenetically-evoked IPSCs by activation of direct pathway terminals showed a significant decrease in amplitude and charge in R6/2 and 12 month-old YAC128, while the kinetics (half-amplitude duration, rise and decay times) were not significantly altered. In GPe neurons, sIPSC frequency was decreased in R6/2 mice while sIPSC kinetics were unchanged in both R6/2 and YAC128 mice. Evoked IPSC responses to optogenetic stimulation of indirect pathway terminals displayed a significant increase in decay time in both R6/2 and 12 month YAC128 neurons. In contrast, sEPSC frequency and kinetics of both SNr neurons and GPe neurons were unchanged in both mouse models. In summary, the decrease in evoked GABAergic response amplitude in the SN (direct pathway) may be due to reduced inputs from MSNs, whereas the increase in GABA response decay time and charge in the GPe may be due to changes in GABA reuptake or postsynaptic receptor subunit composition. In conclusion, the direct and indirect pathways are differentially affected and these differences are consistent across two HD models.

Disclosures: J. Barry: None. C. Cepeda: None. M.S. Levine: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.14/S7

Topic: C.04. Movement Disorders

Support: CHDI Foundation, Inc. A-8462

NIH NS41574

Title: Altered function of Parvalbumin-expressing interneurons in mouse models of Huntington’s disease

Authors: *L. GALVAN, S. M. HOLLEY, C. CEPEDA, M. S. LEVINE;
IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD), a neurodegenerative disorder caused by a mutation in the HD gene, is characterized by dysfunction and ultimate degeneration of striatal medium-sized spiny neurons (MSNs). Although it was thought that interneurons were relatively spared in HD, recent studies have shown significant alterations in parvalbumin (PV)-expressing interneurons. These interneurons display fast-firing properties and mediate feed-forward inhibition in the striatum. Previously we showed using optogenetics to specifically activate PV-expressing interneurons that alterations in GABAergic input from PV-expressing interneurons may contribute to MSN dysfunction in HD. Here we used a similar optogenetic paradigm to further examine alterations in PV-expressing interneuron inputs to MSNs in the R6/2 and the Q175 models. We examined whether PV-expressing interneuron responses in R6/2 mice were differentially affected in direct versus indirect pathway MSNs. R6/2 mice were crossed with PV-Cre mice and D1 tomato mice to identify direct and indirect pathway MSNs. The data show that PV-expressing interneurons induced a larger GABAergic response in direct compared to indirect pathway wildtype (WT) MSNs. In symptomatic R6/2 mice, direct pathway MSNs displayed a smaller response compared to the WT direct pathway response. Q175 mice were crossed with PV-Cre mice to examine the progression of response alterations in MSNs at 2 (presymptomatic) and 8 (beginning of symptomatic stage) months. In 2 month Q175 mice, there were no differences between genotypes. In contrast, in 8 month Q175 mice, activation of striatal PV-expressing interneurons evoked significantly smaller MSN GABAergic responses with a faster decay time than in WTs. We also compared the effects of activation of endocannabinoid receptor 1 (CB1) in the two models. The CB1 agonist, WIN 55,212-2 decreased PV-induced responses in both Q175 and WT MSNs but not in the symptomatic R6/2 MSNs as previously described. Together, these findings suggest that alterations in function of PV-expressing interneurons differ in the R6/2 and Q175 mice but will contribute to neuronal microcircuit alterations in the striatum.

Disclosures: L. Galvan: None. S.M. Holley: None. C. Cepeda: None. M.S. Levine: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.15/S8

Topic: C.04. Movement Disorders

Support: Supported by CHDI A-8348

NIH NS41574.

Title: Impaired functional dynamics of motor cortex microcircuits in mouse models of Huntington's disease

Authors: *A. M. ESTRADA SANCHEZ[1], E. DONZIS[1], T. INDERSMITTEN[1], C. TRAN[1], C. WANG[1], D. CASTRO[1], M. NEDJAT-HAIEM[2], C. CEPEDA[1], P. GOLSHANI[2], M. S. LEVINE[1];
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Abstract: Huntington's disease (HD) is a neurological disorder characterized by motor and cognitive disturbances. Most research on neuronal dysfunction in HD models focuses on the striatum, but it has become clear that the cortex is also affected. Therefore, it is critical to understand functional changes to cortical circuits in HD models. Here, we used the symptomatic R6/2 (2-3 mo) and Q175 (>12 mo) mouse models to examine network changes in pyramidal neurons and interneurons in motor cortex using 2-photon Ca^{2+} imaging. During recordings, the mouse is head-restrained but the body is able to freely move on a spherical treadmill providing comparisons of network activity during periods of locomotion and rest. When measuring activity of individual neurons, we found a shift towards lower amplitude Ca^{2+} transients but higher frequencies in both R6/2 and Q175 mice compared to wildtype littermates (WTs). While locomotion did not affect amplitudes and frequencies, the proportion of Ca^{2+} transients near the initiation of motion was altered. In the R6/2 model, WTs displayed an increase in proportion of Ca^{2+} transients occurring immediately before locomotion initiation but a similar increase occurred immediately after locomotion initiation in R6/2 mice. In the Q175 model, the proportion of Ca^{2+} transients decreased at the initiation of locomotion compared to WTs. To examine network changes we measured significant pairs (determined using a Monte Carlo simulation) of intercorrelations of neurons. Both R6/2 and Q175 had a greater proportion of significantly correlated neuron pairs compared to their respective WTs. Differences between the R6/2 and Q175 model emerged when separating network activity into locomotion and rest periods. In the R6/2 model, the average correlation values decreased during periods of locomotion compared to WTs and increased during rest. In the Q175 model, the average correlation increased compared to WTs regardless of locomotion. This divergence may be due to differences in the amount of time the HD models spent in motion. R6/2 mice spent significantly more time in motion and Q175 mice spent significantly less compared to their respective WTs. Differentiation of interneurons and pyramidal neurons in R6/2 mice showed a significant decrease in interneuron intercorrelations but a significant increase in pyramidal cells. These data suggest an uncoupling of the pyramidal and interneuron network activity resulting in dysregulation of cortical synchronization. Additionally, the disruption in the occurrence of Ca^{2+} transients in relation to locomotion initiation suggests that the HD models may utilize different network dynamics to initiate locomotion.

Disclosures: A.M. Estrada Sanchez: None. E. Donzis: None. T. Indersmitten: None. C. Tran: None. C. Wang: None. D. Castro: None. M. Nedjat-Haiem: None. C. Cepeda: None. P. Golshani: None. M.S. Levine: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.16/S9

Topic: C.04. Movement Disorders

Support: CHDI Foundation, Inc. A-8462

NIH NS41574

Title: Somatostatin interneurons contribute towards increased striatal inhibition in the Q175 mouse model of Huntington's Disease

Authors: *S. M. HOLLEY, L. GALVAN, T. KAMDJOU, C. CEPEDA, M. S. LEVINE; IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington's Disease (HD) is a heritable neurological disorder that affects cognitive and motor function in patients carrying the mutated huntingtin gene. In mouse models of HD, previous reports showed an increase in GABA neurotransmission to striatal medium-sized spiny neurons (MSNs). Here using optogenetics we examined the role of GABAergic, somatostatin (SOM)-expressing interneurons in HD using the Q175 (heterozygote) mouse model. These mice are a slowly progressing knock-in model carrying only one copy of the mutated HD allele and more closely mimic the human condition. Using patch clamp electrophysiology in voltage clamp mode, we recorded from MSNs in brains slices of presymptomatic (2 month) and symptomatic (8 and 12 month) Q175 mice and wildtype (WT) littermates. The amplitudes of responses evoked by activating SOM-expressing interneurons were similar in MSNs at both ages but differences in response kinetics occurred in MSNs from symptomatic mice. Since SOM interneurons are constitutively active in striatal brain slices, we also examined the effects of acutely silencing these neurons with halorhodopsin (eNpHR). Optically silencing SOM-expressing interneurons resulted in a decrease in the frequency of spontaneous IPSCs (sIPSCs) in a subset of MSNs from both WT and Q175 mice. The sIPSC frequency decrease during eNpHR activation was more pronounced in symptomatic Q175 MSNs compared to WT MSNs, suggesting that SOM interneurons contribute to the overall increased inhibition in HD MSNs. Additionally, CB1 receptors were activated with the agonist WIN 55,212-2 to determine if alterations in endocannabinoid-regulated neurotransmitter release is changed when SOM-

expressing interneurons are activated. WIN-55 effectively decreased the overall sIPSC frequency in both WT and Q175 MSNs. When selectively activating SOM-interneurons in the presence of WIN-55 the magnitude of the evoked IPSC in MSNs was decreased and this change was significantly reduced in symptomatic Q175 MSNs. Overall, these findings show that aberrant neurotransmitter release from SOM interneurons contributes to the increased striatal inhibition found in HD mouse models and that dysregulation of the endocannabinoid system may contribute to this effect.

Disclosures: S.M. Holley: None. L. Galvan: None. T. Kamdjou: None. C. Cepeda: None. M.S. Levine: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.17/S10

Topic: C.04. Movement Disorders

Support: CHDI contract A-8047

Title: Synaptic alterations in direct and indirect pathway spiny projection neurons in the Q175 mouse model of Huntington's disease.

Authors: J. SANCHEZ-PADILLA[1], G. TOMBAUGH[1], S. GELMAN[1], K. KRETSCHMANNOVA[1], J. PALMA[1], A. GHAVAMI[1], V. BEAUMONT[2], *R. CACHOPE[2]; [1]Psychogenics Inc, Tarrytown, NY; [2]Translational Biol., CHDI Mgmt. / CHDI Fndn., Los Angeles, CA

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from an extended number of CAG repeats in the *Huntingtin* (*Htt*) gene, for which no disease-modifying therapy is currently available. HD comprises several cognitive and affective symptoms, as well as uncontrolled movement (chorea), which have been hypothesized to arise from a preferential vulnerability of indirect pathway spiny projection neurons (iSPNs) preceding dysfunction of the direct pathway SPNs (dSPNs) in the striatum. In order to characterize this possible imbalance, we performed in vitro brain slice whole patch-clamp recordings from the Q175 heterozygous knock-in HD mouse model expressing GFP under the control of the D2 receptor promoter to identify dSPNs (GFP-negative) and iSPNs (GFP-positive). We asked to what extent alterations in intrinsic and synaptic properties in Q175 mice were selectively affected in each SPN subtype. Using whole-cell path clamp, both dSPNs and iSPNs from Q175 6-month old mice showed elevated membrane resistance and reduced rheobase current,

consistent with previous reports of SPN hyperexcitability in mouse models of HD. Membrane resistance and rheobase in both dSPN and iSPNs from 2-month old Q175 mice did not significantly differ from Wild-type (WT) dSPN and iSPNs respectively, suggesting that deficits in SPN excitability emerged largely in parallel in each subtype. Analysis of mEPSC properties in SPNs revealed a significant decrease in mEPSC frequency only in iSPNs from 6-month old Q175 mice, with no genotypic differences in mEPSC amplitude in either cell type. Amplitude and frequency of mEPSCs were unchanged in 2-month old Q175 mice compared to WT. Finally, we examined LTP at corticostriatal synapses in 6-month old mice using a Hebbian protocol consisting of 4 trains of 100 Hz presynaptic stimulation paired with postsynaptic depolarization. We found that corticostriatal LTP was decreased in dSPNs, while LTP expression in iSPNs was unchanged. Altogether, these results suggest that HD progression is not equally expressed in the indirect and direct pathways. Additional studies are, however, necessary to better understand such differential impairment.

Disclosures: **J. Sanchez-Padilla:** 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 sponsored by CHDI Foundation. **G. Tombaugh:** 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 sponsored by CHDI Foundation. **S. Gelman:** 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 sponsored by CHDI Foundation. **K. Kretschmannova:** 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 sponsored by CHDI Foundation. **J. Palma:** 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 sponsored by CHDI Foundation. **A. Ghavami:** 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 sponsored by CHDI Foundation. **V. Beaumont:** None. **R. Cachope:** None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.18/S11

Topic: E.03. Basal Ganglia

Support: CONACyT 180660

DGAPA-PAPIIT IN216515

Title: NT-3 effects on corticostriatal plasticity

Authors: *E. HERNANDEZ-ECHEAGARAY, Dr[1], V. GÓMEZ[2], E. MENDOZA[2];
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Abstract: In previous work we have described that during the first week of postnatal development in mice, neurotrophin-3 (NT-3) is present in corticostriatal pathway, and after 42 days old, NT-3 is expressed in cell somas of mice striatum. We also have shown that expression levels of mRNA and immune localization of NT-3 and its receptor, the TrkC are altered in striatal damage induced with an *in vivo* treatment with 3-Nitropropionic Acid (3-NP) a mitochondrial toxin used to mimic the histopathology of Huntington's disease (HD). Therefore, we were interested in evaluating modulatory effect of NT-3 on corticostriatal synaptic transmission, as well as, striatal synaptic plasticity induced with high frequency stimulation (HFS; 100Hz), in both control and in damaged striatum. Our results indicated that NT-3 modulates both; synaptic transmission and plasticity in the striatum, nonetheless synaptic plasticity is modify with the 3-NP treatment, instead of producing striatal LTD, LTP is obtained, moreover the administration of NT-3 in the recording bath recovered the plasticity observed in control conditions (LTD) in this model of striatal degeneration. The mechanisms responsible for the striatal plasticity recovery are discussed.

Disclosures: E. Hernandez-Echeagaray: None. V. Gómez: None. E. Mendoza: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.19/S12

Topic: C.04. Movement Disorders

Support: CHDI Foundation, Inc.

Title: *In vivo* characterization of the cortico-basal ganglia connectivity in the zQ175 heterozygous knock-in mouse model of Huntington's disease

Authors: *S. ZHONG[1], A. GHAVAMI[1], R. CACHOPE[2], V. BEAUMONT[2];
[1]In Vivo Electrophysiology, PsychoGenics Inc., Montvale, NJ; [2]CHDI Foundation, Inc./CHDI Management, Inc., Los Angeles, CA

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from an extended number of CAG repeats in the Huntingtin (Htt) gene, for which no disease-modifying therapy is currently available, and comprises several cognitive and affective symptoms, as well as uncontrolled movement (chorea). HD symptoms are thought to be the result of impaired cortico - thalamo - basal ganglia (BG) function; especially a misbalance between the direct and indirect pathways, while the potential dysfunction of the hyperdirect pathway has not been examined. The purpose of the current study was to characterize hyperdirect pathway transmission and its relationship with direct and indirect pathway (dys)function as a contributing factor to HD. We performed in vivo anesthetized electrophysiological extracellular recordings in the anesthetized Q175Het mouse model of HD vs. WT (6 months old).
First, we identified a decreased response of Q175 STN neurons to electrical stimulation of the motor cortex (M1), suggesting impaired hyperdirect pathway transmission. Next, we explored the triphasic response patterns (excitation-inhibition-excitation) of single units in the SNr and/or GP evoked by M1 stimulation, which have been proposed to represent hyperdirect, direct and indirect pathway function, respectively. Our key finding is that in Q175Het mice, the amplitudes for both early and late excitation of SNr neurons to cortical stimulation were significantly decreased compared to WT mice, as revealed from population peristimulus histograms. Our results also revealed that the inhibition phase of SNr neurons was increased in Q175Het but with no change in either latency or duration of the evoked response. For GP neurons in Q175Het mice, amplitudes for both early and late excitation were decreased as in SNr neurons but this did not reach statistical significance. No differences in firing inhibition, response latency or duration were observed. The proportion of neurons showing triphasic responses were decreased for both SNr (39% to 22%) and GP (23% to 19%) in Q175 mice compared to WT mice. Spontaneous firing rates in Q175Het mice showed a modest increase in both SNr and GP neurons compared to the WT but did not reach statistical significance.
These results indicate that a complex dysfunctional interplay between the hyperdirect, direct, and indirect pathways occurs in HD. Genotypic differences established here can be used to monitor basal ganglia circuitry dysfunction in HD models with the potential to evaluate pathway-selective effects of pharmacological treatments.
Study funded by CHDI Foundation, Inc.

Disclosures: S. Zhong: None. A. Ghavami: None. R. Cachope: None. V. Beaumont: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.20/S13

Topic: C.04. Movement Disorders

Title: Characterization of ipRGC impairment in Huntington's disease

Authors: *M.-S. LIN[1], C.-P. CHANG[1], S.-K. CHEN[2], Y. CHERN[1];
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Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs), which express the photopigment melanopsin, respond to light stimulation and regulate the non-image forming visual functions including the photoentrainment of circadian rhythm, pupil light reflex, and sleep. ipRGCs are reported as the resistant cell types in some inherited blindness, but are affected in glaucoma optic neuropathy and aging, which is associated with impaired circadian rhythm regulation. It is still unclear whether the functional properties of ipRGC are altered during neurodegenerative conditions. Previous studies showed that mice (R6/2 and $_{Z}$Q175) with Huntington's disease (HD) exhibited disrupted circadian behaviors without abnormal oscillation of PER1 and PER2 in the suprachiasmatic nucleus (SCN). We hypothesized that dysfunction of ipRGC might attribute to such circadian disruption during HD pathogenesis. We found that the number of melanopsin-expressing ipRGCs was significantly decreased during disease progression in two HD mouse models (R6/2 and N171-82Q). Interestingly, the five different subtypes of ipRGC (M1-M5) appeared to be differentially inflicted during HD progression. Under bright light stimulation, R6/2 mice showed attenuated pupil light reflex and enhanced activity in the light phase after 4-hour light dark (LD) phase advance during jet lag. It appeared that R6/2 mice are relatively insensitive to light stimulation compared to WT mice, and this behavioral change might be associated with the progressive loss of ipRGCs. In summary, we uncovered the degeneration of ipRGC in HD, which might cause the impairment of non-image forming visual functions, and contribute to the non-motor behavioral deficits (such as sleep problem) in HD.

Disclosures: M. Lin: None. C. Chang: None. S. Chen: None. Y. Chern: None.

Poster

226. Huntington's Disease Mechanisms I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 226.21/S14

Topic: C.04. Movement Disorders

Support: PSC-BHE Research Awards Program

Title: The relationship between adult neurogenesis and interval timing is altered in a transgenic Huntington's Disease rat model

Authors: *A. PEREZ[1], J. FISCHETTI[2], D. GARCES[1], S. WODINSKY[2], A. TOROSSIAN[2], C. TSIRIS[1], J. ROJAS[2], B. BROWN[1,2], N. HEMMES[1,2], C. L. PYTTE[1,2], J. ARONOWITZ[2]; [1]Psychology, The Grad. Center, City Univ. of New York, New York, NY; [2]Psychology, Queens Col. of the City Univ. of New York, Flushing, NY

Abstract: The hippocampus processes experience in space and time and contributes to the frontal-striatal system in modulating time perception on the seconds-to-minutes scale. In certain neurodegenerative disorders, such as Huntington's disease (HD), there is an impaired ability to perform tasks that require intact interval timing. The neuropathology of HD is characterized by neurodegeneration in the striatum, cerebral cortex, and hippocampus, as well as altered new neuron addition to the striatum and hippocampus. Here we examined the relationship between hippocampal neurogenesis and measures of temporal discrimination in a transgenic Huntington's disease (tgHD) rat model. Temporal discrimination was measured using a peak interval (PI) paradigm. The peak interval procedure is a variant of the fixed interval (FI) schedule of reinforcement. Peak interval trials were non-reinforced trials presented for 3x the FI-criterion duration. We divided the peak rate of lever press responding by the average smoothed response rate across the PI trial to obtain a ratio measure. Ratios equal to 1 indicate a lack of temporal discrimination (e.g., a flat function when response rate is plotted as a function of elapsed time into the trial) and ratios greater than 1 indicate stronger temporal discrimination. New neurons were labeled with antibody to the neuron-specific protein doublecortin, and numbers of new neurons in the dorsal hippocampus were compared with behavior. Interestingly, we found that in wild-type rats, there was an inverse relationship between new neurons and temporal discrimination. More new neurons corresponded to worse discrimination. This relationship did not exist in tgHD rats. Rather, there was a positive correlation between new neurons and temporal discrimination. These findings suggest that the relationship between new neurons and timing behavior are differentially associated in tgHD rats and healthy, control rats.

Disclosures: A. Perez: None. J. Fischetti: None. D. Garces: None. S. Wodinsky: None. A. Torossian: None. C. Tsiris: None. J. Rojas: None. B. Brown: None. N. Hemmes: None. C.L. Pytte: None. J. Aronowitz: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.01/T1

Topic: C.05. Neuromuscular Diseases

Support: Milton Safenowitz Post-Doctoral Fellowship for ALS Research to YL

NIH grants NS061867 and NS068179 to RB

Title: IP-MS and CLIP-seq define an extensive RBM45 interactome network

Authors: *Y. L. LI[1], M. COLLINS[2], J. AN[2], K. GARCIA[3], P. PIRROTTE[3], R. BOWSER[2]; [1]Neurol., [2]Barrow Neurolog. Inst., Phoenix, AZ; [3]TGen, Phoenix, AZ

Abstract: The pathological accumulation of RNA-binding proteins (RBPs) within inclusion bodies is a hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). RBP aggregation results in both toxic gain and loss of normal function. Determining the protein and RNA binding partners and normal functions of disease-associated RBPs is necessary to fully understand molecular mechanisms of RBPs in disease.
RBM45 is a recently characterized RBP found in inclusions in ALS, FTLD and AD [1]. These inclusions are positive for TDP-43, and RBM45 physically interacts with TDP-43 and FUS in vitro [2]. To delineate protein binding partners of RBM45 and putative biological functions of the protein, we used an immunoprecipitation coupled to mass spectrometry (IP-MS) approach to comprehensively characterize RBM45 protein-protein interactions (PPIs) within HEK293 cells [3]. We identified 132 RBM45 PPIs by IP-MS, including PPIs with many RBPs. Select PPIs were validated by immunoblot and immunocytochemistry, demonstrating that RBM45 associates with a number of other RBPs primarily via RNA-dependent interactions in the nucleus. Analysis of the biological processes and pathways associated with RBM45-interacting proteins indicates enrichment for nuclear RNA processing/splicing and cytoplasmic RNA translation. Moreover, several other ALS-linked RBPs, including TDP-43, FUS, Matrin-3, hnRNP-A1, and hnRNP-A2/B1, interact with RBM45, consistent with prior observations of these proteins within intracellular inclusions in ALS/FTLD. We also used CLIP-seq approach to study the RNA binding targets of RBM45. The RNA targets bound by RBM45 and several ALS-linked RBPs are compared to determine RNA targets co-regulated by RBM45 and these RBPs. Collectively, our results shed new light on RBM45 interactome, biological functions, and contributions to neurodegeneration in ALS/FTLD. **References:** 1. Collins, M., et al., *The RNA-binding motif 45 (RBM45) protein accumulates in inclusion bodies in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) patients.* Acta neuropathologica, 2012. **124**(5): p. 717-32. 2. Li, Y., et al., *RBM45 homo-oligomerization mediates association with ALS-linked proteins and stress granules.* Sci Rep, 2015. **5**: p. 14262. 3.

Li, Y., et al., *Immunoprecipitation and mass spectrometry defines an extensive RBM45 protein-protein interaction network.* Brain Res, 2016. **Acknowledgement:** Funding support by the Milton Safenowitz Post-Doctoral Fellowship for ALS Research to YL, and the NIH grants NS061867 and NS068179 to RB.

Disclosures: Y.L. Li: None. M. Collins: None. J. An: None. K. Garcia: None. P. Pirrotte: None. R. Bowser: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.02/T2

Topic: C.05. Neuromuscular Diseases

Title: The development of therapeutic intrabody against misfolded TDP-43 for ALS

Authors: *Y. TAMAKI[1], A. SHODAI[1], R. HIKIAMI[1], S. MINAMIYAMA[1], T. AYAKI[1], R. TAKAHASHI[1], M. URUSHITANI[1,2];
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Abstract: [Purpose] Accumulating evidence suggests that detergent-resistant, ubiquitinated, and phosphorylated TAR DNA-binding protein of 43 kDa (TDP-43) is a major pathogenic protein in sporadic amyotrophic lateral sclerosis (ALS) when misfolded and mislocalized in the cytosol. The aim of this study is to investigate the therapeutic potential of intrabody against pathogenic TDP-43 species. [Methods] We have recently reported that a novel monoclonal antibody, 3B12A, against peptides containing Asp247 in TDP-43 reacts specifically with TDP-43 inclusions in sporadic ALS patients and with misfolded or mislocalized TDP-43 in cultured cells. To introduce 3B12A efficiently inside cells, we constructed single chain variable fragment of 3B12A (scFv-3B12A) as intrabody against misfolded TDP-43. We also constructed scFv-3B12A containing proteasome localizing signal (scFv-3B12A-CL1) or chaperone-mediated autophagy localizing signal (scFv-3B12A-CMA) for protein disassembling signals. We investigated the specificity and the clearance effects of those scFv-3B12A intrabodies in cultured cells. [Results] Immunoprecipitation and confocal microscopy analysis showed that scFv-3B12A interacted specifically with mislocalized or aggregated forms of TDP-43. CL1 and CMA signals promoted the degradation of scFv-3B12A at the proteasomes and the lysosomes, respectively. In HaloTag-recombinant TDP-43 chase assay, scFv-3B12A and scFv-3B12A-CMA degraded mislocalized cytosolic TDP-43 significantly, but not wild-type or aggregated TDP-43. For the next step, we sought to explore several reported molecular chaperone inducers to refold aggregated forms of

TDP-43 to be targeted by the scFv-3B12A. Some molecular chaperone inducers showed a remarkable reduction of aggregated species of TDP-43 in cultured cells. [Conclusion] Our findings indicated that scFv-3B12A functioned as an intrabody to react with and degrade misfolded/mislocalized TDP-43 inside cells, so far as to be the pre-aggregated form. The combinations therapy with molecular chaperone inducers might be a more promising strategy to step forward the therapy.

Disclosures: Y. Tamaki: None. A. Shodai: None. R. Hikiami: None. S. Minamiyama: None. T. Ayaki: None. R. Takahashi: None. M. Urushitani: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.03/T3

Topic: C.05. Neuromuscular Diseases

Support: German BMBF

Title: Antifibrotic approach targeting the tgfß system

Authors: *S. KUESPERT, S. PETERS, E. ZITZELSPERGER, R. HEYDN, T.-H. BRUUN, U. BOGDAHN;
Dept. of Neurol., Univ. Hosp. Regensburg, Regensburg, Germany

Abstract: TGF-ß is involved in a number of processes like e.g. cell proliferation, migration, wound healing, angiogenesis and cell-cell interactions. It´s known from several studies, that this factor is often elevated during pathogenesis of several disorders including neurodegenerative diseases, pulmonary fibrosis and diabetic nephropathy. These disorders are related to pathogenic modifications of extracellular matrix (ECM) and the actin-cytoskeleton. Often, these observed alterations correlate with severity of disease progression. Connective tissue growth factor (CTGF) is a downstream-mediator of TGF-ß and transfers fibrotic effects of TGF-ß. It has been shown that CTGF leads to an induced deposition of ECM and modulates reorganization of actin-cytoskeleton.
To investigate whether TGF-RII specific antisense-oligonucleotides (ASOs) contribute to a resolution of fibrotic processes by inhibiting TGF-ß signaling, CTGF levels were evaluated in addition to Fibronectin (FN) and Collagen IV (ColIV), representing two main components of ECM in several different organ systems. Furthermore, effects of ASOs on CTGF, FN, actin-cytoskeleton and proliferation were examined in different cell lines as neural precursor (ReNcell CX™) and human lung cancer (A549) cells. Data were obtained by quantitative real-time RT-

PCR, immunoblotting, immunocytochemistry and cell counting. Results indicate that blocking cellular TGF-ß signaling by selective ASO`s leads to reduction of fibrotic deposition and a re-modulation of actin-cytoskeleton. Thus, TGF-RII-ASO`s seem to be promising candidates for an anti-fibrotic treatment approach.

Disclosures: S. Kuespert: None. S. Peters: None. E. Zitzelsperger: None. R. Heydn: None. T. Bruun: None. U. Bogdahn: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.04/T4

Topic: C.05. Neuromuscular Diseases

Support: Target ALS, NIH Grant RO1-NS074886

Title: Glutamate receptors mediate p-glycoprotein upregulation at the blood brain barrier in amyotrophic lateral sclerosis

Authors: *L. A. MOHAMED, S. MARKANDAIAH, P. PASINELLI, D. TROTTI; Deparment of Neurosciences, Thomas Jefferson Univ., Philadelphia, PA

Abstract: Recent studies showed that the ABC transporter, P-glycoprotein (P-gp), expressed at the blood brain barrier (BBB) and the blood spinal cord barrier (BSCB) may contribute to progressive pharmacoresistance in amyotrophic lateral sclerosis (ALS). Riluzole, the only drug which has been approved by the food and drug administration (FDA) for treatment of ALS, has been shown to improve survival by a few months in some patients; however, progressive pharmacoresistance has been reported. Spinal cord tissue samples from ALS patients showed higher P-gp expression specifically at the capillary endothelials of the BSCB compared to healthy individuals. Our lab has shown previously that Riluzole brain penetration is higher in P-gp knockout mice, and P-gp deletion in an ALS mouse model increased brain level of Riluzole and improved survival. We have also demonstrated one of the underlying mechanisms of P-gp mediated pharmacoresistance using in vitro models of BBB. We found that P-gp overexpression was associated with activation of NF-kB-dependent pathways and was regulated by astrocyte secreted factors. Following our previous findings, our overall goal is to fully characterize the molecular mechanisms of P-gp overexpression in ALS utilizing our optimized patient-derived induced pluripotent stem cells (iPSC) BBB cellular model. We have tested the hypothesis that high extracellular glutamate level observed in ALS may induce P-gp expression through stimulation of N-methy-D-aspartic acid (NMDA) receptors at the BBB. Using our BBB model,

our initial findings indicated that co-culture of endothelial cells with patient-derived astrocytes induced P-gp expression and enhanced transport of P-gp probe substrate LD800. This increase in LD800 transport was completely abolished when cells were pre-treated with 100μM MK801, an NMDA receptor antagonist. Our findings suggest that P-gp expression at the BBB could be regulated by several upstream molecular pathways that eventually converge into activation of NF-kB.

Disclosures: L.A. Mohamed: None. S. Markandaiah: None. P. pasinelli: None. D. Trotti: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.05/T5

Topic: C.05. Neuromuscular Diseases

Support: MIUR (PRIN project 2006058401)

Title: Altered mechanisms underlying the abnormal glutamate release in the spinal cord of pre-symptomatic SOD1^{G93A} mouse model of ALS

Authors: *G. BONANNO[1], T. BONIFACINO[1], E. GALLIA[1], L. MUSAZZI[2], M. MILANESE[3], L. CATTANEO[3], M. SEGUINI[2], A. MARTE[4], F. ONOFRI[4], M. POPOLI[2];
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Abstract: Glutamate(Glu)-mediated excitotoxicity plays a major role in motor neurons (MN) degeneration in amyotrophic lateral sclerosis (ALS). Impaired glial uptake and abnormal exocytotic release are responsible for the excessive extracellular Glu. The main goal of this work was to investigate the mechanisms that support the excessive Glu exocytosis in pre-symptomatic SOD1^{G93A} mice. Synaptic terminals were isolated from the spinal cord of SOD1 and SOD1^{G93A} mice and studied for Glu release, intra-terminal Ca^{2+} concentration, synaptic protein expression and phosphorylation state. The basal release of Glu is increased in pre-symptomatic SOD1^{G93A} mice compared to control animals. Exposure to high KCl or ionomycin provoked a Ca^{2+}-dependent exocytotic Glu release that was augmented in SOD1^{G93A} mice. Also the hypertonic sucrose-induced Ca^{2+}-independent Glu exocytosis was abnormally elevated in SOD1^{G93A} mice, suggesting the involvement of the readily releasable pool (RRP) of glutamatergic vesicles. Molecular biology experiments revealed an increased number of assembled SNARE complexes

at the nerve terminal membranes, with no changes of the three SNARE proteins involved. Also the expression of Synaptotagmin-1 and β-Actin, but not that of many other pre-synaptic proteins involved in exocytosis was increased. We found elevated the presynaptic cytosolic Ca^{2+} levels and the phosphorylation of Synapsin-I at the relevant sites for synaptic vesicle mobilization to the RRP of vesicle. Also glycogen synthase kinase-3 phosphorylation at the inhibitory sites was higher in $SOD1^{G93A}$ mice, an event that may favor SNARE protein assembly. The excessive glutamate release was prevented by blocking Synapsin-I phosphorylation with specific antibodies, thus supporting the role of Synapsin-I hyper-activation in the excess of Glu release. Our results highlight that the abnormal Glu exocytosis in the spinal cord of pre-symptomatic $SOD1^{G93A}$ mice is mainly based on the increased size of the RRP of vesicles and is supported by plastic changes of specific presynaptic mechanisms. The precociousness of the event support the idea that the increase of Glu release, also recorded later during pathology, may represent a causative factor rather than a consequence of disease progression.

Disclosures: G. Bonanno: None. T. Bonifacino: None. E. Gallia: None. L. Musazzi: None. M. Milanese: None. L. Cattaneo: None. M. Seguini: None. A. Marte: None. F. Onofri: None. M. Popoli: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.06/T6

Topic: C.05. Neuromuscular Diseases

Support: Italian Mitofusin 2 association

Title: Selective mitochondrial depletion and enhanced mitophagy in human Charcot-Marie-Tooth 2A motor neurons

Authors: F. RIZZO, 20122[1], D. RONCHI[1,2], S. SALANI[1], M. NIZZARDO[1,2], F. FORTUNATO[1], A. BORDONI[1], G. STUPPIA[1], R. DEL BO[1], D. PIGA[1], N. BRESOLIN[1], G. COMI, 20099[1,2], *S. CORTI[1,2];
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Abstract: Charcot-Marie-Tooth 2A (CMT2A) is a common inherited polyneuropathy, characterized by motor and sensitive neuron degeneration, and caused by mutations in Mitofusin 2 gene (*MFN2)*, which encodes a mitochondrial membrane protein critical in maintaining mitochondrial network. Since MFN2 is ubiquitously expressed, the reason for selective motor

neuron (MN) degeneration in CMT2A is unknown. To address this question, we differentiated MNs from induced pluripotent stem cells (iPSCs) derived from CMT2A patients, developing an *in vitro* disease model. CMT2A iPSC-derived MNs (CMT2A-MNs) presented a reduction in mitochondrial content and localization, without significant differences in survival and axon elongation. RNA sequencing and proteomic analyses of key components of the apoptotic program demonstrated that CMT2A-MNs are more resistant to apoptosis than wild-type MNs. Analyzing the balance between mitochondrial biogenesis and the regulation of autophagy-lysosome transcription, we detected an augmented autophagic flux in CMT2A-MNs that was linked with up-regulation of PINK1, PARK2, BNIP3, and a splice variant of BECN1 that was recently showed to be a trigger for mitochondrial autophagy. Our data suggest that the mitochondria reduction in MNs expressing mutant MFN2 is not the result of impaired biogenesis, but more likely the consequence of enhanced mitophagy. Thus, these pathways represent potential novel molecular therapeutic targets for the development of effective cure for CMT2A.

Disclosures: F. Rizzo: None. D. Ronchi: None. S. Salani: None. M. Nizzardo: None. F. Fortunato: None. A. Bordoni: None. G. Stuppia: None. R. Del Bo: None. D. Piga: None. N. Bresolin: None. G. Comi: None. S. Corti: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.07/T7

Topic: C.05. Neuromuscular Diseases

Support: Target ALS GT005287

Title: Mitochondria associated er membranes in amyotrophic lateral sclerosis

Authors: *D. LARREA[1], E. RHODES LOWRY[2], J. SMERDON[2], H. WICHTERLE[2], E. AREA-GOMEZ[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is characterized by progressive degeneration of primary motor cortex neurons, anterior horn cells and lower motor neurons. Mutations in specific genes, such as *SOD1, FUS, VAPB, C9orf72, TARDBP, and SIGMAR1* cause familial forms of ALS, representing only the 5-10% of cases. While implicated in different pathways, these mutations share common pathogenic mechanisms that result in motor neuron degeneration and muscle denervation. Among these, endoplasmic reticulum (ER) stress and mitochondria

dysfunction, alterations in calcium and lipid homeostasis, defective autophagy and protein aggregation have been described, however the cause of these seemingly unrelated phenotypes is unknown. Mitochondria-associated ER membranes (MAMs) are a subdomain of the ER connected physically and biochemically to mitochondria, and are involved in the regulation of several key cellular functions such as lipid metabolism, calcium homeostasis, mitochondrial function, ER-stress and unfolding protein response regulation. Alterations of these functions have been shown to occur early in ALS, although the role of ER-mitochondria interactions has not been systematically studied in this disease. We have studied MAM function measured as (i) the synthesis and conversion of phosphatidylserine to phosphatidylethanolamine (ii) cholesterol esterification by ACAT1 (acylCoA:cholesterol acyltransferase; gene *SOAT1*) activity, (iii) lipid droplet accumulation and mitochondrial respiration in cells and tissues from ALS mouse models and iPS-derived motor neurons from ALS patients. Our results showed decreased levels of (i) phospholipid synthesis (ii) ACAT activity and (iii) significantly lower amounts of lipid droplets. Also, oxygen consumption was decreased in ALS mouse mutant cells and in isolated mitochondria, when compared with controls. Altogether our studies indicate a significant and early alteration in MAM activity and ER- mitochondria cross talk in ALS pathogenesis.

Disclosures: D. Larrea: None. E. Rhodes Lowry: None. J. Smerdon: None. H. Wichterle: None. E. Area-Gomez: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.08/T8

Topic: C.05. Neuromuscular Diseases

Title: Histopathological findings in an adult Down syndrome patient presenting with ALS

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Abstract: A connection between Alzheimer's disease and Down syndrome has been described extensively in the literature. The higher risk for Alzheimer's disease in people with Down syndrome has been attributed to increased production of amyloid beta due to an extra copy of chromosome 21, but other genes on chromosome 21 may also play a role, such as superoxide dismutase (SOD1). Little is known about the consequences of trisomy 21 for other neurodegenerative diseases. We present the case of a 49-year-old male diagnosed with trisomy 21 at an early age, who developed severe intellectual deficiency and progressive muscle weakness of the upper limbs at the age of 47. He also complained of pain in the shoulders, and

became unable to move his right arm within months. The symptoms worsened over the following year. He progressively stopped walking, developed dysphagia, further impairment of the lower limbs and eventually died. At the autopsy, the patient presented characteristic craniofacial morphological features of Down syndrome. Macroscopic examination of the brain showed atrophy of the superior temporal gyrus and the frontoparietal cortex, a small hippocampus and dilatation of the lateral ventricles. Microscopic examination of the brain showed typical features of Alzheimer's disease with amyloid deposits in the cerebral cortex, basal ganglia and cerebellar cortex. Gallyas staining showed the presence of numerous neuritic plaques at the level of the hippocampus. Immunohistological stains for alpha- synuclein did not reveal the presence of Lewy bodies. CD68 immunohistochemistry revealed abundant macrophages in the medullary pyramids and lateral columns and an associated microglial reaction. TDP-43 immunohistochemistry showed a filamentous staining in the cytoplasm and a loss of nuclear staining within motor-neurons. Ubiquitin immunohistochemistry showed weak staining of some spinal nerve roots. SOD1 immunohistochemistry showed a staining in the cytoplasm colocalizing with TDP-43. The present case shows that neurodegenerative disease in Down syndrome patients can take other forms besides Alzheimer’s disease, including amyotrophic lateral sclerosis. In familial ALS (FALS), any one of the reported SOD1 inherited mutations in the SOD1 gene can lead to misfolding of the protein exerting its toxic gain of function. As cytoplasmic TDP-43 inclusions in mutant SOD1-related FALS cases are relatively rare, the presence of cytoplasmic misfolded SOD1 colocalizing with TDP43 suggests that the present case is sporadic in nature and that non-native conformers of SOD1-linked ALS and SALS might converge on a common pathogenic pathway.

Disclosures: B. Paré: None. N. Dupré: None. P. Gould: None. F. Gros-Louis: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.09/T9

Topic: C.05. Neuromuscular Diseases

Support: China Schorlarship council

IWT Grant for SBO project

Title: Motor neurons derived from ALS patients with FUS mutations mimic disease-related phenotypes *In vitro*

Authors: W. GUO[1,5], S. PATEL[2], L. FUMAGALLI[1,5], D. BOHL[6], V. BENOY[1,5], P. VANDEN BERGHE[3], W. ROBBERECHT[1,5,7], *P. DUPONT[4], P. VAN DAMME[1,5,7], C. VERFAILLIE[2], L. VAN DEN BOSCH[1,5];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder which is due to the selective loss of motor neurons in the motor cortex, brainstem and spinal cord. In 10% of patients, ALS is a familial disease. One of the important genetic causes of familial ALS are mutations in the FUS gene. How these mutations cause the disease is still unknown. To model FUS-ALS in a dish, we generated integration-free induced pluripotent stem cells (iPSCs) from ALS patients with different FUS mutations. Fibroblasts from three ALS patients with an R521H and one patient with a P525L point mutation in FUS, as well as from their unaffected family members were infected with the CytoTune-2 Sendai-virus reprogramming kit. Immunostaining, quantitative RT-PCR, teratoma formation analysis and integration assays were used to identify the pluripotency of the iPSC, and the absence of Sendai viruses. Motor neurons were derived from the iPSCs by inhibiting the SMAD pathway and by activating the Hedgehog signaling (SHH) pathway. Highly pure motor neurons were successfully generated from these iPSCs. Mislocalisation of FUS was observed in the ALS patient derived motor neurons, especially in the ones derived from the P525L line. Axonal transport was measured using MitoTracker Red. The relative amount of moving and stationary mitochondria, as well as transport velocities could be analyzed on kymographs. This clearly showed a progressively lower amount of moving mitochondria as a function of the time of differentiation in the ALS patient samples. These results indicate that mutations in FUS lead to axonal transport defects which is in line with the ‘dying back theory’ as this axonal dysfunction could lead to the retraction of the motor axon.

Disclosures: W. Guo: None. S. Patel: None. L. Fumagalli: None. D. Bohl: None. V. Benoy: None. P. Vanden Berghe: None. W. Robberecht: None. P. Dupont: None. P. Van Damme: None. C. Verfaillie: None. L. Van Den Bosch: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.10/T10

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant 5K08NS082364

Title: Pharmacological profiling of human and mouse motor neurons on microelectrode arrays

Authors: *D. F. MOAKLEY[1], J. D. PEREIRA[1], J. S. GAL[1], A.-C. DEVLIN[1], Y. SAPIR[1], L. A. WILLIAMS[2], N. ATWATER[2], D. BAKER[2], O. WISKOW[2], S. LEE[3], K. ROET[3], K. EGGAN[2,4], C. J. WOOLF[3,4], B. J. WAINGER[1,4];
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Abstract: Like all neuronal types, lower motor neurons have a particular electrophysiological identity according to their synaptic inputs and ion channel constitution. Disease modeling using human pluripotent stem cell-derived motor neurons (hPSC-MNs) has shown that there are abnormalities in the intrinsic excitability of hPSC-MNs from patients with amyotrophic lateral sclerosis (ALS), suggesting a change in ion channel function. While the phenotypes seen in ALS patient-derived hPSC-MNs are promising avenues for further investigation, a roster of the specific channels that determine motor neuron excitability is needed. In addition, it is important to confirm that hPSC-MNs functionally resemble *bona fide* motor neurons, given that they are derived in an artificial environment and little broad functional characterization has been performed in hPSC-derived neurons. To better elucidate the determinants of motor neuron excitability, we cultured hPSC-MNs and primary mouse motor neurons (mMNs) on microelectrode arrays (MEAs), grids of extracellular electrodes that allow us to record the baseline firing and response to a host of drugs in large motor neuron populations. Using receptor and channel-selective pharmacological agents as well as ALS candidate therapeutics, we developed a profile of responses that indicate both synaptic and voltage-gated channel contributions to excitability. Spike rate, burst rate, and network burst rate were examined before and after treatment with each agent. When neurotransmitter receptors were investigated, we found that both glutamate and nicotinic acetylcholine receptor blockers stopped the network activity in the cultures. Among the ion channel blockers, Kv7 channel, L-type calcium channel, and HCN channel blockers all consistently modulated activity metrics measured from the hPSC-MNs and mMNs. The pharmacological profile that is emerging through these experiments provides a framework for comparing motor neuron models and identifying ion channel families underlying motor neuron physiology and disease phenotypes.

Disclosures: D.F. Moakley: None. J.D. Pereira: None. J.S. Gal: None. A. Devlin: None. Y. Sapir: None. L.A. Williams: None. N. Atwater: None. D. Baker: None. O. Wiskow: None. S. Lee: None. K. Roet: None. K. Eggan: None. C.J. Woolf: None. B.J. Wainger: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.11/T11

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant R21-NS090912

Muscular Dystrophy Association

Farber Family Foundation

Title: Cell-to-cell transmission of C9orf72-linked dipeptide repeat proteins

Authors: *T. R. WESTERGARD, B. JENSEN, X. WEN, P. PASINELLI, D. TROTTI; Thomas Jefferson Univ., Philadelphia, PA

Abstract: Intronic hexanucleotide repeat expansions in the *C9orf72* gene are the most common genetic cause for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). RNA transcripts of these expansions can undergo RAN translation to form five dipeptide repeat proteins (DPRs): poly(GA), poly(GP), poly(GR), poly(PA), and poly(PR). DPR aggregates are found throughout the CNS of C9orf72-ALS/FTD patients and cause neuronal degeneration and dysfunction in cell and animal models. While DPR toxic mechanisms continue to be investigated, the potential for DPR aggregates to spread has yet to be determined. Utilizing different experimental platforms, we found evidence for cell-to-cell transmission for each DPR with varying frequencies and modality of spreading. One mechanism behind transmission is *via* exosomes. Exosomes isolated from DPR-expressing cells showed varying amounts of DPRs and evidence of transmission to neurons. DPR transmission also occurred through exosome-independent pathways. These studies demonstrate cell-to-cell transmission of C9-DPRs, which is potentially relevant to disease.

Disclosures: T.R. Westergard: None. B. Jensen: None. X. Wen: None. P. Pasinelli: None. D. Trotti: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.12/T12

Topic: C.05. Neuromuscular Diseases

Support: Farber Family Foundation

Title: Astrocyte dysfunction in fus-linked amyotrophic lateral sclerosis

Authors: *K. MCAVOY, K. KRISHNAMURTHY, D. TROTTI, P. PASINELLI;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Mutations in Fused in Sarcoma (FUS) are linked to Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease affecting both upper and lower motor neurons. FUS mutations account for approximately 5% of familial (fALS) and rare cases of sporadic ALS (sALS) while FUS-positive pathological inclusions have been identified in fALS and sALS. It is well established that in ALS, non-neuronal cell types such as astrocytes contribute to motor neuron degeneration and disease progression. We have previously reported that FUS mutations alter astrocyte function and cause astrocytes to become neurotoxic. We identified NF-kB, TNFα signaling, and neuronal AMPA receptors as important mediators of this toxicity. Here, we explore the mechanisms and molecular changes underlying mutant FUS-linked astrocyte dysfunction *in vitro*. Further, we tested the consequences of astrocyte-specific expression of mutant FUS *in vivo* using AAV-mediated delivery of the FUS variant R521G into the cervical spinal cord of adult mice.

Disclosures: K. McAvoy: None. K. Krishnamurthy: None. D. Trotti: None. P. Pasinelli: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.13/T13

Topic: C.05. Neuromuscular Diseases

Support: CIHR

Title: Investigating the cortical aspects of ALS in mouse and human stem cell models

Authors: ***M. C. FRANQUIN**[1], H. PENG[2], B. PONROY[1], C. ERNST[2], D. STELLWAGEN[1]; [1]Ctr. For Res. In Neurosci., McGill Univ., Montreal, QC, Canada; [2]Psychiatry, Douglas Mental Hlth. Univ. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disease involving neurodegeneration of upper (cortical) and lower (spinal and brainstem) motor neurons, as well as other cortical areas. Data from ALS patients indicates cortical hyperexcitability and the most commonly used mouse model of ALS ($SOD1^{G93A}$) shows cortical alterations at the pre-symptomatic stage of the disease in the layer V of primary motor cortex (M1) including hyperexcitability and increased miniature excitatory post-synaptic current (mEPSC) frequency. This could be driven by changes in astrocyte function, as neuronal-only expression of mutant SOD1 does not lead to an ALS phenotype and reactive astrocytes have been observed in patients with ALS. Reactive astrocytes would produce inflammatory signaling, which could trigger changes in homeostatic synaptic plasticity and lead to a perturbed E/I balance in the cortical cells and hyperexcitability.
We are studying this possibility in two models of ALS: the $SOD1^{G93A}$ mouse model and a human model based on the differentiation of induced pluripotent stem cells (iPSCs) from patients into neurons and astrocytes. We are performing electrophysiological as well as morphological analysis in layer V of M1 in the $SOD1^{G93A}$ mouse model to determine the contribution of inflammatory signaling to the changes in synapses. Further, we are characterizing the electrophysiology changes in iPSC-derived neurons, grown in conjunction with wildtype or mutant iPSC-derived astrocytes. The homeostatic synaptic plasticity capacities of neurons from patients with ALS will also be assessed, as well as the contribution of inflammatory signaling particularly tumor necrosis factor alpha (TNF).
Overall, this work would provide new insights into the cortical aspects of ALS and the implications of the different cell types in the pathogenesis with allegations found in the pre-symptomatic mouse model being verified in a human model of the disease.

Disclosures: M.C. Franquin: None. H. Peng: None. B. Ponroy: None. C. Ernst: None. D. Stellwagen: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.14/T14

Topic: C.05. Neuromuscular Diseases

Support: NHMRC Project Grant 1107644

Title: Functional consequences of amyotrophic lateral sclerosis-linked Cyclin F mutations in cultured neuronal cells and post-mortem tissues

Authors: *S. YANG[1], K. Y. ZHANG[1], N. FARRAWELL[2], V. SUNDARAMOORTHY[1], S. T. WARRAICH[1], A. RÁBANO[3], A. GARCÍA-REDONDO[4], J. D. ATKIN[1], J. J. YERBURY[2], G. A. NICHOLSON[5], I. BLAIR[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating fatal neurodegenerative disorder with no effective treatment or direct diagnosis. A proportion of patients also develop clinical or subclinical frontotemporal dementia (FTD). Genetic defects are the only proven cause of ALS, but the primary molecular pathways underlying ALS remain unclear. Recently, we identified ALS-linked mutations in cyclin F (encoded by CCNF), an E3 ubiquitin ligase that mediates ubiquitination of protein substrates for degradation by the proteasome. The objective of this study is to investigate the functional consequences of cyclin F mutations in post-mortem tissue and cell models. To investigate whether ALS-linked cyclin F mutations lead to proteostatic dysfunction, we performed a flow cytometry based ubiquitin-proteasome system (UPS) GFPu reporter assay. The GFPu reporter consists of a 16 amino acid CL1 degron sequence that ensures rapid degradation via the UPS. Thus over-accumulation of GFPu, as measured by GFP fluorescence intensity, indicates UPS dysfunction or overload. The GFPu construct and either mutant cyclin F, wild type cyclin F, or cyclin F with known polymorphisms identified in ALS/FTD patients, including those in public databases were co-transfected into the motor neuron-like NSC-34 cell line. Cells were harvested 48 hr after transfection and the GFP fluorescence intensity was analysed using flow cytometry. Significantly higher levels of GFPu fluorescence were observed for cyclin F with ALS/FTD mutations ($P<0.05$), indicating UPS dysfunction. It was found that the presence of mutant cyclin F, but not wild type cyclin F, nor the cyclin F polymorphisms, caused GFPu to aggregate in the cytoplasm, and cyclin F was sometimes present in these GFPu positive inclusions. Using a flow cytometry based cytotoxicity assay, we also found that cells expressing mutant cyclin F showed a significantly higher percentage of cell death compared to wild type cyclin F and the cyclin F polymorphisms. Ubiquitin-positive cytoplasmic inclusions are a characteristic pathological feature seen in the affected neurons of the brain and spinal cord of both familial and sporadic ALS and ALS-FTD patients. Using immunohistochemical staining, we observed mislocalisation of TDP-43 from the nucleus to cytoplasm, and ubiquitin- and TDP-43-positive cytoplasmic inclusions in post mortem brain stem and spinal motor neurons of a patient with an ALS-linked CCNF mutation. Taken together, we have provided in vitro observations of aberrant cyclin F function that mimic pathogenic features seen in familial and sporadic ALS and support dysfunction of the proteostasis network in ALS pathogenesis.

Disclosures: S. Yang: None. K.Y. Zhang: None. N. Farrawell: None. V. Sundaramoorthy: None. S.T. Warraich: None. A. Rábano: None. A. García-Redondo: None. J.D. Atkin: None. J.J. Yerbury: None. G.A. Nicholson: None. I. Blair: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.15/T15

Topic: C.05. Neuromuscular Diseases

Title: Development and functional applications of human iPSC-derived spinal motor neurons

Authors: E. JONES[1], C. CHAVEZ[1], B. MELINE[1], J. LIU[1], M. MCLACHLAN[1], T. BURKE[1], C. MCMAHON[1], *L. CHASE[2], W. WANG[1];
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Abstract: The aim of this study was to produce spinal motor neurons from human induced pluripotent stem cells (iPSCs) with sufficient purity for use in a multitude of downstream assays including electrophysiological recordings using a multi-electrode array (MEA) system. In particular we wanted to produce motor neurons that could be cultured in defined conditions over long periods of time, without being hampered by outgrowth from proliferative cell types. Using an optimized 3D differentiation protocol that improves upon published methods, we were able to produce motor neurons from iPSCs at greater than 60% purity as measured by Isl 1/2 and Tuj1 positive staining. These cells can be stored frozen, thawed, and cultured in media without glia for extended periods, simplifying experimental design and data interpretation. We collected ICC, qPCR and MEA data to characterize the motor neuron cells and used iPSC lines from multiple donors to demonstrate a robust protocol that produces motor neurons independent of donor iPSC line. In addition, genetically modified iPSC lines were generated to create an isogenic disease model for Amyotrophic Lateral Sclerosis. These data show the characteristics and utilization of motor neurons produced from iPSC.

Disclosures: E. Jones: None. C. Chavez: None. B. Meline: None. J. Liu: None. M. McLachlan: None. T. Burke: None. C. McMahon: None. L. Chase: None. W. Wang: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.16/T16

Topic: C.05. Neuromuscular Diseases

Support: Target ALS

William Randolph Hearst

Title: Identification of potassium channel subtypes involved in human ALS hyperexcitability by patch-seq and patch-RT-qPCR

Authors: *S. LEE[1,2], O. WISKOW[3], S. GHOSH[3], K. ROET[1,3], X. HWANG[1], B. P. BEAN[2], B. J. WAINGER[4], K. EGGAN[3], C. J. WOOLF[1,2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is associated with hyperexcitability in cortical and spinal motor neurons. We have demonstrated a hyperexcitability phenotype in motor neurons derived from ALS patient iPSC lines that harbor SOD1 and FUS mutations and C9orf72 repeat expansions and have shown that inhibition of this hyperexcitability using the potassium channel (Kv7) opener retigabine, reduces motor neuron cell death, supporting a pathogenic role (Wainger et al., Cell Reports, 2014). However, the mechanisms responsible for the hyperexcitability remain unresolved. To tackle this we have recently developed patch-seq and patch-RT-qPCR techniques for linking neuronal excitability and gene expression at the single cell level, collecting RNA after whole cell patch clamp recordings from motor neurons and performing single cell next generation sequencing or high-throughput multiplex qPCR. Single cell gene expression profiling in functionally characterized neurons was conducted on HB9::GFP sorted iPSC-derived motor neurons from an ALS patient carrying the SOD1 A4V mutation (39b) and isogenic control motor neurons with the mutation corrected (39b-cor). We found by whole cell current clamp recording that 39b motor neurons have larger cell sizes and robust hyperexcitability compared to 39b-cor motor neurons. Both single cell RNA-seq and ion channel targeted multiplexed high throughput qPCR analysis independently show a significant down-regulation of a K channel in hyperexcitable ALS motor neurons from this patient and its expression is negatively correlated with neuronal excitability. The hyperexcitability and the K channel subtype down-regulation were replicated in an independent batch of motor neurons from the same line. These results suggest the reduced expression of a K channel as a molecular mechanism for the increased excitability in familial ALS.

Disclosures: S. Lee: None. O. Wiskow: None. S. Ghosh: None. K. Roet: None. X. Hwang: None. B.P. Bean: None. B.J. Wainger: None. K. Eggan: None. C.J. Woolf: None.

Poster

227. Pathogenic Mechanisms of Motor Neuron Disease

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 227.17/T17

Topic: C.05. Neuromuscular Diseases

Title: Dynactin mutations associated with amyotrophic lateral sclerosis and their effect on axonal transport and neuromuscular junction formation

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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, which is mainly sporadic in nature. This progressive pathology has an estimated incidence of 1:1000 and generally leads to death within 2-5 years of diagnosis due to muscle wasting and severe motor neuron loss. Over the last years, mutations have been identified in both sporadic and familial ALS patients, interfering with the function of many genes, including *DCTN1*, which encodes for a subunit of the motor protein complex component dynactin. The dynactin complex serves as an adaptor for the dynein motor complex, responsible for retrograde axonal transport, and it is believed to regulate dynein activity and the binding capacity for cargos. Interestingly, axonal transport deficits have been reported in various neurodegenerative diseases owing to the fact that neurons are highly polarized cells that depend on active axonal transport for growth, establishment and maintenance of synapses.
Defects in transport of material for development or clearance of detritus in the axon can lead to neuronal stress and cell death and could arise from different causes: preferential type of transport, varying load size, and depletion or dilution of the motor protein populations.
In order to determine how retrograde axonal transport is involved in the pathogenesis of ALS, we are characterizing a mutant zebrafish line for *dctn1* with regard to axonal development of primary motor neurons, formation and stability of the neuromuscular junction (NMJ) and the behavioral phenotype produced in embryos.
Fast axonal transport defects are quantified in primary motor neurons using the GAL/UAS bipartite system and fusion protein tracking *in vivo* by confocal timelapse microscopy. We are investigating the transport dynamics of cargos such as endosomes, mitochondria, synaptic vesicles and neurotrophic receptors in the motor neurons of wild-type versus *dctn1* mutant

embryos *in vivo*, and over time. As dynactin was reported to be essential to synapse stability, we are examining the formation and maintenance of the NMJ with techniques such as immunohistochemistry, GCaMP calcium imaging, and muscle-motor neuron paired recordings. We hope to elucidate key molecular mechanisms in ALS etiology by revealing the role of dynein in NMJ maintenance and identifying novel regulatory events in axon degeneration and muscle atrophy along disease progression.

Disclosures: V. Bercier: None. T.O. Auer: None. K. Duroure: None. F. Del Bene: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.01/T18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KU Grant No. SL03/14

KFAS 2013-1207-01C

Title: Catfish skin preparations ameliorates neurobehavioral and histopathological alterations of the sciatic nerve following crush injury

Authors: *W. M. RENNO[1], M. AFZAL[2], B. M. PAUL[2], J. M. AL-HASSAN[2];
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Abstract: Our preliminary investigations showed that fractions from catfish skin preparations (CSP) from the Arabian Gulf Catfish (Arius bilineatus, Val.) exhibit potent anti-inflammatory and healing properties as shown in our previous clinical trials for the healing of non-healing diabetic foot ulcers, chronic back pain, and some other neurological disorders. In this study, we examined the neurobehavioral parameters and histomorphological changes in the sciatic nerve crush injury model following CSP treatment. **Methods:** Adult (4 months) male Wistar rats (n= 8) were randomly assigned to five groups: (i) Control naïve (NAIVE) group; (ii) Control Sham (SHAM) group; (iii) Sciatic nerve crush+Saline (CRUSH) group; (iv) Sciatic nerve crush+CSP1 subcutaneously (s.c.) injected (CRUSH+CSP1) group; (v) Sciatic nerve crush+CSP2 intraperitoneally (i.p.) injected (CRUSH+CSP2) group. Rats in CRUSH+CSP1 and two groups underwent surgery where in sciatic nerve was crushed but treated with 6 mg/Kg CSP s.c. and i.p., respectively, for 11 days. **Results:** Administration of CSP s.c. or i.p. to the nerve injured rats significantly improved their performance on different neurobehavioral functional tests. CRUSH+CSP1 and CRUSH+CSP2 groups showed significant recovery in foot position and toe-

spread analyses following injury. Likewise, CSP-treated animals exhibited improvements in motor recovery as measured by the extensor postural thrust (EPT) and hopping response. The CRUSH+CSP groups showed a significant reduction in the mechanical hyperalgesia threshold, heat withdrawal reflex and tail flick withdrawal latency tests compared to the CRUSH group. CSP treatment remarkably ameliorated the neurodegenerative changes seen in Cresyl violet stained ventral gray horn region of the spinal cord sections ipsilateral to the nerve injury. Further, CSP treatment significantly prevented the decrease in the number of the NeuN immunoreactive spinal neurons ipsilateral to the crush injury. The stereological evaluation revealed significant histomorphological evidence of neuroregeneration in the sciatic nerve of the CRUSH+CSP groups compared to controls at 4 and 6 weeks postoperatively. CSP treatment increased the axon area, myelin thickness, fiber diameter to axon diameter ratio and the myelin thickness to axon diameter ratio. **Summary:** The data analysis supports our hypothesis that CSP treatment lessens neurobehavioral deficits and stimulates regeneration of the axons and histomorphological alterations following nerve injury. Further, CSP protects spinal neurons and enhances subcellular recovery after peripheral nerve injury and thus improves nerve regeneration.

Disclosures: W.M. Renno: None. M. Afzal: None. B.M. Paul: None. J.M. Al-Hassan: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.02/U1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KAKENHI 25460056

KAKENHI 16K07048

Fund from Nukada Inst Med Biol Res

Title: Galectin-3 as a cytoprotective molecules in Schwann cells under diabetic conditions

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Abstract: Accumulation of advanced glycation end products (AGEs) is involved in the pathogenesis of diabetic complications, such as neuropathy, nephropathy and retinopathy. Galectin-3 (GAL-3), a member of a family of β-galactoside binding animal lectins, is identified as an AGE-binding protein and appears to play a protective role against diabetic nephropathy by the removal of the increased amounts of glomerular AGEs. However, functional roles of GAL-3

in the peripheral nervous system (PNS) remain unclear. In this study, we aimed to elucidate the precise localization and possible functions of GAL-3 in the PNS under normal and diabetic conditions. Immunohistochemical analysis revealed that the GAL-3 was predominantly expressed in isolectin B4-binding small non-peptidergic neurons and some of S100-positive Schwann cells in the sections of adult rat dorsal root ganglia (DRG). Next, we conducted western blot analysis to investigate the protein expression of GAL-3 in immortalized adult rat Schwann cells IFRS1 under normal and diabetic conditions. Exposure of the IFRS1 cells to 30 mM of glucose or 0.2 mM of an AGE precursor 3-deoxyglucosone (3-DG) for 7 days up-regulated the expression of GAL-3, 2.1-fold and 2.5-fold as compared with control (5.6 mM of glucose), respectively. The high glucose-induced up-regulation of GAL-3 was suppressed by co-treatment with an anti-glycated agent aminoguanidine (0.2 mM) or an antioxidant trans-resveratrol (20 nM). In addition, treatment of the IFRS1 cells with recombinant GAL-3 (1 μg/mL) for 2 days significantly up-regulated the expression of an anti-apoptotic marker Bcl-2 and down-regulated the expression of an oxidative stress marker 4-hydroxynonenal, 1.5-fold and 0.7-fold as compared with control, respectively. Furthermore, knockdown of GAL-3 mRNA expression by RNA interference resulted in the significant decreases in the cell viability ratios after 7 days exposure to the diabetic conditions as compared with mock (transfected control siRNA); from 0.83 to 0.69 at 30 mM of glucose, from 0.72 to 0.57 at 0.2 mM of 3-DG, and from 0.64 to 0.37 at 0.5 mM of 3-DG, respectively. These findings imply that GAL-3 induced by glycation and oxidative stress under the diabetic conditions exhibit a cytoprotective property in Schwann cells.

Disclosures: H. Yako: None. N. Niimi: None. M. Tsukamoto: None. K. Sango: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.03/U2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work is supported by a GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 160813).

Title: A small heat shock protein protects against vincristine-induced peripheral axonal damage in mice.

Authors: *V. B. CHINE[1], G. KUMAR[1], C. H. E. MA[1,2];
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Abstract: Development of sensorimotor painful peripheral neuropathy is evident in cancer patient treated with Vincristine often results in the withdrawal of cancer treatment. Vincristine is commonly used for treatment of lung cancer, leukemia, and blood-related disorders. Vincristine depolymerizes microtubules resulting in disruption of microtubule dynamics. This disruption leads to inefficient axonal transport and thereby causing peripheral nerve degeneration. Our previous study reported the reduced axonal growth and disruption of cytoskeleton in dorsal root ganglia (DRG) neurons after Vincristine treatment. Vincristine causes terminal arbor degeneration and axonal loss which results in decreasing intra-epidermal nerve fiber density (IENFD). Reduction in sensory nerve action potential (SNAP) amplitude, nerve conduction velocity (NCV) and compound muscle action potential (CMAP) were observed, which was consistent with the development of mechanical allodynia detected in mice after Vincristine treatment. Heat shock protein 27 (Hsp27) is a ubiquitously expressing chaperone acting as anti-oxidant and anti-apoptotic in cellular stress conditions. Previously we have shown accelerated axonal regeneration in mouse with better motor and sensory functional recovery by overexpression of human Hsp27 (hHsp27) specifically in both sensory and motor neurons. Vincristine induced peripheral neuropathy model was developed by injecting vincristine sulfate intraperitoneally to hHsp27 transgenic (hHsp27 Tg) and their littermate (LM) mice while vehicle control (VH) mice were injected with saline. We assessed mechanical and cold allodynia and found that hHsp27 Tg group recovered significantly from allodynia while in LM group both mechanical and cold allodynia persisted till day 28. Moreover immunohistochemical evaluation of IENFD, myelin basic protein and neuromuscular junctions analysis also showed significant protection in hHsp27 Tg mice than in LM mice. A number of electrophysiological parameters such as SNAP, NCV and CMAP were better preserved in hHsp27 Tg compared with LM controls after treated with vincristine. Our results clearly showed that overexpression of hHsp27 can protect against vincristine-induced peripheral neuropathy in mice. Further studies offer possible therapeutic opportunities to prevent the devastation caused by CIPN.

Disclosures: V.B. Chine: None. G. Kumar: None. C.H.E. Ma: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.04/U3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Calico Life Sciences LLC

CTRC- UTHSCSA Pilot Project

Title: Neuroprotective effects of P7C3-A20 in a model of paclitaxel-induced peripheral neuropathy

Authors: P. M. LOCOCO, H. R. SMITH, J. C. ZAMORA, T. A. CHAVERA, K. A. BERG, *W. P. CLARKE;
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Abstract: Paclitaxel (PTX), a microtubule-targeting anticancer agent, produces a debilitating peripheral neuropathy that is accompanied by neuropathic pain. Because effective therapies do not exist, patients often choose to reduce life-saving chemotherapy to cope with the pain. We have reported that the aminopropyl carbazole, P7C3-A20, prevents the development of PTX-induced neuropathic pain in rats. To further characterize the neuroprotective efficacy of P7C3-A20, we performed a dose-response study using our rat behavioral model of PTX-induced peripheral neuropathy. P7C3-A20 (2.2, 6.6, or 20 mg/kg, i.p., q.d.) or vehicle was administered to male Sprague-Dawley rats over a 16-day period. After two days of treatment, PTX (11.7 mg/kg, i.p.) or vehicle was injected every other day for three days. Nociceptive thresholds to mechanical and cold stimuli were measured periodically throughout the experimental period. P7C3-A20 dose-dependently attenuated PTX-induced mechanical and cold allodynia. Immunohistological analysis of intraepidermal nerve fiber (IENF) density in paw biopsies revealed that the PTX-induced reduction of IENF density was also dose-dependently reduced by P7C3-A20. Statistical analyses indicated strong correlations between IENF density and nociceptive threshold to mechanical and cold stimuli. Previously, P7C3-A20 was shown in vitro to stimulate NAMPT, a critical enzyme in the NAD salvage pathway. Thus, we sought to determine whether the protective effects of P7C3-A20 could be antagonized in vivo by the selective NAMPT inhibitor, FK866. Using the same paradigm, we incorporated daily injections of FK866 (0.5 mg/kg, i.p., b.i.d.) along with P7C3-A20 (10 mg/kg). As before, P7C3-A20 prevented the development of mechanical allodynia compared to PTX controls. By contrast, rats co-injected with FK866 and P7C3-A20 displayed persistent mechanical allodynia, indicating that FK866 blocked the protective effects of P7C3-A20. Analysis of IENF densities revealed that FK866 also blocked preservation of nociceptive fibers by P7C3-A20. Collectively, these data suggest that the protective effects of P7C3-A20 require function of NAMPT.

Disclosures: P.M. LoCoco: None. **H.R. Smith:** None. **J.C. Zamora:** None. **T.A. Chavera:** None. **K.A. Berg:** None. **W.P. Clarke:** 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; Calico Life Sciences.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.05/U4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Air Force Office of Scientific Research (EOARD, London, UK),

Air Force Material Command, USAF, under grant number FA8655-05-1-3065

Swedish Medical Research Council (Nr 2710-HSS)

Swedish Strategic Research Foundation, Stockholm, Sweden

India-EU Research Program Neuroscience Research

India-EU Grant Support, Ministry Of Biotechnology, Govt. of India

The Indian Medical Research Council, New Delhi, Govt of India

Title: Diabetes exacerbates methamphetamine induced blood-brain barrier breakdown, edema formation, oxidative stress and myelin damage. Neuroprotective effects of nanowired delivery of antioxidant compound H-290/51

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Abstract: Methamphetamine (Meth) is the most abused drug and often consumed for pleasure in healthy and/or people suffering from endocrine or cardiovascular diseases. Meth induces severe oxidative stress in the brain leading to brain pathology. Likewise oxidative stress also induces brain damage in diabetics. Whether Meth use in diabetics results in exacerbation of brain damage is still unclear. In this investigation we examined Meth induced brain damage and oxidative stress in diabetes in our rat model.
Rats were made diabetic by administration of streptozotocine (75 mg/kg/day for 3 days, i.p.)

resulting in blood glucose level to 22 to 26 mM/L within 4 to 6 weeks. In these diabetic rats Meth was administered 10 mg/kg, s.c. and brain pathology, e.g., blood-brain barrier (BBB) breakdown to Evans blue albumin and radioiodine ($^{[131}$-I]), brain edema formation and neuronal, glial and axonal injuries were examined. Immunostaining for Myelin basic protein (MBP) and histological staining of Luxol Fast Blue (LFB) to study axonal degradation, glial fibrillary acidic protein (GFAP) for astrocytic activation and histopathological techniques of Nissl or H&E stain for neuronal injuries were carried out on paraffin sections. Immunohistochemical analyses of enzymes responsible for free radical gas nitric oxide i.e., neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) were examined on paraffin sections.
Our observations showed about 3- to 5 fold higher BBB breakdown and 2- to 4-fold greater volume swelling and brain edema formation was seen in diabetic rats after Meth exposure as compared to healthy animals. Interestingly, neuronal, glial cell and axonal injuries were exacerbated by 4- to 6-fold in Meth treated diabetic rats as compared to normal healthy animals. Thus, neuronal damage, astrocytic activation and myelin vesiculation were most prominent in the hippocampus, thalamus, hypothalamus, cerebellum and cerebral cortex. These brain areas also exhibited marked increase in nNOS and iNOS expression. The magnitude and severity of iNOS and nNOS expression was 2- to 3-fold higher in diabetic rats after Meth administration. These neuropathological changes and NOS expression in diabetic rats were significantly reduced by TiO2-nanowired delivery of H-290/51 (50 mg/kg, i.p.) 30 min after Meth administration in diabetic rats. Whereas normal H-290/51 was enough to induce neuroprotection in Meth treated healthy animals. These observations are the first to point out that diabetes exacerbate Meth induced neuropathology via oxidative stress and nanodelivery of antioxidant compound is needed for neuroprotection in such situation, nor reported earlier.

Disclosures: J.V. Lafuente: None. A. Sharma: None. E.A. Kiyatkin: None. D. Muresanu: None. A. Nozari: None. P. Sjoquist: None. R. Patnaik: None. A. Ozkizilcik: None. R. Tian: None. H.S. Sharma: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.06/U5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDDK Grant DK017844

DK95449

DK95440

DK059803

Title: Central insulin attenuates diet-induced hypothalamic inflammation

Authors: *A. MAY[1], N. D. BEDEL[2], D. J. CLEGG[3], M. WORTMAN[2], S. WOODS[2], M. LIU[2]; [1]Pathology, [2]Univ. of Cincinnati, Cincinnati, OH; [3]Cedars-Sinai, Los Angeles, CA

Abstract: Inflammation of the hypothalamus promotes weight gain in rodent models of diet-induced obesity and directly contributes to the co-morbidities of obesity, including type II diabetes and cardiovascular disease. Insulin acts within the brain to prevent inflammation in mouse models of LPS-induced sepsis and Alzheimer's disease. However, the effect of insulin on diet-induced inflammation is not yet known. Several clinical studies have demonstrated that intranasal insulin directly enters the brain and effectively reduces body weight and improves cognition in humans. This same approach might also be effective at preventing diet-induced inflammation. Here, we tested the hypothesis that centrally-administered insulin acts as an anti-inflammatory signal in the hypothalamus of rodents during diet-induced inflammation. First, we examined the effects of insulin on inflammatory signaling in cultured hypothalamic neurons. Physiological levels of Insulin increased the phosphorylation of the inflammatory signaling molecule, IKKβ, which is indicative of increased activation of the NF-κB signaling pathway. Since cell culture exposes neurons to levels of glucose and other nutrients that are not physiologically-relevant, we followed-up on these findings *in vivo*, by injecting insulin directly into the third cerebral ventricle to target the hypothalamus of conscious rats. The dose of insulin used in this study has been demonstrated to reduce food intake and body weight in our lab. In contrast to our findings from cell culture, insulin did not induce inflammation relative to controls injected with artificial cerebrospinal fluid (aCSF). In contrast, rats injected with LPS at the same time had significantly higher levels of inflammatory cytokines within this same timeframe. These results provide strong evidence that insulin does not induce hypothalamic inflammation. We next examined the effects of insulin administration into the brain during high-fat diet feeding. Several rodent studies have demonstrated that a 60% high-fat diet reliably induces hypothalamic inflammation within just 1-3 days, relative to controls fed a standard chow diet. We surgically implanted rats with intracerebroventricular cannulas targeting the lateral ventricle and provided a 2-week recovery period prior to the study. As expected, short-term high-fat-diet feeding increased hypothalamic inflammation relative to chow-fed rats. Under these conditions, repeated insulin administration attenuated hypothalamic inflammation relative to aCSF-injected controls, suggesting that insulin may provide clinical benefits by reducing hypothalamic inflammation.

Disclosures: A. May: None. N.D. Bedel: None. D.J. Clegg: None. M. Wortman: None. S. Woods: None. M. Liu: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.07/U6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grants-in-Aid for Scientific Research 16K07076

Title: Dietary restriction promotes cell survival in a mouse model of normal tension glaucoma

Authors: *X. GUO, K. NAMEKATA, A. KIMURA, T. NORO, G. AKIYAMA, Y. AZUCHI, C. HARADA, T. HARADA;
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Abstract: Glaucoma, one of the leading causes of vision loss in the world, is characterized by progressive degeneration of retinal ganglion cells (RGCs) and their axons. Glaucoma is usually associated with elevated intraocular pressure, but there is a subset of glaucoma termed normal tension glaucoma (NTG) that presents with statistically normal intraocular pressure. We previously reported that loss of glutamate transporters (EAAC1 or GLAST) in mice leads to RGC degeneration that is similar to NTG and these animal models have been useful in examining potential therapeutic targets.
Dietary restriction has been reported to increase longevity and has some benefits in injury and disease. Among them, every-other-day fasting (EODF), a form of dietary restriction, has been shown to be neuroprotective when being implemented after rat cervical spinal cord injury. Here we investigated the effects of EODF on RGC protection in EAAC1 KO mice.
In the EAAC1 KO mouse retina, the number of RGCs was significantly decreased and this effect was suppressed with EODF treatment. We also visualized retinal layers in living mice using spectral-domain optical coherence tomography (SD-OCT), a noninvasive imaging technique that can be used to acquire cross-sectional tomographic images of the retina in vivo. The average thickness of the inner retinal layers was decreased in EAAC1 KO mouse eyes, but it was unchanged in EODF treated eyes. Consistent with histological analyses, the visual impairment observed in EAAC1 KO mice, as assessed by multifocal electroretinograms (mfERGs), an established noninvasive method for reliably measuring visual function, was inhibited with EODF treatment, indicating the functional significance of the neuroprotective effect of EODF. We then explored possible mechanisms associated with EODF-mediated neuroprotection. As expected, blood β-hydroxybutyrate levels, a ketone known to be neuroprotective and a histone deacetylase inhibitor, were increased significantly on the fasting days. Consistently, acetylation of histones was upregulated in the EODF retina, and so were the expression levels of neurotrophic factors such as the basic fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF), and catalase, an antioxidant gene. Furthermore, 4-hydroxynonenal (4-HNE), which represents

oxidative stress levels, was reduced in the EODF retina. Our findings suggest that EODF, a safe, non-invasive, and low-cost treatment, may be available for RGC protection during glaucoma.

Disclosures: X. Guo: None. K. Namekata: None. A. Kimura: None. T. Noro: None. G. Akiyama: None. Y. Azuchi: None. C. Harada: None. T. Harada: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.08/U7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Intramural Research Program of the National Eye Institute, NIH

Title: PDGF-AA-mediated retinal ganglion cell neuroprotection results from its interaction with retinal astrocytes and/or a subtype of amacrine cells

Authors: *S. I. TOMAREV[1], S. TAKAHAMA[1], M. O. ADETUNJI[1], T. ZHAO[2], W. LI[2]; [1]SRGCB, LRCMB, [2]RNS, NEI, NIH, Bethesda, MD

Abstract: Glaucoma, a group of chronic, degenerative optic neuropathies, is a major cause of blindness worldwide. Retinal ganglion cell (RGC) death is one of the pathogenic features of glaucoma. Our previous study showed that treatment of retinae with platelet-derived growth factor AA (PDGF-AA) rescued RGCs from death in a rat glaucoma model and retinal explant cultures. PDGF-AA acts through interaction with PDGF receptor alpha (PDGFRα). However, the molecular mechanisms of PDGFRα-mediated neuroprotection in the retina are poorly understood. To elucidate these mechanisms, we used RGC primary cultures and PDGFRα-EGFP mice in which nuclear-targeted EGFP is expressed under the control of the PDGFRα promoter. RGCs isolated using a standard immunopanning protocol from postnatal day 4-6 mice did not express PDGFRα, and addition of PDGF-AA to RGC cultures did not protect RGCs, while addition of a mixture of known neuroprotective factors, BDNF and CNTF, improved survival of RGCs. Similarly, no expression of PDGFRα was detected in RGCs in the adult mouse retina. PDGFRα was detected in astrocytes in the RGC layer and in a GABAergic subtype of amacrine cells localized to the inner nuclear layer. These data suggest that the neuroprotective effect of PDGF-AA is mediated by astrocytes and/or a subtype of amacrine cells that secrete factors protecting RGCs.

Disclosures: S.I. Tomarev: None. S. Takahama: None. M.O. Adetunji: None. T. Zhao: None. W. Li: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.09/U8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: ANPCYT PICT 1563

CONICET PIP 0446

Title: Visual imput is a necessary condition in enriched environment induced neuroprotection against acute retinal ischemia

Authors: *D. DORFMAN, M. L. ARANDA, M. F. GONZÁLEZ FLEITAS, M. S. CHIANELLI, M. I. KELLER SARMIENTO, H. H. DIEGUEZ, P. H. SANDE, R. E. ROSENSTEIN;
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Abstract: Enriched environment (EE) constitutes a strategy that boosts locomotor, exploratory, visual, and cognitive activities, as well as social interaction and voluntary physical exercise. Ischemia is a key component of several retinal diseases that are leading causes of irreversible blindness. In previous work, we have shown that the exposure to EE protects the retina against acute unilateral ischemia in adult rats. In this work, we analyzed the involvement of visual processing in the protection induced by EE against retinal ischemic damage. Methods: Adult male Wistar rats were submitted to acute unilateral or bilateral ischemia by increasing intraocular pressure to 120 mm Hg for 40 min. After unilateral ischemia, animals were exposed to a standard environment (SE), a novelty environment (NE), or an EE for 3 weeks. NE consisted in standard laboratory cages, housing 2 animals and containing novelty objects that were weekly substituted, while EE consisted of big cages housing 6 animals, and containing food hoppers, wheels and different objects repositioned once/day and fully substituted once/week. Animals submitted to bilateral ischemia were exposed to SE or EE for 3 weeks. Retinal function (electroretinography, ERG), and histology were analysed at 3 weeks post-ischemia. Locomotor activity in EE was analysed in animals exposed to unilateral or bilateral ischemia. The expression of c-fos (immunofluorescence) in the retino-recipient layers of the superior colliculi (SC) was assessed in control animals, and animals submitted to unilateral or bilateral ischemia at 24 h of exposure to EE. Results: Unilateral ischemia induced a significant decrease in scotopic ERG response, and clear histopathological alterations. NE was ineffective in protecting the retinal function and structure against unilateral ischemia. EE, which preserved retinal function and histology against unilateral ischemia, did not protect the retina when ischemia was bilaterally induced, regardless that locomotor activity did not differ among groups. In animals submitted to unilateral ischemia, EE induced c-fos expression in the SC superficial layers receiving projections from control and

ischemic eyes. However, when ischemia was bilaterally induced, c-fos expression was absent in the superficial layers of the SC receiving projections from both retinas. Conclusions: These results suggest that, at least for the visual pathway, the protection induced by EE could involve visual processing.

Disclosures: D. Dorfman: None. M.L. Aranda: None. M.F. González Fleitas: None. M.S. Chianelli: None. M.I. Keller Sarmiento: None. H.H. Dieguez: None. P.H. Sande: None. R.E. Rosenstein: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.10/U9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grant Number 15K06781

Title: Neuroprotective functions of apolipoprotein E-containing lipoproteins in neurons and glia of retina

Authors: *H. HAYASHI, M. MORI, Y. BAN, B. YUAN, N. TAKAGI;
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Abstract: Lipoproteins play crucial roles for maintaining lipid metabolism and transport in the peripheral circulation as well as the central nervous system (CNS). In the CNS, glia secrete lipoproteins in high density lipoprotein-like particles containing apolipoprotein E (apo E). It has been reported that cholesterol included in apo E-containing lipoproteins induces synaptogenesis in CNS neurons and apo E-containing lipoproteins produced by glia promote axon growth of retinal ganglion cells (RGCs) in a low density lipoprotein receptor-related protein 1 (LRP1)-mediated process. It has also been known that alpha2-macroglobulin (a2M), one of the LRP1 ligands, is increased in aqueous humor of glaucoma patients, and we also detected the increase of a2M in vitreous humor of glutamate-aspartate transporter-deficient mice, an animal model of glaucoma. Here, we show evidence that apo E-containing lipoproteins protect RGCs via LRP1 from apoptosis induced by glutamate in vitro and in vivo and decrease a secretion of alpha2-macroglobulin (a2M) from primary cultured glia. The neuroprotective effect of apo E-containing lipoproteins via LRP1 was provided by blocking calcium overload through *N*-methyl-D-aspartate receptor in primary cultured RGCs. This protective effect was interfered with a2M, but exogenous treatment of apo E-containing lipoproteins overcame the inhibition. In addition, intravitreal administration of apo E-containing lipoproteins prevented retinal degeneration led by

N-methyl-D-aspartate injection into vitreous humor of rats. On the other hand, apo E-containing lipoproteins attenuated mRNA and protein expressions of a2M in primary cultured retinal glia. A2M secretion from cultured glia to conditioned medium was also decreased by addition of apo E-containing lipoproteins. These findings indicate a potential therapeutic approach of apo E-containing lipoproteins or LRP1 agonists as treatment for nerve injuries and neurodegenerative disorders in the retina.

Disclosures: H. Hayashi: None. M. Mori: None. Y. Ban: None. B. Yuan: None. N. Takagi: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.11/U10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG Grant GRK 1885

Title: Tubastatin A, a specific inhibitor of histone deacetylase 6, protects retinal cells against oxidative stress

Authors: *J. LEYK, U. JANSSEN-BIENHOLD, C. RICHTER-LANDSBERG;
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Abstract: Retinal degenerative disorders, such as age-related macular degeneration and retinitis pigmentosa (RP) are characterized by progressive loss of photoreceptors. Recent studies suggest a role for histone deacetylases (HDACs) in neuro- and retinal degeneration. In a mouse model for RP excessive HDAC activation was observed and inhibition of HDAC activity prevented photoreceptor cell death. Within the HDAC family, HDAC6 is unique since it mainly deacetylates non-histone proteins like α-tubulin, heat shock protein (HSP) 90 and the redox regulatory protein peroxiredoxin 1 (Prx1), which is involved in the reduction of hydrogen peroxide (H_2O_2). HDAC6 plays a role in cellular stress responses and its inhibition has been demonstrated to be protective in models for neurodegeneration. The aim of the present study was to elucidate the influence of HDAC6 inhibition by tubastatin A (TST) in 661W cells, a cell line with characteristics of cone photoreceptors, and its protective role during oxidative stress. The presence of HDAC6 was investigated in retina lysates and slices of C57BL/6 mouse retinae. Immunohistochemistry revealed that HDAC6 is present in the retina of C57BL/6 mice and prominently expressed in photoreceptor inner segments as well as in the outer plexiform layer. It is present in 661W cells and its inhibition by TST results in hyperacetylation of α-tubulin. In

661W cells oxidative stress, exerted by treatment with H_2O_2, caused cytotoxic responses. Preincubation with TST promoted cell survival after H_2O_2 treatment and enhanced the expression of HSP25 and HSP70 by activating HSF1 (heat shock factor 1). The protective effect, however, is not causally related to the induction of HSF1, as demonstrated by incubation with KRIBB11, an HSF1-inhibitor. Immunoblot analysis revealed that H_2O_2 treatment leads to a strong overoxidation and thereby to the inhibition of Prx1. This effect was significantly reduced after preincubation with TST. Thus, HDAC6 inhibition by TST is protective against oxidative stress by regulating the activity of Prx1. In conclusion, HDAC6 inhibition provides a protective means against a stress situation which occurs in retinal degenerative diseases.

Disclosures: J. Leyk: None. U. Janssen-Bienhold: None. C. Richter-Landsberg: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.12/U11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS081333

NIH Grant EY013933

Title: Caspase-9 inhibition abrogates retinal edema and provides functional neuroprotection

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Abstract: Macular edema results from fluid accumulation in the macula due to leaky blood vessels, and is a common complication of diabetes and retinal vein occlusion (RVO). Despite available therapies, an estimated 50% of patients do not respond to treatment. We have identified caspase-9, a protease traditionally associated with apoptosis, as an essential component in edema formation. To test the therapeutic potential of modulating caspase-9 activity in retinal edema, we developed a highly-specific caspase-9 inhibitor. We employed a mouse model of central retinal vein occlusion (CRVO). CRVO was achieved by tail-vein injection of rose bengal, followed by laser photocoagulation of retinal veins. In vivo analyses – optical coherence tomography (OCT), fluorescein angiography and electroretinography (ERGs) - were conducted with the Micron IV system (Phoenix Research Labs). CRVO induces acute retinal edema, which peaks during the first 24 hours following injury. Expression of caspase-9 and VEGF are increased as measured by

western blot and immunohistochemistry. Caspase-9 localizes with endothelial cell markers. Over a 7 day time course the edema resolves, revealing a permanent retinal thinning. At 2 days post-CRVO, TUNEL+ cells indicate significant cell death in the outer nuclear layer. To inhibit caspase-9 activity we employed our novel cell permeant caspase-9 inhibitor and delivered it topically using eyedrops. Topical delivery provides rapid uptake of the inhibitor by the retina. Treatment of animals with the caspase-9 inhibitor eyedrops immediately after induction of CRVO provides morphologic, biochemical and functional protection. Caspase-9 inhibition abrogates the increase in retinal thickness, fewer TUNEL+ cells are detected, and ERGs show functional neuroprotection. Our studies indicate that active caspase-9 plays an essential role in regulating edema pathogenesis. Moreover, our novel cell permeant caspase-9 inhibitor abrogates the edema and may be a potential novel therapy for individuals suffering from macular edema.

Disclosures: M.I. Avrutsky: None. Y.Y. Jean: None. S. Snipas: None. G.S. Salvesen: None. C.M. Troy: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.13/U12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P30 EY006360

NIH Grant R01 EY004864

Department of Veterans Affairs Rehabilitation R&D Service Merit Award E0951-R

Research Career Scientist Award C9257

Research to Prevent Blindness (Departmental Award)

Title: Brain-derived neurotrophic factor-mediated neuroprotection of retinal and cognitive function in exercised diabetic rats

Authors: *A. HANIF[1], R. ALLEN[1], M. GOGNIAT[1], B. PRALL[1], R. HAIDER[1], M. AUNG[1], M. PRUNTY[1], L. MEES[1], C. SIDHU[2], L. HE[2], M. IUVONE[2], M. PARDUE[1,2,3];
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Abstract: While exercise has been shown to have protective effects in the retina and in diabetes, the effects of exercise on retinal and cognitive function in diabetes have not been studied. We hypothesized that treadmill exercise would reduce early visual, retinal and cognitive deficits in the STZ (streptozotocin) rat model of Type I diabetes. We further hypothesized that if BDNF mediates the protective role of exercise in diabetes, treatment with the TrkB inhibitor ANA-12 would abolish this protective effect. Long-Evans rats were assigned to one of four diabetic or control groups (n = 7-36): active + vehicle, inactive + vehicle, active + ANA-12, and inactive + ANA-12. Hyperglycemia was induced using STZ (100mg/kg) with fed glucose levels greater than 250 mg/dL considered diabetic. Rats received treadmill exercise for 30 minutes a day, 5 days per week, at a speed of 0 m/min (inactive groups) or 15 m/min (active groups) for 8 weeks. ANA-12, a TrkB BDNF receptor antagonist, or vehicle was injected 2.5 h before exercise training. Spatial frequency and contrast sensitivity were assessed via optokinetic tracking at 0, 2, 4, 6, and 8 weeks post-STZ. Retinal function was assessed via electroretinogram at 4 and 8 weeks. Cognitive function and exploratory behavior were assessed via y-maze at 8 weeks. Compared with non-diabetic controls, diabetic rats showed reduced spatial frequency ($p < 0.001$) and contrast sensitivity ($p < 0.001$) on an optokinetic tracking task by 4 wks post-STZ, delays in oscillatory potential ($p < 0.05$) by 4 wks post-STZ, and flicker ERG implicit times ($p < 0.05$) by 8 wks post-STZ. Reduced cognitive function ($p < 0.001$) and exploratory behavior ($p < 0.01$, Main effect) on a y-maze task were observed by 8 weeks post-STZ in the diabetic rats. Exercise significantly reduced these deficits in diabetic rats for all tests ($p < 0.05$ to 0.001), with the exception of exploratory behavior. Furthermore, treatment with ANA-12 prior to exercise reduced the protective effects of treadmill exercise in diabetic rats ($p < 0.05$ to 0.001).Exercise treatment protected against early visual, retinal and cognitive dysfunction in a Type 1 rat model of diabetes. Blocking the TrkB pathway reduced exercise's protective effect, suggesting that BDNF signaling may mediate exercise protection in diabetes. Exercise is a promising candidate for treatment of early visual, retinal and cognitive defects in diabetes.

Disclosures: A. Hanif: None. R. Allen: None. M. Gogniat: None. B. Prall: None. R. Haider: None. M. Aung: None. M. Prunty: None. L. Mees: None. C. Sidhu: None. L. He: None. M. Iuvone: None. M. Pardue: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.14/U13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 2R256M060507

NIH Grant 5P20MD006988

Title: DHA regulation of PMP22 in Schwann cells during lipotoxicity.

Authors: *M. A. SERRANO ILLAN, J. D. FIGUEROA, K. CORDERO, M. DE LEON; Loma Linda Univ., Loma Linda, CA

Abstract: Traumatic neuronal injury induces major cellular dysfunction which may include lipid overload and lipotoxicity. These disturbances in turn can adversely affect Schwann cell (SC) viability and disrupt normal myelination. However, it is the type of long-chain fatty acid (LCFA) that reaches the PNS that determines the effect on SCs. Palmitic acid (PA), a saturated LCFA, induces cell death, whereas docosahexaenoic acid (DHA), a polyunsaturated LCFA, promotes survival. This study expands on these findings and examines the regulation of Peripheral myelin protein 22 (PMP22), a protein important for normal SCs maintenance and myelination during this procces. Our studies address the question of whether DHA neuroprotection of SCs during lipotoxicity can modulate PMP22 gene expression.
Quantitative real time polymerase chain reaction (QRT-PCR) analysis showed that Schwann cells treated with DHA (DHA50: BSA 150uM) significantly repressed PMP22 mRNA in a time dependent manner after 24H of exposure. This decrease in PMP22 mRNA results in an increase in cell proliferation. In contrast, treatment of these cultures with a palmitic acid (PA) overload (PA300:BSA150uM) significantly upregulates PMP22 mRNA, a decrease in cell proliferation and an increase in cell death at 48H. Co-treating these cultures with DHA and PA stabilizes the expression of PMP22 mRNA levels and restores normal cell viability.
In vivo experiments involved the use of sciatic nerves from Sprague-Dawley rats on diets rich in omega-3 fatty acids or rich in omega-6 fatty acids for 60 days before undergoing contusive SCI or a spinal cord laminectomy (sham). QRT-PCR and Western blot analyses showed a significant downregulation of PMP22 in rats on the omega-3 PUFA diet. Taken together these data suggest that DHA confers neuroprotection in the event of lipotoxicity, and that this is positively correlated with the downregulation of PMP22 both *in vitro* and *in vivo*.

Disclosures: M.A. Serrano Illan: None. J.D. Figueroa: None. K. Cordero: None. M. De Leon: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.15/U14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPq

CAPES

FAPERJ

Ministerio da Saude

Title: Cell therapy increases the survival and regeneration of retinal ganglion cells after optic nerve injury in adult rats

Authors: L. A. MESENTIER-LOURO, L. C. TEIXEIRA-PINHEIRO, A. J. DA SILVA-JUNIOR, F. GUBERT, P. DOMIZI, G. NASCIMENTO-DOS-SANTOS, C. TEIXEIRA, F. TOVAR-MOLL, M. F. SANTIAGO, *R. MENDEZ-OTERO;
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Abstract: The effects of one intravitreous injection of mesenchymal stromal cells were investigated after optic nerve crush (ONC), in adult pigmented rats. Lister Hooded rats underwent unilateral ONC followed by an intravitreal injection of 5 x 10^5 MSC (treated) or the vehicle (untreated). MSCs were labeled with iron-oxide particles for *in vivo* and *ex vivo* cell tracking. RGC number was estimated by counting $Tuj1^+$ and/or $Brn3a^+$ cells, and optic nerve regeneration was investigated after anterograde labeling with cholera toxin B conjugated to Alexa 488. The reconnection of RGC axons to the neurons in the superior colliculus (SC) was analyzed by the expression of the nerve growth factor-induced gene A (NGFI-A, driven by light stimulation) and the visual function was analyzed using an optomotry apparatus to evaluate the optokinetic reflex. The retinal expression of FGF-2, IL-1β was analyzed 14 days after ONC. MSCs remained mostly in the vitreous body for 18 weeks and protected ~2x more RGC when compared to vehicle injection, for at least 60 days after crush (dac). Regenerated axons up to 1.0 mm beyond the injury site were ~2x more numerous in MSC-treated animals (28 dac) and, in one one of them, there were visible axons up to the SC, 60 dac. $NGFI\text{-}A^+$ cells were 2-fold increased in the MSC-treated groups (60 dac), while the optokinetic reflex was partially recovered in 30% of MSC-treated and in only 9% of untreated animals (80 dac). MSCs increased the expression of FGF-2 and IL-1β in the retina. Our results suggest that RGC degenerate overtime in both groups, but the number of cells is higher in the treated animals for at least 60 days after injury. In addition, NGFI-A expression was increased in the SC of treated animals, suggesting that axons regenerated and made synapses to their target cells in the brain. Further analysis will be performed at 240 days after treatment.

Disclosures: L.A. Mesentier-Louro: None. L.C. Teixeira-Pinheiro: None. A.J. da Silva-Junior: None. F. Gubert: None. P. Domizi: None. G. Nascimento-dos-Santos: None. C. Teixeira: None. F. Tovar-Moll: None. M.F. Santiago: None. R. Mendez-Otero: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.16/U15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACyT 288760

CONACyT 233854

CONACyT 138663

CONACyT 129303

Title: Reproductive experience improves memory and learning of the spontaneously hypertensive female rats

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Abstract: Hypertension (HT) is a disease characterized by a sustained increase in blood pressure. This condition is a major risk factor for suffering cerebrovascular disease, such as ischemic or hemorrhagic stroke, microvascular injury, cognitive impairment and vascular dementia. Experimentally, the model of spontaneously hypertensive (SH) rats has been widely used to study alterations at the anatomical, physiological and behavioral items, which develop by the progression of HT. The SH animals have been showed an increase in the blood pressure after second month affecting brain areas such as hippocampus and prefrontal cortex, important areas for congnition. Specifically, it has shown that the pyramidal neurons of these areas exhibited retraction of the dendritic arborization and reduced number of the dendritic spines (Vega et al., 2004). In females, its well known that neuroprotective effects of hormones and stimuli associated with reproduction, protect against neuronal damage and enhance cognitive processes such as learning and memory in females rats, both short and long-term (Gatewood et al., 2005; Kinsley et al., 2005). Previously, we have shown that Cerebrolisyn treatment, a drug with neurotrophic properties, improves cognitive performance on a task learning and spatial memory and also, increases the length, arborization and density of dendritic spines in prefrontal cortex and hippocampus (Solis et al. 2016). In the present report, we compared the effect of reproductive experience on the memory and learning measured by Water-Morris test, and neural morphology of neurons from CA1 and dentate gyrus (DG) of the dorsal hippocampus, and piramidal neurons from layers III and V of prefrontal cortex by using Golgi-Cox stain, in spontaneously

hypertensive female rats CBL or vehicle-treated. Our results shown that RE improved learning and memory in the SH rats, even, better than cerebrolisyn. In addition, pyramidal neurons of layers III and V of the PFC and, CA1 and DG of dorsal hippocampus of the SH rats which received CBL or vehicle, shown more arborization and lenght in rats with RE compares with virgin rats. Also, CA1 shown an increase in the number of dendritic spines. Our results suggest that reproductive experience induce cerebral plasticity to long term and improved cognitive impairment caused by HT.

Disclosures: V. Cabrera Pedraza: None. C. Solis: None. R. Vazquez-Roque: None. F. De la Cruz: None. S. Zamudio: None. M. Gomez-Villalobos: None. G. Flores: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.17/U16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY026662

Title: Source of optic nerve metabolic vulnerability in a mouse model of glaucoma

Authors: *D. M. INMAN[1], A. H. JASSIM-JABOORI[1], V. GEVORGYAN[2], P. G. PALMER[3]; [1]Pharmaceut. Sci., [2]Northeast Ohio Med. Univ., Rootstown, OH; [3]Bio-Med Sci. Acad., Rootstown, OH

Abstract: Physiological changes that precede axon degeneration can be driven by metabolic dysfunction. We observed lack of resilience to oxygen-glucose deprivation to a degree that correlates with magnitude of intraocular pressure exposure in a mouse model of glaucoma. These experiments investigated the source of energy vulnerability by examining energy storage (glycogen and creatine kinase activity), energy exchange (monocarboxylate transporters (MCT), glucose transporters), and metabolic substrate utilization (lactate dehydrogenase activity) in the optic nerve. DBA/2J (D2) mice that develop glaucoma-related optic neuropathy and age-matched DBA/2J$^{wt\text{-}gpnmb}$ (D2G) control eyes were injected with cholera toxin B to assess axon transport status. Mice were grouped by age (3, 6, and 10 months) and extent of transport deficit; mRNA was isolated, and gene expression analyzed in optic nerve by qPCR. Optic nerves were also isolated and analyzed for glycogen levels and creatine kinase activity. Energy storage analysis indicated significant decreases in glycogen (10m D2 versus D2G, $p=0.027$) and creatine kinase activity (10m D2 with axon transport deficit versus D2G, $p=0.0288$). Significant increases in MCT-2 and -4 gene expression levels were observed in 10m D2 optic nerve compared to age-

matched control D2G (t-test, p=0.0036, p=0.0376, respectively); MCT-2 is preferentially expressed in axons, and MCT-4 in astrocytes. Upregulation of Glut3, a glucose transporter specific to axons, was observed in 3m D2 versus D2G (p=0.0007), in 10m D2 with normal transport versus D2G (p=0.0017), and in 10m D2 with transport deficit versus D2G (p=0.0081). Astrocyte-specific lactate dehydrogenase-A (LDH-A) was significantly upregulated in 10m D2 with axon transport deficit compared to 10m D2 with normal transport (p=0.0439). Axon-specific LDH-B was significantly upregulated in 3m D2 versus D2G (p=0.0052), and in 3m D2G versus 10m D2 with ($p<0.0001$) or without axon transport (p=0.0147). Decreased glial-specific expression of MCT-1 in the context of axon transport deficit accompanied increased LDH-A expression, indicating that glial cells may be making more lactate or utilizing it, precluding its transport to dysfunctional axons. In functional 10m D2 axons, increased MCT-2 and -4 expression suggests a drive to obtain lactate. The energy vulnerability observed in D2 optic nerve may be traced to the significant decrease in glycogen storage and creatine kinase activity, despite increased opportunity for lactate exchange through MCT upregulation in functional axons.

Disclosures: D.M. Inman: None. A.H. Jassim-Jaboori: None. V. Gevorgyan: None. P.G. Palmer: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.18/U17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: KAKENHI 25430056

KAKENHI 16K07048

Fund from Nukada Inst Med Biol Res

Title: Establishment of a spontaneously immortalized Schwann cell line IKARS1 from aldose reductase-deficient mice

Authors: *K. SANGO[1], N. NIIMI[1], H. YAKO[1], M. TSUKAMOTO[1], H. MIZUKAMI[2], S. K. CHUNG[3];
[1]Tokyo Met Inst. Med. Sci., Tokyo, Japan; [2]Dept Pathol Mol Med, Hirosaki Univ., Hirosaki, Japan; [3]Dept Anat, Univ. of Hong Kong, Hong Kong, China

Abstract: Aldose reductase (AR), the first enzyme of the polyol pathway, converts glucose to sorbitol, and galactose to galactitol. Sorbitol is further converted to fructose by sorbitol dehydrogenase (SDH). The increased glucose flux into the polyol pathway via AR in Schwann cells and subsequent accumulation of sorbitol and fructose is recognized as a major contributing factor for the pathogenesis of diabetic neuropathy. The accumulation of galactitol as a consequence of galactose feeding in animals can be a cause of 'galactosemic' neuropathy. In contrast to these pathogenic roles, AR contributes to osmoregulation and aldehyde detoxification under normoglycemic conditions; however, little is known about its physiological roles in the peripheral nervous system (PNS). We established spontaneously immortalized Schwann cell lines from long-term cultures of aldose reductase (AR)-deficient and normal C57BL/6 mouse dorsal root ganglia (DRG) and peripheral nerves. These cell lines, designated as immortalized knocked out AR Schwann cells 1 (IKARS1) and 1970C3, showed distinct Schwann cell phenotypes, such as spindle-shaped morphology and immunoreactivity for S100 and p75 low-affinity neurotrophin receptor. Conditioned medium obtained from these cells exhibited potent biological activity for the promotion of survival and neurite outgrowth of cultured adult mouse DRG neurons. Exposure to 25 mM of galactose for 24 h resulted in a drastic increase in intracellular galactitol contents in 1970C3, but not in IKARS1. However, we failed to detect significant up-regulation of sorbitol and fructose contents by exposure to high glucose (30 mM) for 24 h in the both cell lines . By using DNA microarray and subsequent real time RT-PCR analyses, we observed significant down-regulation of mRNA expression for the polyol pathway-related enzymes, such as SDH and ketohexokinase, in IKARS1 as compared with 1970C3. In contrast, significant up-regulation of mRNA expression for aldo-keto reductases Akr1b7 and Akr1b8 in IKARS1 as compared with 1970C3 was detected. Furthermore, exposure to the reactive aldehydes, such as 3-deoxyglucosone (0.5 mM), methylglyoxal (0.25 mM) and 4-hydroxynonenal (2.5 μM), significantly (>2.5 fold) up-regulated the mRNA expression for Akr1b7 in IKARS1, but not in 1970C3. Because we observed no significant differences between the two cell lines in the viability after exposure to these aldehydes, the detoxification function might be taken over by Akr1b7 and other aldo-keto reductases in the absence of AR. These Schwann cell lines can be useful tools for the study of polyol metabolism and functional roles of AR in the PNS.

Disclosures: K. Sango: None. N. Niimi: None. H. Yako: None. M. Tsukamoto: None. H. Mizukami: None. S.K. Chung: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.19/U18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NEI grant EY007360

Schumacher Institute for Vision Research

Title: Blockade of gap junctions preserves structural <and> for> functional integrity of the retina in a mouse model of glaucoma

Authors: *A. AKOPIAN[1], S. KUMAR[1], H. RAMAKRISHNAN[1], S. VISWANATHAN[1], S. BLOOMFIELD[2];
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Abstract: We reported recently that gap junction (GJ)-mediated bystander effect plays a significant role in the propagation of cell loss in retina under a various insult conditions (Akopian et al., 2015). Here we show that GJ-mediated cell death forms a critical mechanism in the progressive loss of inner retinal neurons seen in glaucoma. Experimental glaucoma was induced in adult C57BL/6 (WT) and connexin 36 knockout ($Cx36^{-/-}$) mice by raising IOP with intracameral injections of polystyrene microbeads. Elevated IOP in WT mouse resulted in reactive gliosis, as assessed by GFAP immunolabeling as well as 34% reduction in retinal ganglion cells (RGCs) and a 17-50% reduction in different subpopulations of amacrine cells (ACs). In addition, there was a significant degenerative change in RGC dendritic tree and axons, which preceded cell death. Glaucoma was then induced in WT mouse treated with meclofenamic acid (MFA) to block gap junctions or in $Cx36^{-/-}$ mice, which showed elevated IOP to levels comparable to untreated WT mouse. Blockade of GJs by (MFA) or ablation of Cx36 significantly attenuated reactive gliosis and offered substantial protection of inner retinal neurons. The ERG scotopic threshold response (STR) and oscillatory potentials (OPs), which reflect activity of RGCs and ACs, respectively, were markedly reduced in glaucomatous eyes of untreated WT mice. The time course of ERG changes correlated well with the loss of the inner retinal cells. In contrast, the ERG remained largely unchanged in MFA-treated WT mice or in $Cx36^{-/-}$ mice. Axonal integrity was assessed by confocal microscopy both within retina and in optic nerve (ON) cross sections. The ON of glaucomatous eyes of WT mice showed a disruption of the honeycomb pattern of GFAP-positive astrocytic processes and SMI32-positive axonal bundles with a 45% ($p<0.001$) reduction in the density of axons. In contrast, the glaucomatous ON of $Cx36^{-/-}$ mice showed a staining pattern and axonal density comparable to those in WT mice. Finally, the visual evoked potential (VEP) in glaucomatous WT mice was reduced by 71%, whereas VEPs recorded in MFA-treated mice or $Cx36^{-/-}$ mice had amplitudes comparable to controls. Our data show that experimental glaucoma produced electrophysiological changes that mirrored the loss of inner retinal neurons and axons in the optic nerve. Moreover, blockade or ablation of GJs preserved structural and functional integrity of the retina and ON in glaucomatous eyes. Our results strongly indicate that neuronal GJs form novel therapeutic targets for neuroprotective treatments directed at reducing the progressive loss of neurons and axons in glaucoma, thereby preserving visual function.

Disclosures: **A. Akopian:** None. **S. Kumar:** None. **H. Ramakrishnan:** None. **S. Viswanathan:** None. **S. Bloomfield:** E. Ownership Interest (stock, stock options, royalty, receipt

of intellectual property rights/patent holder, excluding diversified mutual funds); Equity interest in new startup company Connexin Pharmaceutics.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.20/V1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY018606

Research to Prevent Blindness

Glaucoma Research Foundation

Title: JNK-JUN signaling is important for RGC axonal injury induced soma degeneration

Authors: *S. SYC, K. A. FERNANDES, R. T. LIBBY;
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Abstract: Glaucoma is an optic neuropathy characterized by retinal ganglion cell (RGC) death and optic nerve degeneration. The focus of this study was to identify the molecular signaling pathway(s) critical for ocular hypertension-induced RGC degeneration. Previously, we showed that JNK-JUN signaling is a major regulator of RGC death after axon injury, a crucial insult to RGCs after an ocular hypertensive insult. In DBA/2J ocular hypertensive mice, JUN and JNK are expressed in a temporal and spatial pattern consistent with these genes functioning during the window of RGC death. To test the importance of this pathway in glaucomatous neurodegeneration, a floxed allele of *Jun* and *Six3*-cre (a neural retina cre) were backcrossed into the DBA/2J mouse. To ensure genetic background did not alter the protection afforded by $Jun^{-/-}$ for RGC death after axonal injury, controlled optic nerve crush (CONC) was performed in young DBA/2J *Jun* deficient mice (D2.$Jun^{-/-}$) and littermate controls (D2.$Jun^{+/+}$ with and without cre). Similar to the protection from CONC observed on the C57BL/6J background, D2.$Jun^{-/-}$ protected 76% of RGCs 35 days after CONC compared to 25% survival seen in D2.$Jun^{+/+}$ mice ($n=6$, $p<0.001$). To ensure *Jun* deficiency did not alter the intraocular pressure (IOP) profile of DBA/2J mice, IOPs were measured at 3, 9, 10.5, and 12 months. No significant difference was seen between D2.$Jun^{-/-}$ and D2.$Jun^{+/+}$ mice ($p>0.05$; $n\geq36$ for each genotype and time point). To assess the influence of $Jun^{-/-}$ on ocular hypertension induced axonal degeneration, optic nerves from aged D2 mice were stained and graded on a scale measuring optic nerve damage (mild, moderate or severe). Preliminary data suggests the proportion of nerves with mild and moderate

axonal damage was increased in *Jun*$^{-/-}$ mice, but nerves with severe axonal damage were observed in all genotypes at 12 months (16 D2.*Jun*$^{+/+}$, 18 D2.*Jun*$^{-/-}$ nerves). *Jun* deficiency did protect 69% of RGC somas as compared to only 20% survival in control eyes in eyes with severely damaged optic nerves (severe grade; n=7, $p<0.001$). The somal protection conferred by *Jun* deficiency may be even higher since ~17% of RGCs still express JUN (incomplete recombination of the *Jun* allele). As *Jun*$^{-/-}$ did not provide complete protection, mice deficient in both *Jun* and *Chop*, the endoplasmic reticulum (ER) stress target gene previously shown to be a major regulator of axon-injury induced RGC death, were generated. Inhibiting these two pathways appears to have an additive effect on RGC survival, providing near complete protection 60 days after CONC (n=4, $p<0.001$). These results suggest that together JNK-JUN and ER stress signaling are important for axonal injury induced RGC death.

Disclosures: S. Syc: None. K.A. Fernandes: None. R.T. Libby: None.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.21/V2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST104-2320-B-006-004

NCKUH10408005

Title: Profiling mitochondrial defects from nucleoside/nucleotide reverse transcriptase inhibitors in neurons: potential contribution to HIV-associated neurocognitive disorders

Authors: K.-M. HUNG, *M. CALKINS;
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Abstract: A cornerstone of current HIV treatment are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). however patients who receive long term treatment with NRTIs often develop severe side effects, including painful neuropathy. The potential contribution of NRTI-mediated toxicity to HIV-associated neurocognitive disorders (HAND) has not been fully explored. NRTI toxicity is thought to be mediated through mitochondrial DNA polymerase γ (pol γ) inhibition, which impairs mitochondrial DNA (mtDNA) synthesis and leads to various mitochondrial dysfunctions. To evaluate the relationship between NRTI-mediated pol γ inhibition and mitochondrial toxicity in neurons, we systematically investigated mitochondrial regulation in NRTI-treated primary cortical neurons by measuring parameters related to mtDNA

content, retrograde signaling responses and mitochondrial homeostasis. The effects of six different NRTIs with variable pol γ inhibitory activity and mitochondrial toxicity were assessed. The strong pol γ inhibitors, ddC and ddI, abolished mtDNA synthesis and greatly reduced mtDNA content. However, mtDNA transcription was not as severely affected, and only ddC treated cells showed mild deficits in oxidative phosphorylation. Detrimental effects on mitochondrail motility and morphology were observed after AZT and d4T treatment in the absence of mtDNA depletion or inhibition of mtDNA synthesis. The results suggest that individual NRTIs induce distinct profiles of mitochondrial dysfunction in neurons which likely reflect multiple mechanisms of toxicity. Furthermore, primary cortical neurons appear to tolerate major reductions in mtDNA content before mitochondrial dysregulation is detectable. Four of the six NRTIs induced mitochondrial disruptions indicating a clear potential for CNS neurotoxicity and suggesting that HAND etiology may be affected by NRTI treatment.

Disclosures: K. Hung: None. **M. Calkins:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FTC and TDF were supplied by Gilead.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.22/V3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Eisai

NIH S10OD010610

Title: Rapid recovery from several morphological and biochemical effects of chemotherapy-induced neuropathy following paclitaxel and eribulin in mouse sciatic nerves

Authors: *B. M. COOK[1,2], S. J. BENBOW[3,2], K. M. WOZNIAK[4], B. S. SLUSHER[4], Y. WU[4], B. A. LITTLEFIELD[5], M. A. JORDAN[3,2], L. WILSON[3,2], S. C. FEINSTEIN[3,2];
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Abstract: Improvements in cancer treatment with microtubule-targeting agents (MTAs) have led to a growing population of cancer survivors. The reversibility of chemotherapy-induced peripheral neuropathy (CIPN), a disabling and potentially permanent side effect of MTAs is

becoming increasingly important. The time to onset and frequency of severe CIPN varies among different MTAs. Previously, we found that after 2 weeks of maximum tolerated doses (MTD) in mice, paclitaxel treatment resulted in a 30% reduction in sciatic nerve axons per unit area while eribulin generated a 21% reduction compared to controls. Paclitaxel also increased the frequency of myelin abnormalities more significantly than did eribulin (6.6% and 2.2% of total axons, respectively). Biochemically, paclitaxel and eribulin both induced α-tubulin expression (1.9- and 2.5-fold, respectively) and tubulin acetylation (5- and 11.7-fold, respectively). Eribulin but not paclitaxel induced EB1 expression 2.2 fold. We are currently extending these comparative studies to acquire a more detailed understanding of the recovery from drug treatment. Mice were dosed with MTD doses of eribulin (1.2 mg/kg), paclitaxel (30 mg/kg), ixabepilone (2 mg/kg), and vinorelbine (11 mg/kg), or their respective vehicles, each being administered intravenously 3 times per week for 2 weeks and followed for 6 months after the cessation of dosing. Preliminary evaluation of sciatic nerve morphology 2 weeks after cessation of paclitaxel or eribulin treatment indicated that paclitaxel treated animals continued to exhibit a 30% reduction in axons per unit area whereas eribulin treated animals had recovered to axon numbers similar to control. Myelin abnormalities had largely recovered but were still present in both paclitaxel and eribulin treated animals (0.82% and 0.64% of total axons, respectively). Quantification of sciatic nerve biochemistry 2 weeks after cessation of paclitaxel or eribulin indicated that axonal levels of α-tubulin, acetylated tubulin, and EB1 rapidly return to control levels. In summary, we found that (i) morphologically, sciatic nerve axons recover more rapidly in eribulin treated animals than in paclitaxel treated animals and (ii) biochemically, drug-induced increases in gene expression following paclitaxel and eribulin treatment are relatively transient. Overall our data in mice suggest a milder onset and faster recovery with eribulin treatment than for paclitaxel. Ongoing analyses of animals 3 and 6 months after cessation of treatment will reveal the extent to which these effects persist.

Disclosures: **B.M. Cook:** 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; Eisai. **S.J. Benbow:** 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; Eisai. **K.M. Wozniak:** 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; Eisai. **B.S. Slusher:** 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; Eisai. **Y. Wu:** 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; Eisai. **B.A. Littlefield:** 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; Eisai. **M.A. Jordan:** 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; Eisai. **L. Wilson:** 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; Eisai. **S.C. Feinstein:** 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; Eisai.

Poster

228. Neuroprotective Mechanisms: Diabetes, Neuropathy, and Retina

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 228.23/V4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 EY0 12245

Title: Mitochondrial complex I deficiency decreases canonical Wnt signaling in retinas

Authors: *L. SONG, A. YU, G. CORTOPASSI;
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Abstract: Mitochondrial complex I deficiency is the most common mitochondrial disorder and contributes to neurodegenerative diseases such as Leber's hereditary optic neuropathy (LHON), Leigh syndrome, and Parkinson's disease, yet the mechanism by which complex I deficiency causes neurodegeneration remains poorly understood. Wnt signaling pathway plays a critical role in retinal development and neurodegeneration. To address whether the altered activity of the Wnt signaling pathway is responsible for neurodegeneration induced by mitochondrial complex I deficiency, we use Ndufs4 knockout (Ndufs4-/-) mice as a model of neurodegeneration. Ndufs4 is a key complex I subunit and its deletions cause complex I deficiency. RNA-seq analysis showed that Wnt2b was gradually decreased in retinas of Ndufs4-/- mice from p23 to p30, which precedes retinal ganglion cell loss. Furthermore, we found that both total β-catenin and active β-catenin were dramatically decreased in retinas of Ndufs4-/-mice and LHON cybrid cells with complex I deficiency. In addition, we found that the enhanced activation of Wnt signaling increases the viability of cybrid cells untreated and treated with complex I inhibitor. These data strongly suggest that Wnt signaling may play a crucial role in retinal neurodegeneration induced by mitochondrial dysfunction.

Disclosures: A. Yu: None. G. Cortopassi: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.01/V5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR FRN93603

Weston Brain Institute

Title: Focused ultrasound-mediated delivery of intravenous immunoglobulins modulates neuroinflammation in an amyloidosis mouse model

Authors: *S. DUBEY[1,2], B. KIM[2], A. BURGESS[1], J. MCLAURIN[1,2], D. R. BRANCH[2], K. HYNYNEN[1,3], I. AUBERT[1,2];
[1]Biol. Sci. Res., Sunnybrook Res. Inst., Toronto, ON, Canada; [2]Lab. Med. & Pathobiology, [3]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: Intravenous immunoglobulins (IVIg), natural antibodies collected from the plasma of thousands of healthy blood donors, are currently being tested for efficacy in the treatment of Alzheimer's disease (AD). Its ability to target different pathological markers in AD, combined with its excellent safety profile in humans, makes it an attractive treatment option for AD. IVIg is a limited resource with a high demand for treating several different diseases, which necessitates the requirement of a therapy that will increase IVIg efficacy in the brain while minimizing the therapeutic dosage. To enhance the delivery of systemically administered IVIg to brain regions most affected by Aβ pathology, we used transcranial focused ultrasound (FUS), guided by magnetic resonance imaging (MRI). Previous data from our laboratory indicate that combined FUS and IVIg treatment significantly increases IVIg delivery, reduces amyloid plaque load and increases neurogenesis in the hippocampus. The objective of the present study was to further investigate the effect of combination FUS and IVIg therapy on neuroinflammatory markers in the brain and cytokine levels in the periphery. Using a mouse model of amyloidosis, we administered IVIg intravenously, with or without application of FUS. FUS was performed once a week, for two consecutive weeks, and was targeted bilaterally to the hippocampus. After treatment, blood and tissue was collected for biochemical analysis of mRNA and protein expression using qPCR and western blot analysis. Our results indicate that compared to animals treated with FUS or IVIg alone, FUS and IVIg treated animals have altered levels of cytokines and chemokines in the brain and peripheral blood. Levels of pro-inflammatory chemokines such as CXCL-10 and CXCL-1KC were unchanged while anti-inflammatory chemokines such as RANTES were increased. These findings shed light on the inflammatory effects of combinatorial FUS and IVIg therapy in a mouse model of AD.

Disclosures: S. Dubey: None. B. Kim: None. A. Burgess: None. J. McLaurin: None. D.R. Branch: None. K. Hynynen: None. I. Aubert: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.02/V6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT GRANT 222854 (FONDO 113111

Title: Experimental exposure to ozone causes oxidative damage to lipids and proteins in rat hippocampus. Dietary curcumin inhibits the damage.

Authors: M. A. RAMIREZ-HERRERA[1], J. J. RAMIREZ-VAZQUEZ[2], *M. L. MENDOZA-MAGANA[3], S. D. NERI-FLORES[1], A. CASTILLO-ROMERO[1], L. HERNANDEZ[1], G. CAMARGO[1], M. M. J. ROMERO-PRADO[1], C. R. CORTEZ-ALVAREZ[1], A. A. RAMIREZ-MENDOZA[1];
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Abstract: For decades the ozone (O_3) has been recognized as dangerous pollutant which causes respiratory, cardiovascular and neurodegenerative diseases. The oxidant power of O_3 affects lipids, proteins, carbohydrates and nucleic acids. Curcumin (CUR) is a natural antioxidant isolated from the rhizome of *Curcuma longa Lynn*. Its activity has been tested *in vitro* and *in vivo* assays. The aim of this work is to determine the level of oxidized lipids and proteins during exposure to O_3 and analyze the antioxidant activity of CUR. Methods: Fifty male Wistar rats were distributed into five groups. The groups intact, CUR (5.6 mg/Kg) and exposed to O_3 served as controls. The experimental groups were defined as: the therapeutic group exposed to 0.7 ppm of O_3 seven days before administration CUR in the diet and the preventive group that received CUR seven days before to O_3 exposure. All groups were divided into the acute exposure that lasted 15 days and the chronic exposure lasted 60 days. At the end of the acute and chronic exposure the animals were sacrificed and the hippocampus was dissected to prepare a homogenate in PBS with antiprotease cocktail and butylhydroxytoluene. For lipid peroxidation (LPO) the concentration of malondialdehyde and 4-Hydroxynonenal was measured by espectrophotometry. For protein oxidation (PO) the kit of Oxyblot was used to detect carbonilation. Results of LPO were analyzed by ANOVA and Kruskal-Wallis as post-hoc test. Results of oxidized proteins were analyzed by estimated marginal means (EMM) and the

Bonferroni's post-hoc test. The acute and chronic exposure to O_3 increased significantly LPO and PO when compared versus the intact and CUR groups. The dietary administration of CUR decreased significantly the LPO and PO during the acute and chronic exposure to O_3. As well, the therapeutic and preventive administrations of CUR were effective to revert and prevent the oxidative damage caused by O_3. Thus, CUR could be effective to confer neuroprotection against damaged caused by O_3.

Disclosures: M.A. Ramirez-Herrera: None. J.J. Ramirez-Vazquez: None. M.L. Mendoza-Magana: None. S.D. Neri-Flores: None. A. Castillo-Romero: None. L. Hernandez: None. G. Camargo: None. M.M.J. Romero-Prado: None. C.R. Cortez-Alvarez: None. A.A. Ramirez-Mendoza: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.03/V7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH RO1 CA155293

NIH T32 OD010957

NIH U19 AI67798

Title: Altered gene expression following whole brain irradiation in nonhuman primates

Authors: R. ANDREWS, R. HAMPSON, L. METHENY-BARLOW, D. HANBURY, J. D. BOURLAND, A. PEIFFER, J. M. CLINE, *S. A. DEADWYLER;
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Abstract: Fractionated Whole Brain Irradiation (fWBI) is used in the treatment of intracranial neoplasia in nearly 200,000 people per year. Despite delivering the total radiation dose in smaller fractions spaced over days to weeks, the principal complication is delayed injury to normal (non-neoplastic) tissues, cognitive/memory impairment, cerebrovascular injury and white matter degradation.
To evaluate molecular effects of radiation-induced brain injury, quantitative RT-PCR was performed on dorsolateral prefrontal cortex (DLPFC), hippocampus and temporal white matter from 4 male Rhesus macaques (age 6-10 years) that had received fWBI (40 Gy total, 8 fractions @ 5 Gy each, 2x/week). All animals developed neurologic impairment (decline in working memory performance) between 4-12 months post WBI, and brain injury was confirmed by

histopathology revealing neurovascular lesions and necrosis of white matter. Ninety target genes were selected for RT-PCR based on their roles in neurotransmission, blood brain barrier integrity, inflammation, angiogenesis, extracellular matrix deposition and hypoxia. Findings were compared to sex and age matched non-irradiated control animals (n=3). Changes in relative expression ≥2-fold, and p-values <0.01 were considered biologically and statistically significant, respectively.
With respect to PFC neural function and white matter injury, the most prominent changes were decreased expression of synaptophysin ($p < 0.005$), NMDA receptor 2-alpha ($p < 0.009$), and AMPA4 receptor ($p < 0.006$), which may contribute to impaired excitatory neurotransmission. Surprisingly, there was no significant change in these genes in hippocampus. Markers of macrophage/microglial-mediated neuroinflammation were increased within the DLPFC (MCP1, $p < 0.002$) and white matter (MCP1: $p < 0.004$; CD68: $p < 0.003$). PECAM1, associated with endothelial inflammation was upregulated within white matter ($p < 0.0007$). A three-fold up-regulation of lactate dehydrogenase A was suggestive of white matter hypoxia ($p < 0.002$). Factors associated with vascular remodeling were up-regulated within white matter (SERPINE1: $p < 0.0007$; fibronectin: $p < 0.0001$; vascular endothelial cell growth inhibitor (VEGI): $p < 0.003$) but not in DLPFC or hippocampus.
We infer from these data that impaired hippocampal function was largely due to impaired connectivity with other brain areas via white matter degradation and impaired neurotransmission in DLPFC. These changes were coupled to pathologic vascular remodeling, due to neurovascular and endothelial inflammation and hypoxia, yielding combined neurological and vascular pathologies following fWBI.

Disclosures: R. Andrews: None. R. Hampson: None. L. Metheny-Barlow: None. D. Hanbury: None. J.D. Bourland: None. A. Peiffer: None. J.M. Cline: None. S.A. Deadwyler: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.04/V8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Weston Brain Institute

CIHR Operating Grants MOP-84501

Title: shRNA-mediated knockdown of α-synuclein in brain regions targeted by MRI-guided focused ultrasound.

Authors: *F. NABBOUH[1], K. XHIMA[2], K. MARKHAM-COULTES[2], P. NAGY[2], A. BURGESS[3], K. HYNYNEN[3], I. AUBERT[2], A. TANDON[1];
[1]Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [2]Biol. Sci., [3]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized pathologically by misfolded and aggregated α-synuclein (α-syn) protein. Decreasing the expression of α-syn therefore has considerable therapeutic potential. However, noninvasive drug delivery to affected brain regions presents a significant barrier to the treatment of PD. MRI-guided focused ultrasound (MRIgFUS) combined with microbubbles injected into the bloodstream can briefly increase the permeability of the blood-brain barrier (BBB) in selective brain regions. Using this approach, therapeutics administered through the circulatory system can efficiently cross the BBB without the use of invasive surgery. In this experiment, we used a viral vector expressing short hairpin RNA (shRNA) to knockdown α-syn gene expression in a mouse model of PD. MRIgFUS was targeted to the olfactory bulb and dorsal motor nucleus of the vagus nerve. We quantified α-syn expression levels using immunohistochemistry to evaluate the efficacy of its knockdown. We found that α-syn immunoreactivity was decreased in brain regions targeted with MRIgFUS and α-syn-shRNA in comparison to control mice that received scrambled shRNA. Our results demonstrate that MRIgFUS can effectively deliver shRNA targeting α-syn directly into brain areas that are particularly vulnerable to pathogenesis. In addition, FUS-mediated BBB opening in the olfactory bulb and dorsal motor nucleus were effectively targeted for the first time in this study.

Disclosures: F. Nabbouh: None. K. Xhima: None. K. Markham-Coultes: None. P. Nagy: None. A. Burgess: None. K. Hynynen: None. I. Aubert: None. A. Tandon: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.05/V9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP 2014/10667-5

Title: Temporal modulation of sonic hedgehog after microtubule disruption in the striatum of mice

Authors: *T. DUARTE, G. H. D. ABREU, E. DEL BEL;
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Abstract: There is increasing evidence that microtubule dysfunction plays a role in the neurodegeneration observed in Parkinson's disease (PD). Recent studies, for example, have shown that interruption of the anterograde transport of sonic hedgehog (Shh) results in a progressive model of PD. In this study, we quantify Shh expression in multiple brain regions of mice after unilateral administration of colchicine, a well-known microtubule disruptor, into the striatum. Examination of changes in the chemical signature of specific cell types in the striatum in the injected and control sides was also performed. Briefly, animals were sacrificed at 3, 14 and 21 days post-injection, and the striatum was analyzed immunohistochemically for tyrosine hydroxylase (TH), Chat, nNOS, IBA (microglia), and GFAP (astrocytes). Western Blot experiments were done to assess the time course of changes in Shh in both the striatum and cortical areas. We found that the density of TH-positive fibers in striatum on the colchicine-treated side significant decreased on day 14 and 21 at the same time that we observed massive striatum atrophy. Chat and nNOS staining revealed the loss of these neurons in the colchicine-treated side on day 14 post-injection when compared with the contralateral side. Quantitative study confirmed the significant increase of reactive microglia on day 3 and further activation on day 14 post-colchicine. The reactive astrogliosis was also observed 3 days post-colchicine injection, and this increase was also observed 14 and persists until 21 days. The modulation of Shh protein beginning 3 days post-lesion and reach maximal by 14 days post-lesion, with recovery by 21 days post injection. Experiments are in progress to further clarify the source and the effects of Shh in the adult forebrain after colchicine induced neurotoxicity.

Disclosures: T. Duarte: None. G.H.D. Abreu: None. E. Del Bel: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.06/V10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2013CB530900

81271421

Title: CDK5 activator protein p25 preferentially binds and activates GSK3β

Authors: *J. ZHANG;
Xiamen Univ., Fujian, China

Abstract: Glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinase 5 (CDK5) have overlapping substrate targets and influence many of the same functions. Their 3-dimensional structures are remarkably similar; both are tau kinases and have been proposed to contribute to the pathogenesis of Alzheimer's disease. This led us to hypothesize that both might be capable of bindingcyclin proteins - the activating cofactors of all CDKs. CDK5 is normally activated by the cyclin-like proteins, p35 and p39, but can nonetheless bind all major cyclins. By contrast, we show that GSK3β does not bind to p35, nor cyclin A or D which share similar structures, but unexpectedly binds to calpain cleavage product of p35, p25. Indeed,over-expressed GSK3β out-competes CDK5 for p25, while CDK5 is the preferred p35 partner. FRET analysis in primary cortical neurons reveals nanometer apposition ofGSK3β:p25 in cell soma as well as in synaptic regions. Interaction with p25 aberrantly altered GSK3β substrate specificity. The GSK3β:p25 interaction leads to enhanced hyperphosphorylation of tau. However, *in silico* modeling suggests the docking site for p25 on GSK3β is the Axin-binding domain. Consistent with this idea, p25 inhibits the formation of the gsk3β/axin/apc destruction complex resulting in decreased phosphorylation of β-catenin. Co-expression of GSK3β and p25 in cultured neurons, results in a neurodegeneration phenotype that exceeds that observed with CDK5 and p25. When p25 is transfected alone, the resulting neuronal damage is blocked more effectively with a specific siRNA against gsk3β, than with that against cdk5. We propose that the effects of p25, though normally attributed to activate CDK5, may be mediated in part by elevated GSK3β activity.
Keywords: GSK3β, CDK5, p25 and neurodegeneration

Disclosures: J. Zhang: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.07/V11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: EPSRC PhD studentship

Pfizer Ltd PhD studentship

Title: Zebrafish as a model for translational neurobiology: implications for drug discovery

Authors: *A. SUDWARTS, A. BROCK, C. BRENNAN;
SBCS, Queen Mary, Univ. of London, London, United Kingdom

Abstract: Recent years have seen an increase in the prevalence of many neurological disorders. However, the discovery and development of new drugs to tackle these conditions has been relatively insufficient. This is largely due to the financial and ethical costs incurred by using mammalian model organisms in translational neurobiology. Thus, the development and validation of non-mammalian screening assays would facilitate lead identification.
Here we assessed the potential of adult zebrafish as a model for drug discovery. We have established scalable automated assays of both appetitive and aversive classical conditioning tasks. Analysis of the performance of a mutant hAPP knock-in mutant fish showed reduced aversion learning, following conditioning. We also assessed molecular and behavioural responses to known a neurotoxin, aluminium. qPCR expression analysis in neurotoxin-treated and control animals reveals regulation of conserved molecular pathways. We discuss these results and their implications for drug discovery in light of behavioural analysis.

Disclosures: A. Sudwarts: None. A. Brock: None. C. Brennan: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.08/V12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACyT

Title: Theoretical study of the quantum-chemical behavior of molecules involved in parkinson

Authors: *E. M. GARCIA[1], C. BARRIENTOS-SALCEDO[2];
[1]Univ. Veracruzana, Boca Del Rio, Mexico; [2]Bioanalisis Fac., Univ. Veracruzana, Veracruz, Mexico

Abstract: Parkinson's disease (PD) is a neurodegenerative síndrome and the most prevalent movement disorder amongst the elderly population. Cardinal motor symptoms result from preferential loss of a subset of dopaminergic neurons within the substantia nigra pars compacta. Molecular changes associated with this neuronal cell loss include mitochondrial dysfunction, reduction in protein homeostasis, oxidative and inflammatory damage and mishandling of celular calcium. It's an appealing disease model for expanding research towards a better understanding of the interrelationship between genes and environment (the 'exposome'). Since 2005, exists an

increasing interest in Quantum Information Theory (QIT) using electronic structures and revealing chemically relevant regions shown in energy profiles, while in neurosciences has been used in a regular basis for contrasting experimental data and predictive models. Between DNA, RNA and proteins, the latter have been chosen because of their key involvement in the diseases' pathology. Our objective is to perform a theoretical study of the quantum-chemical behavior of molecules involved in PD. We hipothesize that proteins that converge in their pathways will have similar quantum-chemical behavior.
For methodology, we used databases such as: KEGG, BioGRID, PDGene, etc. and incorporated them using Cytoscape to find relevant pathways. Then, we compiled Protein Data Bank structures, followed by specific regions thought to contribute to these diseases. Energy minimization was achieved employing GROMACS and Gaussian with puntual calculations using set basis HF 6-31+G** B3LYP accompanied by NBO assessment of the extent of amidecarbonyl sigma/pi separation and bondbending. We included characterization of the physicochemical properties, to identify chemical reactivity through some quantum chemical descriptors. Finally, we employed Shannon Entropy, Fisher Information and complexity calculations in order to formulate a prediction algorithm.
We found over 1,000 molecules related to PD, which where narrowed down to those with genome-wide significant ($p < 1x10-5$). In a merge network of PD molecules betweeness centrality gave us 0.261 R-squared, which indicates low control that molecules exert over the interactions of other molecules in the network. Energy minimization varied between GROMACS and Gaussian and ranged from -2.10E+05 to -6.30E+06. The latter showing disparity in both energy minimization and geometry optimization.

Disclosures: E.M. Garcia: None. C. Barrientos-Salcedo: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.09/V13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: ALS Therapy Alliance

Title: Slowing disease progression in ALS with a targeted neuregulin antagonist

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Abstract: ALS is a neurodegenerative disease that spreads up and down the spinal cord until patients succumb. At present time there are no effective treatments to slow disease progression. We previously reported that neuregulin (NRG) receptors are activated on microglia in the ventral horn and corticospinal tracts of ALS patients and in SOD1 mice (1,2). We have developed a targeted NRG antagonist (HBD-S-H4) that is a humanized fusion protein between NRG's heparin-binding domain and a decoy HER4 receptor (3). This fusion protein blocks microglial activation in vivo in a model of chronic pain (4). In the present study, we tested the therapeutic effects of HBD-S-H4 in the ALS-SOD1 mouse model. We found that expression of HBD-S-H4 in the central nervous system (CNS) delays disease onset and prolongs survival in GFAP-tTA:tetO-HBD-S-H4:SOD1 transgenic mice in correlation with different levels of HBD-S-H4 expression in the CNS. We also delivered recombinant HBD-S-H4 into the CNS through implanted intracerebroventricular (icv) cannulas which had no toxic effects, delayed disease onset, and prolonged survival in two strains of SOD1 G93A mice (SJL.B6 and B6 strains). Mechanistically, the drug hit its target producing a significant reduction of both small and large sized microglia in the ventral horn. While the exact mechanisms of how NRG receptor activation modulates microglial activation is still under study, the lack of toxicity and clear therapeutic effect of HBD-S-H4 provides important preclinical data supporting its use in clinical trials in patients with ALS to slow disease progression.

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Disclosures: F. Song: None. J. Liu: None. E.H. Simpson: None. J.A. Loeb: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.10/V14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Childhood Cancer Foundation

The King Gustav V Jubilee Clinic Cancer Research Foundation

The Swedish Radiation Safety Authority

Swedish Governmental Grants to Scientists working in Healthcare

The Frimurare Barnhus Foundation

The Wilhelm and Martina Lundgren Foundation

The Swedish Society of Medicine

Title: Amyloid precursor protein (APP) is modulated after cranial radiotherapy to the developing mouse brain

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Abstract: Objective
Brain tumours are one of the most common childhood cancers. Among young adults, 1 in 600 are childhood cancer survivors in Sweden today. Cranial radiotherapy is one of the strategies used to treat brain cancer, but it can also lead to permanent damage in the survivors and have a negative impact on their quality of life. The so called late effects after irradiation include perturbed growth, late puberty and cognitive impairment. Alleviating these late effects would have a huge impact in the lives of patients. However, the damages induced by radiotherapy have no cure and there are still no effective interventions available to help the survivors. Amyloid precursor protein (APP) is a membrane protein found in tissues throughout the body. Although mostly associated with Alzheimer's disease where it is the main component of the extracellular amyloid plaques, it has many functions in the normal brain where it is suggested to be involved in synaptogenesis, neurite outgrowth and cell adhesion, to name some. In a pilot study the levels of soluble APP decreased in the cerebrospinal fluid following cranial radiotherapy in patients. Here, APP and its potential role after cranial radiotherapy was studied in the developing mouse brain.
Methods
Male and female mice were exposed to a single dose of 8 Gray to the brain at postnatal day 14. Areas involved in memory and learning were investigated when the irradiation-induced injury was well established. Relative expression levels of APP were measured using quantitative reverse transcription polymerase chain reaction. Immunohistochemistry was used to quantify APP in the thalamus after irradiation.
Results
There was no difference between irradiated mice and controls regarding APP gene expression, neither in the thalamic region nor in the hippocampus. However, irradiation caused a significant accumulation of APP in the thalamus where irradiated male and female mice had a higher density of APP-positive cells than controls. The effect was biggest in male mice, where the

density of APP-positive cells increased by 99 % ($P \leq 0.01$) after irradiation. In females the density increased 57 %, but did not reach a significant level. The size of the thalamus was not affected by irradiation in either of the sexes.

Conclusion

There is a higher number of APP-positive cells in the thalamus after irradiation but the expression levels are not increased. These results suggest that it is either a change in APP metabolism or thalamic accumulation of elsewhere produced APP that is responsible for the observed accumulation of APP in the thalamus of irradiated animals.

Disclosures: Y.F. Eriksson: None. M. Kalm: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.11/V15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS073899

NIH Grant MH103848

VHA Grant BX001637

VHA Grant BX002475

Title: Proline isomerization controls toxic amyloid formation

Authors: *J. D. BAKER[1,2], L. B. SHELTON[1,2], D. ZHENG[1], J. J. SABBAGH[1,2], L. J. BLAIR[1,2], A. DARLING[1], C. DICKEY[1,2];
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Abstract: Controlling aberrant amyloid aggregates associated with neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) is critical to their prevention. Understanding the mechanisms by which amyloidogenesis occurs is key to finding new treatments for myriad neurological disorders and further we must determine the toxic species responsible for neurodegeneration. Because it is known that amyloid, rich in ß-sheet secondary structure, often contains structurally essential proline residues, we hypothesize that enzymes capable of isomerizing these important amino acids may affect aggregation kinetics of diverse amyloid substrates. Here we have characterized well-studied peptidyl-prolyl isomerases (PPIases) and report on their enzymatic effect on P301L tau and

A53T α-synuclein, associated with AD and PD respectively. By combining thioflavin T fluorescence assays with nanoparticle tracking analysis (NTA), a technique allowing us to characterize aggregate size profiles, we have found that PPIases have opposing effects not only on aggregation kinetics, but also on aggregate species formation. These tools allow us to determine not only how chaperones interact with amyloid, but more importantly help us to control proteotoxic amyloid formation.

Disclosures: J.D. Baker: None. L.B. Shelton: None. D. Zheng: None. J.J. Sabbagh: None. L.J. Blair: None. A. Darling: None. C. Dickey: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.12/V16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The sigma-1 receptor binds hexanucleotide repeat expansions of C9orf72: Implication in ALS and FTD

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Abstract: The GGGGCC (G_4C_2) hexanucleotide expansions in the non-coding region of *C9orf72* gene have been reported in cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). G_4C_2 RNA repeats cause toxicity and accumulations of dipeptide repeat proteins (DRP) via the repeat–associated non-AUG (RAN) translation. Recent studies from large-scale genetic screen in *Drosophila* have shown that G_4C_2 repeat expansion compromises the nucleocytoplasmic transport at the nuclear pore complex (NPC) that represents a newly discovered mechanism of neurodegeneration. Sigma-1 receptors (Sig-1Rs) are ligand-regulated membrane proteins that function as molecular chaperones in the endoplasmic reticulum (ER) and are known to be a pluripotent modulator in living systems. Previous studies showed that a missense mutation at Sig-1R amino acid residue 102 (E102Q) was seen in familial ALS patients. The cytoplasmic TAR DNA binding protein (TDP-43)-positive inclusions were seen in motor neurons of ALS patients. Overexpression of Sig-1R mutants (E102Q) led to the extra-nuclear localization of TDP-43 via nucleocytoplasmic shuttling in Neuro2A cells. However, whether Sig-1Rs are involved in G_4C_2 repeats-mediated ALS/FTD is unknown. Here we firstly found that Sig-1Rs exist at the ER and plasma membrane but also at the NPC. Further, by using the biotin pull-down assay we found that biotin-labeled $(G_4C_2)_{10}$ RNA repeats binds and interacts

with Sig-1R-YFP in Neuro2A cells. The endogenous Sig-1R in rat liver microsomes was also found to bind the $(G_4C_2)_{10}$ RNA repeats. To further dissect the interaction domains between Sig-1R and G_4C_2 RNA repeats *in vitro*, we generated recombinant glutathione S-transferase (GST)-tagged proteins including full-length and three truncated fragments of mouse Sig-1R. The pull-down assay revealed that full-length of recombinant Sig-1R (amino acids 1 to 223) physically interacts with G_4C_2 RNA repeats, but not GST alone. The majority of the Sig-1R-$(G_4C_2)_{10}$ RNA interaction occurs on the N-terminal (amino acids 1 to 79) and C-terminal fragments (amino acids 174 to 223) of the recombinant Sig-1R. We propose here a novel regulatory mechanism that Sig-1R may participate in the regulation of hexanucleotide repeat expansions in *C9orf72* by serving as a molecular "sponge" in a manner to reduce the toxicity of the RNA repeats. The dysfunction of Sig-1R may thus relate to the disease state of ALS and FTD. (This work was supported by IRP/NIDA/NIH/DHHS)

Disclosures: P. Lee: None. **T. Su:** None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.13/V17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intrathecal delivery of rAAV9-ABCD1 by osmotic pump in a mouse model of X-ALD

Authors: *A. BERENSON[1], Y. GONG[1], R. KOK[1], N. SASIDHARAN[1], D. MU[1], A. VOLAK[1], X. O. BREAKEFIELD[1], C. A. MAGUIRE[1], G. GAO[2], F. EICHLER[1];
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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 (ABC) transporter. The mouse model of X-ALD with ABCD1 deficiency develops phenotype similar to adrenomyeloneuropathy(AMN), the most common phenotype of X-ALD manifesting spinal cord axon degeneration and peripheral neuropathy but so far lacking therapeutic options. We previously reported successful transduction of central nervous system cells in vitro and in vivo using recombinant adeno-associated virus serotype 9 (rAAV9) vector for delivery of the human ABCD1 gene (ABCD1). Unfortunately, intravenous delivery in young mice was associated with cardiac toxicity due to transgene overexpression. We therefore set out to optimize delivery to the spinal cord while minimizing systemic leakage using intrathecal osmotic pump. Self complementary AAV9 GFP(scAAV9GFP) and rAAV9 encoding ABCD1 (rAAV9-ABCD1)

were delivered to Abcd1-/- mice intrathecally (IT) at spine region between L4-L5 using either gas-tight Hamilton syringe attached to a 33-gauge steel needle in 2mins duration, or osmotic pump in 24h duration with PBS injection as sham control. Two weeks after injection, mice were sacrificed and perfused with 4% PFA. Tissues were then collected, sectioned and stained for immunoflurescence analysis. scAAV9-GFP delivered intrathecally by osmotic pump leads to widespread expression across CNS and dorsal root ganglia (DRG) in a dose dependent manner, with spinal cord and DRG showing higher expression compared to brain. rAAV9-mediated ABCD1 gene transfer via intrathecal osmotic pump leads to widespread gene delivery to CNS with reduced leakage into the systemic circulation compared to intrathecal bolus and intravenous injection. rAAV9-mediated ABCD1 gene transfer via intrathecal osmotic pump targets mainly astrocytes and endothelial cells in the SC and both satellite cells and neurons in the DRG. ICV benefits despite localized expression in brain. Therefore we anticipate even better performance using intrathecal pump delivery. We conclude that rAAV9-mediated ABCD1 gene transfer via intrathecal osmotic pump leads to more uniform and widespread gene delivery to CNS with reduced leakage into the systemic circulation compared to intrathecal bolus injection.

Disclosures: A. Berenson: None. Y. Gong: None. R. Kok: None. N. Sasidharan: None. D. Mu: None. A. Volak: None. X.O. Breakefield: None. C.A. Maguire: None. G. Gao: None. F. Eichler: None.

Poster

229. Neurodegenerative Diseases

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 229.14/V18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: SC CTSI (NIH/NCRR/NCATS) Grant KL2TR000131

2014 NIH Big Data to Knowledge (BD2K) Initiative under U54EB020403

Title: Neuroanatomical changes of non-CNS cancer - a cross sectional study using contrast-enhanced clinically-indicated MRIs

Authors: *M. S. SHIROISHI[1,2,3], J. FASKOWITZ[3], F. D'AMORE[1], A. EMAMI[1], D. H. HWANG[1], S. Y. CEN[1], A. LERNER[1], A. W. TOGA[4], R. E. JACOBS[5], B. ZLOKOVIC[6], M. LAW[1], P. M. THOMPSON[3], N. JAHANSHAD[3];
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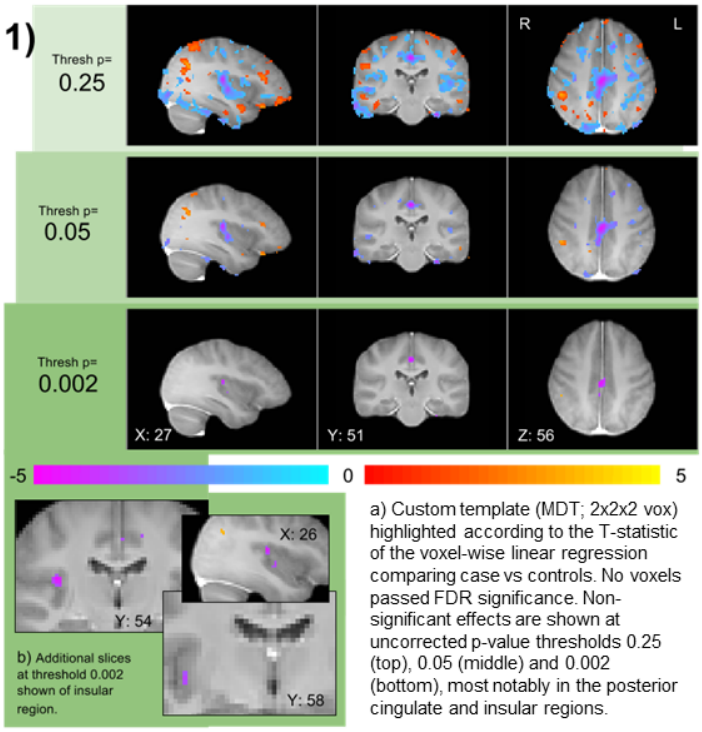
Marina Del Rey, CA; [4]Lab. of Neuro Imaging/USC Mark and Mary Stevens Neuroimaging and Informatics Institute, Keck Sch. of Medicine, Univ. of Southern California, Los Angeles, CA; [5]Caltech, Pasadena, CA; [6]Zilkha Neurogenetic Inst. and Dept. of Physiol. and Biophysics, Keck Sch. of Medicine, Univ. of Southern California, Los Angeles, CA

Abstract: Introduction: With improvements in non-central nervous system (CNS) cancer survival, cognitive deficits associated with cancer itself and its treatment, known as cancer-related cognitive impairment (CRCI), has been increasingly recognized. There is little understanding of the biological mechanisms underlying CRCI as well-powered neuroimaging studies of CRCI have yet to be performed. However, a large amount of clinical brain MRI data performed to exclude the possibility of CNS metastases exists. These MRIs generally use contrast agents and image acquisition parameters that are difficult to use with standard automated imaging protocols. We performed a cross-sectional pilot study to examine neuroanatomical differences in patients with cancers compared to controls using imaging data from clinically-indicated contrast-enhanced MRIs.
Methods: We used whole-brain contrast enhanced T1-weighted (T1w) MRIs of adults < 60 years of age with cancers (cases) and without cancer (controls) performed on 5 different scanners. We performed voxel-wise cortical thickness (CT) estimates and tensor-based morphometry (TBM) with advanced normalization tools (ANTs) CT and registration schemes. T1w scans were initially denoised. Scanner specific minimal deformation targets (MDTs) were made and used to create an overall study MDT. Gray matter parcellations were conducted with FreeSurfer for CT analysis. Full skull stripped T1w scans from all individuals were warped to the MDTs for TBM analysis.
Results: Seventy-one MRI scans were analyzed. No statistically significant differences in CT or TBM were found between cases and controls after voxelwise multiple comparisons correction, however, there were regions of suggestive associations (uncorrected $p < 0.01$) within the posterior cingulate and insula (Figure 1).
Conclusions: Our pilot study suggests pooling heterogeneous scans from multiple different clinical scanners may be a promising approach to perform well-powered neuroimaging studies of CRCI. We are currently analyzing a larger cohort of 213 scans to validate our findings.



Disclosures: M.S. Shiroishi: None. J. Faskowitz: None. F. D'Amore: None. A. Emami: None. D.H. Hwang: None. S.Y. Cen: None. A. Lerner: None. A.W. Toga: None. R.E. Jacobs: None. B. Zlokovic: None. M. Law: None. P.M. Thompson: None. N. Jahanshad: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.01/W1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Wellcome Trust Programme Grant WT094823

Title: Mitophagy in the immature brain after hypoxic-ischaemic injury

Authors: *C. THORNTON[1], K. SOBOTKA[3], R. VONTELL[2], S. NAIR[3], A. A. BABURAMANI[2], P. GRESSENS[4], H. HAGBERG[2];
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Abstract: Perturbation of mitochondrial function is a key hallmark in neonatal hypoxic-ischemic (HI) brain injury, both in animal models and in term infants. Not only is ATP production impaired, but dysregulation of calcium homeostasis and the generation of reactive oxygen species from defective mitochondria result in greater damage than in adult brain due to an immature antioxidant defence system. Mitophagy (mitochondrial recycling) offers a mechanism of efficient disposal of deficient mitochondria which are subsequently replaced through biogenesis. However, unregulated mitochondrial turnover (too much / too little) could be particularly critical in the immature brain as it may affect not only short- and long-term structural and functional recovery but also brain development. This study aims to characterise the molecular players and the efficiency of mitophagy as a protective mechanism after HI in the immature brain. Mitochondrial fission is required as a precursor to mitophagy. We find that after oxygen/glucose deprivation *in vitro* or HI in immature mice *in vivo*, mitochondria undergo rapid fission, characterised by alterations in location and phosphorylation state of the fission mediator Dynamin-Related protein (Drp)1 and a reduction in length and volume of mitochondria. Immunohistochemistry performed on *ex vivo* HI brain sections reveals a colocalisation between mitochondria and autophagosomes (as detected by TOM20 and LC3-II respectively) suggesting an increase in mitochondrial recycling. In addition, we detect changes in both the expression and regulation of mitophagy facilitators such as BNIP3, PINK1 and Parkin. We are currently investigating the temporal regulation and the specific receptors implicated so that we can completely understand the molecular basis of mitophagy in the immature brain. Only then will we be able to determine whether mitophagy offers a new avenue for therapeutic intervention for the babies who suffer life-long disabilities as a result of birth asphyxia.

Disclosures: C. Thornton: None. K. Sobotka: None. R. Vontell: None. S. Nair: None. A.A. Baburamani: None. P. Gressens: None. H. Hagberg: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.02/W2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Drp1 association with Fis1 is a critical mediator in microglial activation and neurodegeneration

Authors: A. U. JOSHI[1], M.-H. DISATNIK[1], *D. MOCHLY-ROSEN[2];
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Abstract: Neuroinflammation is a common feature of many neurodegenerative diseases, including Huntington's disease (HD). Activation of microglia cells in the brain contributes to neurodegenerative processes promoted by many neurotoxic factors such as pro-inflammatory cytokines and nitric oxide (NO). Mitochondria are critical for ATP production, intracellular Ca^{2+} signaling, generation of reactive oxygen species and programmed cell death. A balance between fission and fusion maintains mitochondrial quality control and enables meeting energy demands in various subcellular compartments. Previous studies demonstrated increased proinflammatory cytokines in brain tissue and plasma of HD mice and patients. Increased activities of dynamin-related protein 1 (Drp1), disrupted mitochondrial structure and dynamics have also been observed in HD samples. Hyperphosphorylated Drp1 is recruited to the mitochondria by fission1 (Fis1) protein promoting excessive mitochondrial fragmentation. In this study, we observed increased Drp1/Fis1 interaction in lipopolysaccharide (LPS)-stimulated BV2 microglial cell line. We further found that Drp1 oligomerization occurs as an early event under inflammatory conditions in BV2 microglial cells and eventually increased mitochondrial fragmentation and dysfunction. Inhibition of mitochondrial fragmentation by treatment with P110, that selectively inhibits Drp1/Fis1 interaction alone, effectively suppressed the level of mitochondrial ROS and NO. P110 treatment also significantly prevented LPS-induced increase in the TNF-α, IL-1β, IL-6, iNOS and Cox-2 in BV-2 microglial cells. Furthermore, LPS-induced suppression of mitochondrial ROS generation also regulated inhibitor of kappa Bα (IκBα) activation and nuclear factor-κB (NF-κB) nuclear localization. Notably, P110 treatment did not alter mitochondrial fission and function under basal conditions. In R6/2 mice, inhibiting Drp1/Fis1 interaction reduced activation of astrocytes and microglia. ELISA analyses showed that P110 decreased elevated levels of TNFα and Il-6 in plasma and brain tissue in R6/2 mice. Further, inhibiting Drp1/Fis1 interaction improved survival in R6/2 mice. Collectively, our findings suggest that an effective and selective treatment targeting mitochondrial dysfunction is a potential therapeutic strategy for HD.

Disclosures: A.U. Joshi: None. M. Disatnik: None. D. Mochly-Rosen: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.03/W3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CHRIM

HSCF

Research Manitoba

Title: Cognitive impairment in male offspring exposed to gestational diabetes is associated with hippocampal neuronal changes & neuroinflammation

Authors: *T. M. KAUPPINEN[1,3], B. VUONG[2], G. ODERO[2], M. STEVENSON[2], S. ROZBACHER[2], S. KERELIUK[2], T. PEREIRA[2], V. DOLINSKY[2,4];
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Abstract: Gestational diabetes mellitus (GDM) is the most common complication of pregnancy. Population health studies have demonstrated a link between GDM and impaired cognitive abilities and psychiatric disorders in the offspring. GDM and diets containing excess fats and sugars promote inflammatory responses. Prolonged inflammation can impair the fetal neuronal circuitry development resulting in lifelong effects on cognitive functions. We hypothesized that GDM causes adverse inflammatory responses contributing to derangement of fetal developing neuronal networks, and impairing the neurocognitive abilities of the offspring. We induced GDM by exposing rat dams to diets high in sucrose and fatty acids 6 weeks prior and throughout their pregnancy. Neonatal (20E) and 15 week old (=young adult) male offspring from GDM and lean dams were examined. The neurocognitive abilities of 15 week old offspring were evaluated in Open field, Morris Water Maze and with Novel Object Recognition test, and the brains from both age groups were analyzed by immunohistochemistry. Complementing i*n vitro* experiments involved analyzing microglial responses to elevated levels of glucose and/or fatty acids. Offspring from GDM dams had impaired memory. Fetal GDM exposure combined with a postnatal HFS diet triggered atypical explorative behaviour in open field test. Analysis of brain tissues from neonatal and young adult offspring of GDM dams showed increased astroglial GFAP expression, increased morphological transformation towards amoeboid microglia, and reduced expression of synaptic vesicle protein. Young adult offspring also had disorganized CA1 pyramidal layer and reduced CX3CR1 expression. Postnatal junk food diet further promoted GDM induced abnormalities. Cultured microglia exposed to high glucose and/or fatty acids (palmitate) transformed into activated, amoeboid morphology, significantly increased nitric oxide and superoxide production, and changed cytokine release profile. Both in vitro and *in vivo* data strongly supports our hypothesis that GDM induces chronic inflammatory responses in the offspring brain. Microglia culture experiments confirmed that excess glucose and/or fatty acids induce pro-inflammatory responses. Detrimental pro-inflammatory responses combined with impaired microglial neurotropic functions could explain hippocampal neuronal derangement and synaptic degradation, predisposing offspring for behavior changes, and memory and learning impairments.

Disclosures: T.M. Kauppinen: None. B. Vuong: None. G. Odero: None. M. Stevenson: None. S. Rozbacher: None. S. Kereliuk: None. T. Pereira: None. V. Dolinsky: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.04/W4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: HD070996

HD074593

Title: Possible prolonged endoplasmic reticulum (ER) stress following neonatal hypoxia-ischemia (HI)

Authors: *F. J. NORTHINGTON[1], R. CHAVEZ-VALDEZ[1], D. FLOCK[1], O. AVERITT[1], L. J. MARTIN[2];
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Abstract: ER stress is associated with multiple adult onset neurodegenerative and other chronic illnesses and is considered a therapeutic target for many of these diseases. Sporadically over time, several laboratories have identified ER stress in response to neonatal hypoxia-ischemia and other forms of excitotoxicity. Salubrinal and other small molecules have been shown to inhibit both the markers of ER stress and provide neuroprotection in experimental animal models. Recently we found marked acute ER stress and more prolonged ER pathology extending >5 days following neonatal HI. Whether this ER stress persists into the chronic phase and effects delayed injury and repair after HI is completely unknown. We used the Rice-Vannucci model to induce cerebral HI in C57B6 mice at p10 with unilateral carotid ligation and 45min of hypoxia (FiO_2=0.08). Mice n=6/gp were survived to p40 and perfused for analysis of injury, immunohistochemistry and electron microscopy (EM). Injury was graded on a 0-3 scale in hippocampus, striatum and cortex and brains with score of 3 in any region were excluded from any further analysis. Calcium binding proteins, calreticulin (CR) and calbindin (CB) were assayed immunohistochemically sections and adjacent sections sub-dissected for regions within the ipsilateral hippocampal circuit and processed for ultrastructure. CR is used frequently as an ER marker. CR and CB are expressed throughout the hippocampal circuit but differentially. Multiple regions within the ipsilateral hippocampal circuit displayed enhanced immunoreactivity(IR) for CR compared to their contralateral counterpart, including cigulate

cortex, hippocampus and dentate gyrus, entorhinal cortex and hypothalamus. IR was present in neurons and non-neuronal cells. Within neurons, enhanced IR manifests as thickened-darkened IR in the cell periphery with areas of swelling and in some neurons, the area of the nucleus is IR with swellings contiguous to the nuclear membrane. No such differences were noted in CB expression. EM revealed some neurons with darkened cytoplasm, autophagocitic bodies and mildly swollen ER in the ipsilateral cingulate. Rare dilations of the ER similar to that seen in the subacute phase were noted in the ipsilateral cingulate. Microglia were also frequently seen on EM in the ipsilateral cingulate. No obvious differences in CR IR or ER ultrastructure was noted in the white matter. Chronically altered ER structure within the ipsilateral hippocampal circuit is noted following neonatal HI. Whether this represents ongoing repair and recovery or persistent vulnerability of the hippocampal circuit to further damage following neonatal HI is unknown.

Disclosures: **F.J. Northington:** 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. **R. Chavez-Valdez:** None. **D. Flock:** None. **O. Averitt:** None. **L.J. Martin:** None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.05/W5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Leducq Foundation (DSRRP34404)

ERA-net (EU;VR 529-2014-7551)

Wellcome Trust (WT094823)

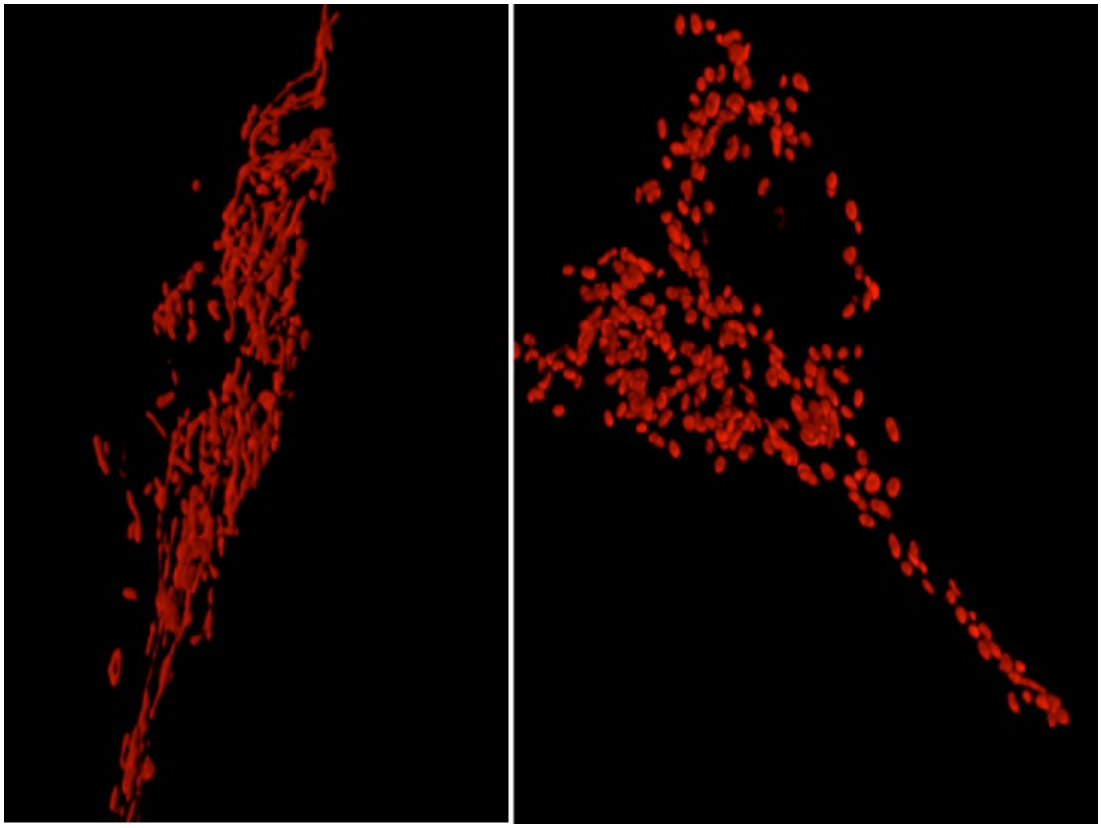
VR 2015-02493

Title: Lipopolysaccharide induces mitochondrial fission and a metabolic shift in microglia

Authors: S. NAIR[1], C. MALLARD[1], C. THORNTON[3], K. SOBOTKA[1], *H. HAGBERG[2,4]; [1]Neurosci. and Physiol., [2]Perinatal Ctr., Göteborg, Sweden; [3]Ctr. for the Developing Brain, [4]Dept. of Perinatal Imaging and Hlth., London, United Kingdom

Abstract: Mitochondrial integrity and inflammation are critical in neonatal brain injury (1). Microglia are nervous system-specific immune cells influencing brain development, inflammation and repair.

The aim was to explore mitochondrial morphology in relation to energy metabolism and cytokine release in primary microglia after stimulation with the TOLL-like receptor 4 agonist lipopolysaccharide (LPS).
We examined specific bioenergetic features of primary microglia under LPS stimulation using doses eliciting a moderate (50 ng/mL) and marked (100 ng/mL) cytokine/chemokine response. LPS administration for 3 to 24 h was followed by measurement of oxygen consumption rate and extracellular acidification rate using the XFp extracellular flux analyzer (Seahorse Bioscience). Changes in mitochondrial morphology in LPS-treated microglia prepared from COX8-GFP mice were studied quantitatively using a Zeiss ELYRA SR-SIM high resolution microscope.
We obtained high resolution visualization of mitochondrial structure using SR-SIM microscopy. LPS (100 ng/mL) induced fission of mitochondria (Figure. left: control; right: LPS) i.e. the number of fragmented (<1 um) was significantly increased whereas tubular (1-3 um) and elongated (>3 um) mitochondria were decreased. These morphological changes were accompanied by a shift from mitochondrial respiration to aerobic glycolysis. We are currently exploring the effect of pharmacological inhibition of mitochondrial fragmentation on the metabolic and inflammatory phenotype. We hypothesize that further understanding of the role of energy metabolism and mitochondrial fusion-fission events in inflammatory cells is critical for development of neuroprotective strategies in the immature brain.
1. Hagberg H, Mallard C, Rousset C, Thornton C. *Lancet Neurol* 13:217-32,2014



Disclosures: S. Nair: None. C. Mallard: None. C. Thornton: None. K. Sobotka: None. H. Hagberg: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.06/W6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: "Progetto di Ateneo" University of Padua, CPDA144389/14

Title: Role of the alpha,beta-unsaturated carbonyl moiety of curcumin and its bis-demethoxy derivative for inhibition of lipopolysaccharide-induced Toll-like receptor 4 activation and inflammatory response in microglia

Authors: *P. F. GIUSTI[1], F. BELLUTI[3], C. MARINELLI[2], E. CARACCIOLO[2], R. LO[4], S. STIFANI[4], S. MORO[2], M. ZUSSO[2];
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Abstract: Toll-like receptors (TLRs) play a key role in the recognition of both invading pathogens and endogenous danger signals to induce inflammatory responses both in peripheral organs and in the central nervous system (CNS). TLR4, a receptor responsible for the recognition of lipopolysaccharide (LPS), serves as the primary mediator of immune responses. An exaggerated inflammatory response can be detrimental to normal CNS functioning and is widely associated with the pathogenesis of numerous neurodegenerative and psychiatric disorders. Identification of molecules capable of interfering with TLR4 activation and understanding the molecular mechanisms modulating TLR4 activity may be a viable strategy for attenuating the severity of CNS inflammatory conditions. An increasing number of studies show the anti-inflammatory properties of curcumin; however, the underlying mechanism has not been clearly identified. In this study, bis-demethoxycurcumin (**GG6**) and its cyclized pyrazole analogue (**GG9**) (which lacks the α,β-unsaturated carbonyl moiety) were synthesized and examined for their binding affinity to the TLR4-MD-2 complex. Molecular docking experiments were carried out to assess the possible LPS-competitive accommodation of both **GG6** and **GG9** into the TLR4-MD-2 complex. As for curcumin, both analogues could conceivably bind to the hydrophobic region of the MD-2 pocket. However, only curcumin and **GG6**, presenting the α,β-unsaturated carbonyl moiety, inhibited LPS-induced TLR4 dimerization, activation of mitogen-activated protein kinase and nuclear factor-κB pathways and secretion of the pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α in primary cortical microglia. To investigate the mechanisms underlying the anti-inflammatory activity of curcumin and **GG6**, microglial cells were cultured in a low Mg^{2+} environment and exposed to curcumin, **GG6** and **GG9** before LPS stimulation. LPS-induced TLR4 dimerization and pro-inflammatory cytokine release were significantly decreased compared to normal Mg^{2+} culture medium. Curcumin and

GG6 also markedly and concentration-dependently reduced cytokine output in contrast to the pyrazole analogue **GG9**. These data demonstrate that the α,β-unsaturated carbonyl moiety of curcumin, by binding divalent cations such as Mg^{2+}, plays a critical role in curcumin anti-inflammatory responses.

Disclosures: P.F. Giusti: None. F. Belluti: None. C. Marinelli: None. E. Caracciolo: None. R. Lo: None. S. Stifani: None. S. Moro: None. M. Zusso: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.07/W7

Topic: C.03. Parkinson's Disease

Support: Crafoord foundation

Multipark

Mexican Research Council

Title: Vra4 locus mediated effects on microglial activation and dopaminergic neurodegeneration induced by overexpression of alpha synuclein.

Authors: *I. JIMENEZ FERRER[1], M. JEWETT[1], A. TONTANAHAL[1], M. ROMERO-RAMOS[2], M. SWANBERG[1];
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Abstract: Increasing evidence supports the role of inflammation as an important contributor to disease pathology in Parkinson's disease (PD). Understanding the genetic link between neurodegeneration and specific immune responses is therefore crucial to identify novel biomarkers of early stages of the disease and to devise tailored neuroprotective interventions by targeting critical inflammatory pathways. Single nucleotide polymorphisms in the MHC-II locus are associated with idiopathic PD, implicating its importance in disease pathogenesis. We have previously identified the Vra4 locus in rats with high linkage to MHCII expression. Vra4 was fine-mapped to the MHCII transactivator gene (*Mhc2ta*), which was shown to modulate susceptibility to neuroinflammation and experimental autoimmune encephalomyelitis as well as to be associated to human inflammatory disease. Microglial activation is prevalent in PD and occurs since early stages of the disease progress, MHCII expression on the surface of activated microglia could modulate the overall quality of the inflammatory response leading to a

differential degree of dopaminergic neurodegeneration. A congenic strain was created, which is identical to the DA parental strain except for having PVG alleles in the Vra4 locus, and thus lower MHCII expression. This Vra4 congenic strain is a highly physiologically relevant tool to study quantitative MHC effects on neurodegeneration and microglial response, since it is not a transgenic or knock-out, but based on a natural allelic variant. We have used the rAAV-alpha-syn PD model in Vra4-congenic and parental rats to determine the impact of naturally varying MHCII expression on microglial activation, degeneration of dopaminergic neurons, axonal pathology and motor impairment. This project is the first to address the impact of physiological differences in MHCII expression on PD-related pathology. By defining the mechanisms behind MHCII effects on microglial activation in PD, we may identify new targets for neuroprotective therapies.

Disclosures: I. Jimenez Ferrer: None. M. Jewett: None. A. Tontanahal: None. M. Romero-Ramos: None. M. Swanberg: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.08/W8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Intramural Funding

Title: Erythropoietin in the brain reduces high fat-diet induced microglial activation and regulates glucose metabolism

Authors: S. DEY[1], J. CABAN[1], Z. CUI[2], O. GAVRILOVA[2], M. GASSMANN[3], *C. T. NOGUCHI[1];
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Abstract: High fat-diet (HFD) feeding adversely affects hypothalamic regulation of energy homeostasis. Saturated fatty acids present in HFD can activate microglial cells of the hypothalamus, and induce them to proliferate, leading to enhanced local inflammatory cytokine production. Microglial cells express the receptor for erythropoietin (Epo), a cytokine produced centrally by astrocytes. Epo, produced peripherally by the kidneys is known to be necessary for erythropoiesis and can inhibit obesity-induced white fat inflammation in mice. However, Epo and its receptor (EpoR) function in microglial cells are not characterized. We hypothesized that

Epo signaling in brain could regulate HFD-induced microglial activation and thereby maintain metabolic homeostasis. To study the effect of Epo in the brain, we examined a mouse model of chronic over-expression of human *EPO* transgene in brain (*Tg21*). The *Tg21* mice exposed to 3 week HFD-feeding gained less fat mass and body weight, and had lower fasting blood glucose compared to wild-type (WT) mice. Moreover, Tg21 mice showed a small but significantly lower food intake and a trend towards higher total activity compared to WT mice. Immunofluorescent staining of the arcuate nucleus region of hypothalamic sections showed lower inflammatory cytokine TNFα and activated microglia marker Iba1 in Tg21 compared to WT mice. Acute intracerebroventricular administration of Epo (Epo ICV) in WT mice also prevented HFD-induced fat mass and weight gain relative to saline-controls (saline ICV). Epo ICV mice also showed lower fasting blood glucose, but hematocrits similar to saline ICV, suggesting no peripheral effects of Epo ICV. In contrast to Tg21 mice, Epo ICV mice did not show any difference in food intake compared to saline ICV, but showed lower gene expression for inflammatory markers *TNFα*, *IL6*, *IL1α*, *SOCS3* and higher expression of anti-inflammatory *IL10*. In summary, our studies suggest an important regulatory role of Epo in the brain in preventing high fat diet-induced fat gain, inflammation and microglial cell activation, combined with modulating food intake and activity in mice.

Disclosures: S. Dey: None. J. Caban: None. Z. Cui: None. O. Gavrilova: None. M. Gassmann: None. C.T. Noguchi: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.09/W9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 Grant NS091242

NIH RO1 Grant NS076715

AHA Grant-in-aid 15GRNT2440158

AHA Fellowship grant 12PRE12050312

Title: Non-invasive mitochondrial modulation: a novel approach to reduce cerebral injury following neonatal hypoxia-ischemia

Authors: *T. H. SANDERSON[1], C. A. REYNOLDS[1], E. GRULEY[1], C. STRUBAKOS[1], M. HUTTEMANN[2];
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Abstract: Insufficient oxygen delivery to the brain can promote significant neonatal brain injury. While prompt re-oxygenation is critical in the management of affected neonates, the reintroduction of oxygen can potentiate injury by promoting reactive oxygen species (ROS) generation within the mitochondria. While mitigating ROS damage may serve as a common therapeutic avenue for cerebral reperfusion injury, traditional attempts to scavenge ROS have failed. This failure is thought to be due to inherent difficulties in delivery to the brain and sub-cellular targets within the early minutes of reflow. Accordingly, we developed a non-pharmacologic therapy that targets cytochrome c oxidase (CcO) using specific wavelengths of infrared light (IRL) that circumvents these delivery barriers. We discovered 2 specific IRL wavelengths that penetrate the brain and reversibly reduce the activity of CcO. We proposed that these wavelengths will inhibit ROS generation by stabilizing the mitochondrial membrane potential following neonatal hypoxia/ischemia. We tested this hypothesis in parallel cell culture and neonatal rat models. All IRL wavelengths that reduce CcO activity reduced neuronal death following oxygen-glucose deprivation (OGD) in cell culture ($n = 7$, $p<0.05$). IRL also directly modulated mitochondrial respiratory rate, reduced $\Delta\Psi_m$ in a switch-like manner, and blocked mitochondrial ROS production, providing preliminary insight into the mechanism of action. Next, the 2 wavelengths that reduce CcO activity were evaluated for neuroprotection in the Vannucci neonatal HIE model with unilateral carotid ligation followed by 180 min of hypoxia (8% O_2). Pups were randomly enrolled into IRL treatment groups (n=21-33), initiated immediately upon relief of hypoxia. Mean infarct volume was reduced 48 hours after injury with treatment of a single IRL wavelength (29.3% infarct) and dual wavelength treatment (23.6% infarct) when compared with untreated-controls (44.4% infarct; $p<0.05$). An additional cohort was randomly enrolled in treatment with dual wavelength IRL or non-treatment for analysis of infarct progression with MRI, behavioral analysis, and long-term (28 day) histology. In agreement with our short-term outcome studies, our preliminary data demonstrate an improvement in infarct volume measured by MRI and histology 28 days post-HIE. In addition, IRL-treated rats also had preservation of function, as demonstrated by a 40% improvement in the rotorod test ($n = 10$-15, $p<0.05$). These data demonstrate the neuroprotective effect of non-invasive reduction of CcO activity with specific IRL wavelengths and may provide a novel strategy for the treatment of neonatal HIE.

Disclosures: T.H. Sanderson: None. C.A. Reynolds: None. E. Gruley: None. C. Strubakos: None. M. Huttemann: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.10/W10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sex differences in microglia morphology, phagocytic marker CD11b, and cytokines in the adult healthy brain among male, female and post-menopause model mice.

Authors: K. YOUNG[1], *H. MORRISON[2];
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Abstract: Microglia cells are constitutively active and attuned to immediately detect and respond to changes in their microenvironment through constant contact with neurons and glia. A highly ramified morphology and phagocytic capacity is central to efficient microglial surveillance and response. It is unknown if, preceding injury, microglia ramified morphology, phagocytic capacity and cytokine concentrations are different between sexes and if additional differences exist within the female population according to estrus cycle and reproductive status. The purpose of this study was to elucidate sex differences in constitutive microglia-associated neuroinflammation: ramified morphology, phagocytosis and brain cytokine concentrations. In addition, we employ a clinically relevant postmenopause mouse model to test for differences among healthy female pre- and postmenopause mice. For postmenopause model, we injected 4-vinylcyclohexene diepoxide (160mg/kg i.p.) into 6wk old female mice for 21 days. Brain tissue was collected from healthy euthanized C57Bl/6 (16wk) male (N=8), diestrus female (N=16), estrus female (N=10) and postmenopause female (N=18) mice for immunohistochemistry (IHC) methods and Multiplex immunoassay. Image analyses of confocal images were used to quantify microglia ramified morphology (anti- IBA-1; number of microglia process endpoints/cell and process length/cell) and phagocytic marker CD11b among groups. Multiplex data allowed for simultaneous measurement of brain cytokine concentrations among groups. Data analysis reveal that summed microglia process length/cell is increased in the female postmenopause mouse versus male ($p<0.002$) and diestrus or estrus females ($p<0.0001$ and $p=0.0002$, respectively). The number of microglia process endpoints/cell is increased in males versus estrus females ($p=0.01$). In females, endpoints/cell is increased in the postmenopause female versus estrus or diestrus females ($p<0.0001$ and $p=0.006$, respectively). Microglia CD11b is increased in diestrus female microglia versus males ($p=0.05$). Concentrations of brain cytokines (IL1β, IL6, IL4, IL10) were increased in males versus all female groups with largest effect size observed between male and postmenopause female mice. In summary, microglia ramified morphology, presence of phagocytic receptor CD11b and brain inflammatory milieu is different among healthy male, female and female postmenopause mice. We suggest that observed sex-differences in

constitutive microglia-associated neuroinflammation are an important consideration when determining the trajectory and magnitude of microglia responses to injury.

Disclosures: K. Young: None. H. Morrison: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.11/W11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: VR Grant 2012-2992

ALFGBG Grant 432291

Leducq Foundation Grant DSRR_P34404

Swedish Brain Foundation Grant FO2014-008

Torsten Söderberg Foundation Grant M98/15

Åhlén Foundation

VINNOVA Grant 2015-01505

Title: CNS effects of neonatal Staphylococcus epidermidis infection

Authors: *C. MALLARD[1], J. LAI[1], P. SVEDIN[1], X. WANG[1], T. STRUNK[2], A. CURRIE[2], M. PETTENGILL[3], O. LEVY[4];
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Abstract: Background and objective: Neonatal sepsis is a serious problem in neonatal intensive care units, causing increased hospitalization costs and prolonged hospitalization stays. The coagulase-negative staphylococcus, Staphylococcus epidermidis (SE), has emerged as the predominant pathogen of late-onset neonatal sepsis in premature infants, accounting for up to 78% of neonatal late-onset sepsis. We previously determined that intravenous injection of SE in postnatal day (PND) 1 mice induces systemic cytokine production and impairs brain development. In this study we have tested the response of older neonatal mice to the bacteria, so

as to determine the vulnerability window for infection-induced brain injury in neonates.
Methods: SE grown to mid-log phase was administered intraperitoneally into PND4 mice. Animals were monitored over time for body weight and temperature changes and long-term survival. Bacterial colony-forming units from the peripheral blood, liver and spleen was determined by spot plating. Cytokine production was determined by multiplex cytokine bead array analysis. Immunohistochemistry of brain sections assessed effects of the infection and inflammation in the brain.
Results: Injection of SE led to bacteremia within two hours in a dose-dependent manner. Pro-inflammatory cytokines were significantly up-regulated in the peripheral blood of infected animals from 2 to 24 hours post infection, and tapered down by 48 hours, in association with the clearance of the bacteria from the blood. Neutrophil and monocyte chemotactic cytokines (CXCL1 and CCL2), granulocyte-colony stimulating factor, and caspase-3 activity were also significantly elevated in the brain by 14 hours post infection. There was a dose-dependent decrease in body weight gain after the infection. No difference in brain gray and white matter volume was detected 10 days after the infection compared to saline injected mice.
Conclusions: Although intraperitoneal injection of *S. epidermidis* in PND4 animals led to a similar infection pattern and response compared to intravenous infection of PND1 animals, no gross morphological injury in the brain was detected. These data raise the possibility that vulnerability of the immature brain to infection and inflammation is age- and/or route-dependent. Comparing and delineating the difference between neonatal mice of different ages will provide insight into factors leading to brain injury upon infection and inflammation, informing development of therapeutic treatments for infants affected by these conditions.

Disclosures: C. Mallard: None. J. Lai: None. P. Svedin: None. X. Wang: None. T. Strunk: None. A. Currie: None. M. Pettengill: None. O. Levy: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.12/W12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research Fund of the Paracelsus Medical University (PMU-FFF) Grant R-13/02/046-KLE

European Union's FP7 Grant HEALTH-F2-2011-278850 (INMiND)

Title: Acute and chronic allergic reactions differentially affect immune signaling in the hippocampus

Authors: *B. KLEIN[1,2], B. ALTENDORFER[1,2], B. HAMMER[1,2], J. THALHAMER[3], S. SCHEIBLHOFER[3], R. WEISS[3], L. AIGNER[1,2];
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Abstract: Allergic diseases cause systemic inflammation and affect almost one quarter of the world-wide population. Although it has been shown that immune responses in the periphery have effects on the brain, the impact of allergies on the CNS needs further investigation. For this reason, we analyzed acute (3 days challenge) and chronic (4 weeks challenge) grass pollen allergy models. Mice were sensitized twice and challenged with the clinically relevant grass pollen allergen Phl p 5. The allergic mice showed no behavioral differences in the Open Field test and the Elevated Plus Maze. Blood samples were obtained 4 days after the second sensitization and at the end of experiment. In the sera, T_H2 cytokines (e.g. IL-4, IL-5) were elevated in the acute and chronic allergy models. Additionally, in the chronic allergy model pro-inflammatory cytokines (e.g. IFN-γ, TNF-α) were increased. In both allergy models, we analyzed the effects of the T_H2-polarized immune responses on the expression of immune mediators in the hippocampus, in comparison to the olfactory bulb and the cerebellum, using RT-qPCR. Results showed a significant down-regulation of immune mediators (e.g. IL-1β, IL-13, H2-Aa) in the hippocampus in the chronic allergy model. On histological level, we observed similar changes, e.g. a reduced number and soma size of microglia/macrophages. This data indicates that chronic allergy modulates CNS immune signaling.

Disclosures: B. Klein: None. B. Altendorfer: None. B. Hammer: None. J. Thalhamer: None. S. Scheiblhofer: None. R. Weiss: None. L. Aigner: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.13/X1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Leducq Foundation (DSRRP34404)

ERA-net (EU;VR 529-2014-7551)

Wellcome Trust (WT094823)

VR 2015-02493

Title: Magnesium pre-exposure induces mitoprotection in the immature brain

Authors: ***G. KONING**[1], C. MALLARD[1], X. WANG[1], S. NAIR[1], C. THORNTON[2], P. GRESSENS[3], H. HAGBERG[1,2,4];
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Abstract: Pre-exposure to sub-threshold hypoxia confers increased tolerance to neonatal cerebral hypoxic-ischemic (HI) injury, a phenomenon known as preconditioning. We have recently found that $MgSO_4$ (0.6 mg/kg) also induces powerful preconditioning in the immature brain. Intraperitoneal $MgSO_4$ was given 6 days, 3 days, 24h or 12h prior to neonatal HI induced in rats at postnatal day 7. This treatment reduced brain injury in all areas, but particularly in the cerebral cortex, by as much as 50-80%. $MgSO_4$ given 3h, 30min prior to or 1h post HI had no effect on brain injury. These results suggest that $MgSO_4$ induces changes that requires some time ($\geq$12h) to develop CNS resistance to a subsequent insult, which may imply that gene transcription or epigenetic alterations are required. In order to explore the mechanisms behind this protective response we initially studied effects on cerebral blood flow. Using iodoantipyrine, we found that cerebral blood flow was not affected by $MgSO_4$ 24h after drug administration, nor after exposure to 30 min or 60 min of HI. We next sampled cerebral cortex 3h and 24h after $MgSO_4$ and analyzed global gene transcription with Affy mRNA array 2.0 STv1 (36,685 genes) and Affy miRNA 4.0 (Genomic Centre, KCL, London) as well as with Next Seq, Illumina (Genetic Center, Gothenburg). The results show that many mRNA and miR related to mitochondria were affected and combined network and pathway analysis (IPA software) revealed that genes upregulating metabolism were suppressed and that those downregulating metabolism were increased. We next isolated mitochondria from cerebral cortex 24h after $MgSO_4$ treatment as well as immediately after HI (30 min or 60 min duration) and finally 3h after the insult. There was no difference in respiratory control ratio (RCR) in $MgSO_4$ vs. control mitochondria 24h after administration. However, after 30 min of HI the RCR was significantly higher after $MgSO_4$ (4.4 ± 0.6) compared to control (2.7± 0.4) $p<0.05$. In summary, intraperitoneal $MgSO_4$ provides profound preconditioning protection to neonatal HI if given 12h-6 days prior to the insult. Global mRNA and MiR analysis and experiments in isolated mitochondria indicate that $MgSO_4$ suppresses mitochondrial metabolism making mitochondria more resistant to respiratory deterioration during HI. We are currently performing metabolomic analysis to better understand the mito-protective effect of $MgSO_4$ pre-administration.

Disclosures: G. Koning: None. C. Mallard: None. X. Wang: None. S. Nair: None. C. Thornton: None. P. Gressens: None. H. Hagberg: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.14/X2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Generation of an *In vivo*-like phenotype of primary murine microglia cells in culture

Authors: *H. CYNIS[1], S. BARENDRECHT[1], H.-U. DEMUTH[1], R. EICHENTOPF[1], B. HIETEL[1], C. PÖSEL[2], S. SCHILLING[1], D.-C. WAGNER[2];
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Abstract: Microglia cells are resident phagocytes of the CNS derived from primitive macrophages of the yolk-sac. The cells populate the CNS early during embryogenesis and are important for brain homeostasis by scanning the surrounding tissue for cellular damage. Microglia cells also support the formation of neural circuits and may contribute to cognitive impairment by actively eliminating complement-tagged synapses. Since these cells operate within the complex environment of CNS tissue, *in vitro* studies are hampered by a rapid loss of the microglial phenotype in culture presumably due to the lack of input from other cells of the CNS.
In the present study, we were interested in microglia gene expression signatures in culture obtained by different isolation methods and the establishment of specific culture conditions to retain and/or restore an *in vivo*-like phenotype.
The applied methods comprised different isolation techniques of primary microglia including FACS sorting from adult and newborn mice, density gradient isolation and shaking from mixed glia cultures. The microglial gene expression was characterized by RNA microarray analysis and qRT-PCR. To influence the microglial phenotype, the cells were co-cultured with primary neurons or stimulated using different combination of cytokines and growth factors and again analyzed by RNA microarray analysis and functional assays such as quantification of microglial phagocytosis.
The microglial signatures obtained by gene expression analysis greatly depend on the method used for cell isolation and culture. Specific combinations of factors important for microglial function, such as CX3CL1, IL-34 and TGF-beta are able to influence gene expression signatures generating a phenotype resembling microglia cells *in vivo*.
The results will help to establish protocols to enable in depth-analysis of native microglia functions *in vitro* such as their contribution to phagocytosis of beta-Amyloid peptides in Alzheimer's disease.

Disclosures: H. Cynis: None. **S. Barendrecht:** None. **H. Demuth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Probiodrug AG. F. Consulting Fees (e.g., advisory boards); Probiodrug AG. **R. Eichentopf:** None. **B. Hietel:** None. **C. Pösel:** None. **S. Schilling:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiodrug AG. F. Consulting Fees (e.g., advisory boards); Probiodrug AG. **D. Wagner:** None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.15/X3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1RF1AG051437

Title: MicroRNAs influence microglia-mediated phagocytosis of $A\beta_{1-42}$ *In vitro*

Authors: *M. S. ALOI[1], W. SU[2], S. FUNG[2], S. DAVIDSON[2], S. JAYADEV[2], G. A. GARDEN[2];
[1]Pathology, [2]Neurol., Univ. of Washington, Seattle, WA

Abstract: Neuroinflammation is a hallmark of acute and chronic CNS injury, including traumatic brain injury and neurodegenerative diseases. Microglia are the innate immune cells of the CNS that effect rapid phenotypic changes by utilizing powerful post-transcriptional modulators, such as microRNAs (miRNAs). Recent studies have implicated two miRNAs known to regulate inflammatory responses, miR-146a and miR-155, in the pathogenesis of several neurodegenerative disorders including multiple sclerosis, ALS, familial Parkinson's disease, and Alzheimer's disease (AD). Expression of both miR-155 and miR-146a is altered in AD mouse models. Interestingly, miR-146a promotes anti-inflammatory functions and deletion of miR-146a leads to an accelerated aging phenotype, which can be abrogated by conditional deletion of miR-155 in T cells. We have also observed that miR-146a expression in microglia is regulated by presenilin 2, a protein mutated in familial AD. We hypothesized that miR-155 and miR-146a modulate microglial inflammatory phenotypes, thereby regulating microglia mediated internalization amyloid beta (Aβ). To evaluate the role of miR-155 and miR-146a in microglia activation, we successfully developed an Adeno-Associated Virus (AAV) serotype 2 vector to introduce CMV-Cre into floxed-miR155 (miR-$155^{flx/flx}$) microglia and flox-stop-flox miR-155 conditionally overexpressing neonatal microglia (*mbic-fsf*). We observed that in cultured microglia, conditional overexpression of miR-155 significantly upregulated miR-146a. Furthermore, conditional deletion of miR-155 in cultured microglia promotes phagocytosis of oligomerized $A\beta_{1-42}$ *in vitro* as measured by flow cytometry, while overexpression of miR-155

decreases oligomerized $A\beta_{1-42}$ internalization. Taken together, our results support the hypothesis that miR-155 and miR-146a modulate microglia-mediated phagocytosis and clearance of $A\beta_{1-42}$. Understanding how miRNAs modulate microglia activation and phagocytosis of $A\beta_{1-42}$ will further elucidate the molecular pathways regulating neuroinflammation in AD pathology.

Disclosures: M.S. Aloi: None. W. Su: None. S. Fung: None. S. Davidson: None. S. Jayadev: None. G.A. Garden: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.16/X4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: AMERICAN HEART ASSOCIATION

Title: The sympathetic side of phagocytes

Authors: *I. SHAKED;
Univ. of California At San Diego, LA Jolla, CA

Abstract: The nervous system and the immune system sustain profound communication, which ensures homeostasis and health. On the other hand, brain to immune miscommunication may lead to neuroinflammation and pathology.
In a recent work we identified the nuclear receptor Nr4a1 as a link between the sympathetic nervous system and the immune system, which controls production of norepinephrine (NE) in monocyte-derived macrophages. Using a mouse model of CNS autoimmunity, we have demonstrated a novel role for NE-producing phagocytes in neuroinflammation. Moreover, Nr4a1-dependent patrolling monocytes monitor and maintain the brain vasculature under normal condition and following laser-induced focal injury to the brain (model of ischemic stroke). Our study suggests a new role for phagocytes as a crucial translators and amplifiers of neuronal signaling to endothelial and immune cells, and which therefore, by “spreading the neuronal word” play a crucial role in controlling neuroinflammation.

Disclosures: I. Shaked: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.17/X5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Institute on Aging Northwestern University Alzheimer's Disease Center (AG013854)

National Institute of Neurological Disorders and Stroke (NS085770)

Title: Concordance between cortical atrophy and distribution of microglia in primary progressive aphasia with TDP-43 inclusions

Authors: G. KIM[1], T. GEFEN[1], Z. PARTON[1], N. LALEHZARI[1], F. RAHMANI[1], *S. WEINTRAUB[2], E. H. BIGIO[1], E. ROGALSKI[1], M.-M. MESULAM[1], C. GEULA[1]; [1]Cognitive Neurol. and Alzheimer's Dis. Ctr., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [2]Northwestern Feinberg Sch. of Med., Northwestern Univ. Med. Sch., Chicago, IL

Abstract: Primary progressive aphasia (PPA) is a neurodegenerative clinical dementia syndrome characterized by a gradual dissolution of language. The asymmetric nature of the language network and focal atrophy render PPA an excellent model for investigation of the relationships between the regional distribution of pathologic markers, cortical atrophy, and clinical phenotype. At autopsy, approximately 30% of PPA patients have TAR-DNA binding protein-43 (TDP-43) pathology (PPA-TDP). We previously reported concordance between microglia activation and cortical atrophy in PPA-TDP participants with progranulin mutations. The current study investigated whether a similar concordance exists in PPA-TDP participants without progranulin mutations. Whole hemisphere sections from three PPA-TDP cases were immunohistochemically stained for HLA-DR, a marker of activated microglia. Using unbiased stereology, densities of activated microglia were quantified in the following regions: inferior frontal gyrus (IFG), middle frontal gyrus (MFG), inferior parietal lobule (IPL), superior temporal gyrus (STG), middle or inferior temporal gyrus (MTG or ITG), entorhinal cortex (ERC), and hippocampus (HIP). Cortical atrophy was assessed using one of three methods: 1) structural MRI scans collected close to death analyzed using the FreeSurfer software, 2) clinical MRI scans, or 3) raw images of the postmortem brain. Case 1, a right-handed male, displayed substantial asymmetry of microglia distribution, greater in the left hemisphere in all cortical areas. This showed concordance with atrophy patterns visualized by quantitative MRI. Case 2, a left-handed male, had reversed asymmetry of atrophy and right hemisphere language dominance; clinical scans exhibited severe atrophy of the right temporal lobe. Microglia density displayed substantial asymmetry in right hemisphere cortical areas, with greatest asymmetry in STG and ITG, concordant with patterns of atrophy on MRI. Microglial counts in Case 3, a right-handed male participant, were fairly

symmetric across all cortical regions, aligning with the absence of visible left-sided asymmetric atrophy in most cortical areas. These findings demonstrate significant microglial activation in PPA-TDP that match patterns of atrophy. It remains to be determined whether microglial activation contributes to cortical damage in PPA-TDP.

Disclosures: **G. Kim:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **T. Gefen:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **Z. Parton:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **N. Lalehzari:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **F. Rahmani:** None. **S. Weintraub:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **E.H. Bigio:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **E. Rogalski:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **M. Mesulam:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine. **C. Geula:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.18/X6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 5F31NS079091-03

Title: Analysis of microglia population dynamics in the healthy and diseased brain

Authors: *D. E. MARZAN[1], W. GAN[3], J. SALZER[2];
[2]Neurosci., [1]NYU Sch. of Med., New York, NY; [3]Dept. of Physiol. and Neurosci., Skirball Inst. of Biomolecular Med., New York, NY

Abstract: Microglia are the resident immune cells of the central nervous system (CNS) that arise from yolk sac progenitor cells and colonize the brain during early embryonic development. In the healthy adult brain, these cells exist as a distinct population without infiltration of peripheral monocytes and macrophages. Although these cells are long lived with a slow turnover, the exact identity of the source(s) of microglia in the adult remains unclear. Here we offer direct evidence that microglia are a self-renewing population and do not arise from a progenitor and/or stem pool. In our studies, we utilized a genetic strategy to differentially label endogenous CNS microglia from myeloid cells in the peripheral immune system. We ablated 99% of microglia in

the brains of these mice by using a well characterized inhibitor of the Colony Stimulating Factor 1 Receptor. As previously reported (Elmore et al., Neuron 2014), there is a rapid repopulation of microglia in the brain upon removal from this inhibitor. We have determined that these repopulated cells originated from the surviving resident microglia. This finding supports the theory that the few microglia surviving ablation are sufficient to repopulate the entire brain. Moreover, using the same labeling strategy in a model of demyelination, we also observed that almost all macrophages in the CNS are microglia in origin with little to no contribution from peripheral myeloid cells. To corroborate this data, experiments are underway in mice that lack the ability for monocytes to be recruited into the inflamed CNS. These studies provide new insights into microglial biology in both the healthy and diseased brain.

Disclosures: D.E. Marzan: None. W. Gan: None. J. Salzer: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.19/X7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant MH64570

NIH Grant P30AI078498

NIH Grant T32AI049815

Title: Optical control of microglial APC function using Cas9 effectors

Authors: *P. MILLER-RHODES, R. WYCALLIS, S.-M. LU, H. GELBARD;
Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Human immunodeficiency virus-1 (HIV-1) associated neurocognitive disorder (HAND) describes the neurocognitive impairment observed in up to half of HIV-1 infected individuals. HAND is refractory to combination antiretroviral therapy; thus, adjunctive therapies for HAND remain a critical unmet medical need. Recent efforts by our laboratories have identified therapeutic strategies that target both innate and adaptive immune effectors, including GM-CSF and vasoactive polypeptide receptor type 2 receptor 2 (VPAC2) agonists. Increasing evidence shows that these agents resolve microgliosis and the correlated destruction of synaptodendritic networks by promoting regulatory T cell (Treg) responses over effector T cell (Teff) responses. While we have shown previously that these agents promote Treg expansion and/or stabilization, we reasoned that this immune transformation is sustained in part by the

direct effects of GM-CSF and/or VPAC2 agonism on APC populations. Specifically, we hypothesized that VPAC2 agonism/GM-CSF induces a tolerogenic APC phenotype in microglia. We approached this hypothesis mechanistically by generating a series of microglia and T cell lines that stably express programmable Cas9 effectors capable of modulating gene expression in a spatially and temporally controlled manner. To this end, we chose two Cas9 effector systems: (1) the "Suntag" effector, which uses a peptide array to recruit ten copies of the transcriptional activator VP64 to genome-targeted, nuclease-deficient Cas9 (dCas9):sgRNA complexes, and (2) the light-activated Cas9 effector system (LACE), which leverages the light-inducible heterodimerization of Cry2 and CibN, which are fused to VP64 and dCas9, respectively, in order to recruit two copies of the transcriptional activator module to the dCas9-sgRNA complex. We used these Cas9-effector cell lines to identify and interrogate genes that are central to the transformation of activated microglia into tolerogenic APCs.

Disclosures: P. Miller-Rhodes: None. R. Wycallis: None. S. Lu: None. H. Gelbard: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.20/X8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS Grant 16K08635

JSPS Grant 16K07076

JSPS Grant 16K11308

JSPS Grant 15H04999

Title: Dock8 deficiency suppresses microglial migration and neuroinflammation

Authors: *K. NAMEKATA, X. GUO, A. KIMURA, C. HARADA, T. HARADA;
Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: The Rho subfamily GTPases (Rac1, Cdc42 and RhoA) are best documented for their roles in actin dynamics, and they regulate a variety of cellular processes, including cell migration, morphogenesis, neuronal development, gene expression and cell division and adhesion. The Dock family proteins (Dock1-11) of atypical guanine nucleotide exchange factors (GEFs) activate Rac1 and/or Cdc42, but not RhoA. The Dock family proteins are involved in various disorders, including Parkinson's disease and Adams-Oliver syndrome. Recently, Dock8

functions and signaling have gained attention due to the discovery of a combined immunodeficiency syndrome caused by Dock8 mutations in humans. Accumulated evidence revealed that Dock8 deficiency leads to multiple defects in immune cells, such as T cells and B cells. Microglia, the resident immune cells in the CNS, have important roles in neuroinflammation. In the CNS, microglia are activated by several disorders and injury, and accumulate at the lesion site in order to initiate immune responses. Activated microglia are associated with the progression of several neurodegenerative diseases, such as Alzheimer's disease. Therefore, microglia are one of the key targets for therapeutic intervention. To date, the function of Dock8 in microglia is unknown. In this study, we generated Dock8-deficient mice and investigated the function of Dock8 in microglia. To examine which cell types abundantly express Dock8 in the CNS, we performed immunoblot analysis using primary cultured cells (neuron, astrocyte and microglia). Immunoblot analysis showed Dock8 is expressed in microglia but not in neurons and astrocytes. The chemotaxis assay revealed the reduced cell migration ability in Dock8-deficient microglia. We also found that the formation of podosomes, actin-associated adhesion structures involved in the cell migration, is suppressed in Dock8-deficient microglia, suggesting that Dock8 regulates microglial migration via actin dynamics. In addition, Dock8-deficiency reduced microglia accumulation at the lesion site and ameliorated the clinical signs in experimental autoimmune encephalomyelitis (EAE) mice, an animal model of multiple sclerosis (MS). These findings suggest that Dock8 stimulates migration of microglia, which leads to severe CNS inflammation. Thus, manipulation of Dock8 signaling might be a novel therapeutic strategy against diseases associated with neuroinflammation including MS and optic neuritis.

Disclosures: K. Namekata: None. X. Guo: None. A. Kimura: None. C. Harada: None. T. Harada: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.21/X9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA

Title: Chronic ethanol increases toll-like receptor 7-induced neuroinflammation and neurodegeneration in C57BL/6 mice

Authors: *L. QIN, F. T. CREWS;
Bowles Ctr. Alcohol Studies, Univ. North Carolina, Sch. Med., Chapel Hill, NC

Abstract: Toll-like receptor (TLR) 7 triggers production of proinflammatory cytokines when activated by viral singe-stranded RNA or the small molecule agonist imiquimod (R837). We investigated the effects of chronic ethanol (ETOH, 5 g/kg/day, i.g., daily for 10 days) on TLR7 expression in brain and imiquimod (2.5 mg/kg, i.p.) induction of neuroinflammation and neurodegeneration in male C57BL/6 mice. ETOH increased levels of TLR7 mRNA and protein, determined by real-time PCR and entorhinal cortex immunohistochemistry. Confocal microscopy revealed that TLR7 was expressed in NeuN+IR neurons and Iba1+IR microglia, but not in GFAP+IR astrocytes. Chronic ethanol treatment increased p-NF-κB p65 immunoreactivity, an indicator of increased neuroimmune signaling and HMGB1, a neuroimmune signaling molecule. Imiquimod, the TLR7 agonist increased TLR7 mRNA and IHC similar to ethanol, whereas ETOH treatment followed by a single imiquimod treatment further increased TLR7 expression. Ethanol pretreatment potentiated imiquimod-induced brain mRNA levels of TNFα (535 %), IL-1β (186%), IL-6 (236%), and MCP-1 (369%), compared with imiquimod alone treatment consistent with ETOH induction of TLR7. Increased levels of brain cytokines coincided with increased microglial activation, NOX $gp91^{phox}$, and markers of neurodegeneration: activated caspase-3 and Fluoro Jade B. Ethanol potentiation of imiquimod was associated with ethanol-increased expression of both TLR7 and the endogenous TLR agonist HMGB1 in the brain. These studies indicate that ETOH increases TLR7 and TLR7 agonist induced increase in brain cytokines, chemokines and NADPH oxidase that contribute to neurodegeneration.

Disclosures: L. Qin: None. F.T. Crews: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.22/X10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: AA020023

AA020024

AA019767

AA007573

Title: The TLR7 agonist imiquimod induces neurotoxicity via HMGB1-let-7 signaling in brain slice cultures: ethanol potentiation and neuroprotection of rifampin

Authors: *J. Y. ZOU, L. G. COLEMAN, F. T. CREWS;
Univ. North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Activation of the RNA-sensing Toll-like receptor (TLR) 7 induces neurodegeneration through neuronal TLR7. The present study used an *ex vivo* model of organotypic hippocampal-entorhinal cortex (HEC) slice cultures to investigate the role of danger signaling molecule HMGB1 and miRNA let-7b in TLR7 agonist imiquimod-induced neurotoxicity as well as the effects of ethanol and MD2 inhibitor rifampin. Imiquimod treatments of HEC slices caused neuronal cell death in concentration-dependent manner, with 10mg/ml and 20mg/ml imiquimod causing a 67% and 152% increase in PI uptake (index of neuronal death) respectively. Measurements of media HMGB1 levels revealed that imiquimod (10mg/ml) caused rapid active release of HMGB1 (3 fold increase at 8hr) and gradually reached peak in correlation with neuronal cell death. Inactivation of HMGB1 with inhibitor glycyrrhizin significantly reduced imiquimod-induced neuronal death, suggesting that actively released HMGB1 contributes to TLR7-activated neuronal cell death. MicroRNA let-7b is known to serve as an endogenous ligand that activates neuronal TLR7 causing neuro-immune activation and neurodegeneration. Measurements of extracellular miRNAs from microparticles isolated from culture media indicated that imiquimod treatments increased release of miR-let-7b and miR-155. Using the assay of HMGB1 immunoprecipation followed by RT-PCR for miRNAs, we detected that HMGB1 is mainly associated with let-7b but not miR-155, suggesting HMGB1 coupling with let-7b leading to activation of intracellular neuronal TLR7. Eliminating microglia with CSF-1 receptor inhibitor PLX3397 appears not to reduce release of extracellular let-7b and the expression of HMGB1 mRNA level as well as imiquimod-induced neuronal cell death, further indicating neuronal origin of HMGB1 and let-7b. We further test the effects of ethanol and rifampin on imiquimod-induced neuronal death. Ethanol (100mM) prime for 48hrs significantly enhanced neuronal vulnerability to imiquimod (10mg/ml), causing a 38% increase in PI uptake relative to imiquimod alone. Combination of ethanol and imiquimod also increased increase the association of let-7b with HMGB1 by 50% above control. Furthermore, the presence of rifampin (10uM) completely blocked imiquimod-induced neurotoxicity as well as the release of HMGB1 and let-7b. Together, these novel results identify the mechanisms underlying activation of intracellular neuronal TLR7.

Disclosures: J.Y. Zou: None. L.G. Coleman: None. F.T. Crews: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.23/X11

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH 042767

NIH 080585

Title: The injury-induced up-regulation of glial aromatase is cyclooxygenase dependent in the zebra finch (Taeniopygia guttata).

Authors: *C. J. SALDANHA[1], A. L. PEDERSEN[2], C. J. GOULD[2];
[1]Biol. and Psychology, [2]Behavior Cognition & Neurosci., American Univ., Washington, DC

Abstract: Aromatase expression is induced in astrocytes following brain damage in homeotherms. The consequences of induced astrocytic aromatization are well studied, but little is known about the factor(s) that induce glial aromatase. Central application of an inflammagen is sufficient to induce astrocytic aromatase, suggesting a causative link between inflammation and glial aromatase expression. To test this hypothesis, we administered lipopolysaccharide (LPS) or vehicle peripherally to adult zebra finches of both sexes and observed their behavior 2 or 24 hr later. The birds were then sacrificed, and the central transcription of pro-inflammatory cytokines (TNFα, IL-1β and IL-6) and aromatase was measured, as was telencephalic aromatase activity (24hr time point only). Two hours post LPS resting behavior increased ($p = 0.002$) and locomotor behaviors decreased ($p = 0.03$) in both sexes relative to controls and pretreatment observations. But 24 hours later, the behavior of LPS birds was similar to controls ($p = 0.81$). Two hours post LPS injection, IL-1β was higher in both sexes ($p = 0.0003$), but only females showed higher TNFα mRNA ($p =0.03$). However, 24 hours post LPS, cytokines had returned to baseline, but aromatase mRNA ($p = 0.004$) and activity were elevated in both sexes ($p = 0.01$). No changes in IL-6 were detected in either sex following treatment. These data suggest that neuroinflammation following peripheral LPS administration may be sufficient to induce central aromatase expression. To test the necessity of this association, we measured the neural aromatase expression and estradiol content (E_2) of adult females injected with the cox1/2 inhibitor (indomethacin) or vehicle into contralateral telencephalic hemispheres, 6 or 24hr post injury. An EIA for prostaglandin E_2 (PGE_2) revealed lower PGE2 in the lobe injected with indomethacin relative to the contralateral lobe at both time points ($p=0.004$). Next, aromatase transcription was measured relative to GAPDH using qPCR and revealed lower aromatase expression in the lobe treated with indomethacin at 6 ($p = 0.006$), but not 24 hours post injury ($p = 0.39$). Correspondingly, the lobe injured in the presence of indomethacin had lower levels of E_2 relative to the vehicle-treated hemisphere at 6 ($p=0.02$), but not 24hr post injury ($p=0.82$). These data suggest that inflammatory signaling, specifically cyclooxygenase activity and consequent PGE2 synthesis and secretion, may be necessary for injury-induced increases in aromatization and central E_2. Future studies will test the cell-specificity of aromatase expression following peripheral LPS and central inhibition of PGE2 synthesis.

Disclosures: C.J. Saldanha: None. A.L. Pedersen: None. C.J. Gould: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.24/X12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: ICMR

Title: Estrogen therapy modulates inflammatory and apoptotic protein expression in estrogen receptor knock down female rat hippocampus

Authors: *P. KUMAR, P. DHAR;
Anat., AIIMS, New Delhi, India

Abstract: Background: Estrogen is an essential female sex hormone known to exert pleiotropic effects on different tissues including the central and the peripheral nervous system. It also plays a significant role in immune functions by influencing the inflammatory response. Decreased levels of this hormone have long been associated with cognitive decline and neurodegenerative changes in the brain areas associated with learning and memory. It is also known that neurodegenerative changes and inflammatory responses in the brain go hand in hand.
Aim and Objectives: To evaluate the immunoregulatory and protective role of estrogen replenishment therapy in estrogen receptor knock down female rat hippocampus with the help of immunohistochemical, western blot and molecular biology techniques.
Materials and Methods: Adult female wistar rats (4-5 months) were divided into various groups (n=6/group) such as gp-I-ovary intact control; gp-II ERα knock down; gp-III ER β knock down; gp-IV ERα and ER β knock down; gp-V ERα knock down + 17β-estradiol; gp-VII ERα and ER β knock down + 17β-estradiol. 17β-estradiol (E2) was administered daily by s.c. injection (0.1 mg/kg body wt. in 0.1 ml sesame oil) for 30 days. shRNA/siRNA Lentiviral particles was used for inhibition of expression of corresponding estrogen receptors.
Results: Depletion of the ovarian hormone (E2) resulted in upregulation of Bax, complement proteins (C3 and C1q), pro-inflammatory cytokine (TNF-α) and microglial activation in hippocampus. However, the anti-apoptotic protein (Bcl2) expression was down regulated. E2 therapy to ovx rats reversed the expression of all above mentioned immuno and apoptotic marker proteins to nearly the ovary-intact-like state. Concomitant changes in the ERα and β expression with hormone depletion/replacement therapy substantiate it to be a hormone-mediated process. In conclusion, the results indicate that the conservation of immune function in brain may be affiliated with the hormone interactions.

Disclosures: P. Kumar: A. Employment/Salary (full or part-time): AIIMS. **P. Dhar:** A. Employment/Salary (full or part-time): AIIMS.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.25/X13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant MH109165

Title: Peripheral chronic Interleukin-1 exacerbates neuroinflammation via endothelial Interleukin-1 Receptor 1

Authors: *D. NEMETH, X. LIU, D. DISABATO, N. QUAN;
Inst. For Behavioral Med. Res., Columbus, OH

Abstract: Peripheral inflammation is known to induce neuroinflammation which can contribute to the neuropathologies present in neurodegenerative diseases, such as Alzheimer's disease and Multiple Sclerosis. In addition, these neurodegenerative diseases can also upregulate Interleukin-1β (IL-1β) expression in the central nervous system (CNS). Although central and peripheral IL-1β are known to contribute to the development of inflammation, peripheral IL-1β alone is insufficient to induce neuroinflammation. In the present study, we examine the effects of chronic central IL-1β expression and concurrent central and peripheral IL-1β induced inflammation. Human IL-1β expressing adenoviral vector (adIL-1) was injected into the CNS alone or simultaneously into both CNS and circulation. Central, but not peripheral, adIL-1 was able to induce dose dependent microglial activation, leukocyte infiltration, and increased blood brain barrier permeability at the injection site. We then utilized transgenic mouse lines, in which Interleukin-1 Receptor 1 (IL-1R1) is restrictively expressed on endothelial cells, peripheral myeloid cells, microglia, astrocytes or not expressed (Tie2Cre-IL-1R1$^{r/r}$; LysMCre-IL-1R1$^{r/r}$; CX3CR1Cre -IL-1R1$^{r/r}$; GFAPCre-IL-1R1$^{r/r}$; IL-1R1$^{r/r}$, respectively). Results show chronic IL-1β expression can induce localized neuroinflammation through the abovementioned cell types. Interestingly, extensive distribution and magnitude of microglia activation and leukocyte infiltration was observed in the Tie2Cre-IL-1R1$^{r/r}$ mice, indicating endothelial cells may be a crucial mediator of IL-1β induced neuroinflammation. The addition of peripheral adIL-1 simultaneously with central adIl-1 exacerbated aforementioned pathologies in WT and Tie2Cre-IL-1R1$^{r/r}$ but not IL-1R1$^{r/r}$ animals. This study suggests that peripheral inflammation can augment the magnitude of existing central neuroinflammation via endothelial IL-1R1.

Disclosures: **D. Nemeth:** A. Employment/Salary (full or part-time): The Ohio State University, College of Dentistry. **X. Liu:** None. **D. DiSabato:** None. **N. Quan:** None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.26/X14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Elevated dopamine levels and reduced dopamine turnover in the striatum after Toxoplasma gondii infection

Authors: *J. B. EELLS[1], S. GUO-ROSS[1], D. BRAMLETT[1], D. S. LINDSAY[2], A. VARELA-STOKES[1];
[1]Mississippi State Univ., Mississippi State, MS; [2]Dept. of Biomed. Sci. & Pathobiology, Virginia-Maryland Regional Col. of Vet. Med., Blacksburg, VA

Abstract: Infection with the protozoan parasite *Toxoplasma gondii* has been linked with an increased risk of several neuropsychiatric disorders, including schizophrenia, obsessive compulsive disorder and attempted suicide. Additionally, *T. gondii* infection is associated with more severe symptoms and exacerbated pathological changes in the brains of schizophrenia patients. In rodents, *T. gondii* infection alters behaviors that include elevated activity, reduced levels of anxiety and loss of aversion to cat urine. One mechanisms proposed through which *T. gondii* alters behavior is via effects on dopamine neurotransmission. Previous studies have shown that *T. gondii* can increase whole brain dopamine levels and that two tyrosine hydroxylase enzymes, the rate limiting step in the synthesis of dopamine, are expressed by *T. gondii*. The current study was designed to investigate the relationship between striatal dopamine levels and localization of *T. gondii* cysts. Mice were infected with *T. gondii* and euthanized 8 weeks later. The striatum was isolated from one side of the brain for the measurement of dopamine and metabolites. Tissue sections through the striatum from the other side of the brain were stained with H&E and examined for *T. gondii* cysts. *Toxoplasma gondii* infected mice had significantly higher tissue dopamine levels but reduced dopamine turnover (DOPAC/Dopamine) as compared to uninfected controls. On tissue sections, *T. gondii* cysts were identified in the cortex, but no cysts were observed in the striatum of infected mice. These data demonstrate that *T. gondii* infection can alter dopamine neurotransmission in the striatum. Therefore, *T. gondii* cysts are unlikely to be the direct source of the elevated dopamine levels as a substantial number of cysts would be needed to significantly add to the high dopamine production in the striatum. Neuroinflammation or direct effects on dopamine neurons are other potential mechanisms through which *T. gondii* infection could alter dopamine neurotransmission.

Disclosures: J.B. Eells: None. S. Guo-Ross: None. D. Bramlett: None. D.S. Lindsay: None. A. Varela-Stokes: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.27/X15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Lipopolysaccharide-induced changes in neurotransmitters in the striatum and prefrontal cortex of freely moving animals.

Authors: *N. MOORE, A. RASSOULPOUR, A. ARANOV, H. KOOIJKER, Y. CHANG, L. YU, H. JANSSENS, C. CIARDELLO, M. VAN DER HART;
Brains On-Line LLC, South San Francisco, CA

Abstract: Systemic administration of the endotoxin lipopolysaccharide elicits a rapid innate immune response. This systemic inflammatory response also impacts the central nervous system, including changes in cytokines and possibly neurotransmitters. The changes in specific neurotransmitters, however, remains unclear (Mohankumar etal, 1999, Van Heesch et al,2014). The current set of experiments was designed to further explore regional effects of systemic LPS treatment on changes in dopamine (DA), serotonin (5HT), norepinephrine (NE), gamma-aminobutyric acid (GABA), glutamate (Glu) and glycine (Gly) in the prefrontal cortex (PFC) and striatum of the same animals. Adult male mice were bi-laterally implanted with microdialysis probes in the PFC and striatum, and after the collection of a stable baseline were treated with 0.3 mg/kg of LPS. LPS treatment resulted in a differential response among neurotransmitters and brain regions. NE, DA, and 5HT were all elevated to varying degrees in the prefrontal cortex (97%, 117%, 36% respectively). However, striatal DA levels were not affected by LPS treatment while NE and 5HT increased similar to the PFC. The levels of Glu, GABA, and Gly were unchanged in the 4 hour period after treatment in both brain regions.. Future studies will extend out the current study design to 24 hour to determine if an increase in the amino acids may occur at a later time point. Together, the results demonstrate a clear regional control of monoamines in the CNS of mice following systemic LPS treatment.

Disclosures: **N. Moore:** A. Employment/Salary (full or part-time): Full. **A. Rassoulpour:** A. Employment/Salary (full or part-time): fuul. **A. Aranov:** A. Employment/Salary (full or part-time): full. **H. Kooijker:** A. Employment/Salary (full or part-time): full. **Y. Chang:** A. Employment/Salary (full or part-time): full. **L. Yu:** A. Employment/Salary (full or part-time): Full. **H. Janssens:** A. Employment/Salary (full or part-time): full. **C. Ciardello:** A. Employment/Salary (full or part-time): full. **M. van der Hart:** A. Employment/Salary (full or part-time): full.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.28/X16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIGMS COBRE: 1P20GM103653

Title: Microglial activation in the developing rodent brain following single-day moderate binge-alcohol exposure

Authors: *M. J. RUGGIERO, K. BOSCHEN, A. KLINTSOVA;
Univ. of Delaware, Newark, DE

Abstract: Developmental exposure to alcohol has been shown to activate microglia, which may contribute to deficits in cognitive functions in humans with Fetal Alcohol Spectrum Disorders (FASDs) and behavioral deficits in animal models of FASDs. This current study focuses on the brain's short-term immune response to neonatal alcohol exposure (AE), by expanding the timeline of microglial activation through measurement of microglia cell number and pro-inflammatory cytokine expression in the developing rat hippocampus. We hypothesized that neonatal AE would lead to an increase in microglial cell counts and inflammatory cytokine release, and that sex differences would be observed. Male and female rat pups were given alcohol (5.25 g/kg/day) through intragastric intubation on postnatal day (PD) 4 to model a single binge-like exposure during the third trimester in humans. Suckle control (SC) rats remained with the dam undisturbed, and sham-intubated (SI) animals were intubated without receiving alcohol to account for potential stress effects of the procedure. Rats were sacrificed on PD5 or PD8 and the hippocampus processed for the various assays. Cell number was estimated in three subregions of each of the three hippocampal subfields: DG, CA1, and CA3. The results support our hypothesis: on PD5, an increase in microglial number was seen in AE male rats in the hilus and molecular layer of the dentate gyrus compared to SC. Males generally had higher levels of microglia in all hippocampal subregions. Interestingly, female pups exhibited a decrease in microglia number following AE in the dentate gyrus granule cell layer and molecular layer when compared to SI controls. For gene expression assays, preliminary data shows that expression of the pro-inflammatory cytokines CCL4 and IL-1β in AE animals on PD5 were not statistically significant compared to controls when male and female pups were analyzed separately. When the data from both sexes were combined, significant increases in CCL4 expression were seen in AE and SI animals compared to SC. In summary, we are observing immediate, short-term effects of AE on microglial activation as seen by cell counts in males, and pro-inflammatory cytokine gene expression in both males and females. These findings are significant as they add to our knowledge of specific sex-dependent effects of alcohol on microglia in developing brain.

Supported by NIH/NIGMS COBRE: The Delaware Center for Neuroscience research grant 1P20GM103653 to AYK.

Disclosures: M.J. Ruggiero: None. K. Boschen: None. A. Klintsova: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.29/X17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Centre for Neuroscience and Regenerative Medicine

USUHS intramural grant

Title: Angiotensin II receptor blockers differentially regulate LPS-mediated microglial activation

Authors: *Z. C. JANATPOUR[1], K. AFFRAM[2], R. SHARMA[2], A. J. SYMES[2];
[2]Pharmacol., [1]Uniformed Services Univ., Bethesda, MD

Abstract: Traumatic brain injury (TBI) results in acute inflammation that impacts on the ability of the brain to recover from the initial insult. Thus, pharmacologic interventions that reduce neuroinflammation should be beneficial. Angiotensin II (Ang II), signaling through its AT1 receptors (AT1R), has pro-inflammatory effects in many different organ systems including the brain. AT1R antagonists, angiotensin receptor blockers (ARBs), have anti-inflammatory actions in addition to reducing other negative sequelae of Ang II signaling. Using LPS-stimulation of microglia as a model of microglial activation, we have shown that the ARB telmisartan reduced expression of the pro-inflammatory genes iNOS and IL-1β in primary microglial culture. However, direct Ang II treatment of microglia did not result in induction of pro-inflammatory mediators, and telmisartan maintained its anti-inflammatory activity in microglia cultured from AT1R knockout mice, suggesting that blockade of angiotensin was not the mechanism through which telmisartan reduced expression of pro-inflammatory genes.
Several ARBs are FDA approved to treat hypertension. They all block signaling through the AT1R. However, their effects on other pathways vary significantly. For example, telmisartan, candesartan, and losartan, but not eprosartan, have PPARγ agonist activity, which may reduce inflammation. However, we did not observe any reduction of telmisartan's anti-inflammatory effects in the presence of the PPARγ antagonist, T0070907. A recent publication showed that telmisartan's ability to inhibit microglial activation is dependent on AMPK activation. We show

that candesartan has anti-inflammatory activity in LPS-stimulated microglia. The mechanisms through which this and other ARBs reduce inflammation in microglia are not known. We therefore treated primary rat microglia or the murine microglial cell line, BV2, with LPS in the presence or absence of either telmisartan, candesartan, olmesartan, losartan, ibresartan, valsartan or eprosartan. All ARBs reduced the LPS-induced expression of iNOS and IL-1β mRNA, showing that they share anti-inflammatory activity, though telmisartan was the most potent anti-inflammatory agent. However, we found that only telmisartan stimulated AMPK activity, whereas valsartan, irbesartan and olmisartan decreased it. This dissociation between anti-inflammatory activity and AMPK activation suggests that there are AMPK independent mechanisms through which ARBs act to reduce microglial activation. Understanding the mechanisms through which different ARBs act will assist in determining which ARB is the best candidate for treating TBI.

Disclosures: Z.C. Janatpour: None. K. Affram: None. R. Sharma: None. A.J. Symes: None.

Poster

230. Mechanisms of Microglia Activation

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 230.30/X18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Peruvian Ministry of Education - Postgraduate Scholarship Programme

Title: Microglial activation and associated pathways as therapeutic targets for Alzheimer's disease

Authors: *C. N. VILLEGAS LLERENA, M. MATARIN, J. HARDY, J. POCOCK;
Mol. Neurosci., UCL Inst. of Neurol., London, United Kingdom

Abstract: Neuroinflammation is one of the main pathological hallmarks of Alzheimer’s disease (AD) and it is well recognized that microglia are pivotal for the immune response observed in AD. In the brain, microglia act as sentinel cells attacking and removing pathogens and cell debris, but have been shown to become reactive in AD. A likely connection between microglia and AD neurodegeneration is Amyloid Beta (Aβ). This protein accumulates and forms the characteristic plaques that are present in the brains of AD patients and has been reported as a potent activator of microglia. Despite this, little is known about how Aβ alters microglial genetic expression and impacts their reactivity.
In recent times, Genome Wide Association Studies (GWAS) have allowed identification of more than 20 genetic risk associations for AD. Many of the associated genes highlight the importance

of immune pathways in AD pathology. Even more recently, the identification of mutations in TREM2 - a gene almost exclusively expressed by microglia in the brain - as AD risk factors has increased the efforts to understand neuroinflammation and microglia in the context of AD. Our study focuses on understanding how microglial activation status (M1, M2 or other) may influence the expression of genes that have been identified as AD risk factors. In particular we are interested in how different Aβ conformations can alter microglia whole-genome expression profile. To this end, we used different gene expression profiling approaches and the BV2 murine cell model to assess gene expression changes during activation using different stimuli, including Aβ and other classic M1/M2 activators. In this study, we found that different microglial activators can modify gene expression profiles of many AD associated genes in a manner that could potentially contribute to AD progression. This study revisits possible roles for microglial activation in AD pathogenesis and progression and focusses on candidate pathways as therapeutic targets for AD.

Disclosures: C.N. Villegas Llerena: None. **M. Matarin:** None. **J. Hardy:** 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; ESAI. **J. Pocock:** 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; ESAI.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.01/Y1

Topic: C.07. Ischemia

Support: The National Science and Engineering Research Council (Canada)

Research Manitoba

The Winnipeg Health Sciences Centre

Title: Oxygen-glucose deprivation reduces cortical arteriole lumen diameter by generating 20-hydroxyeicosatetraenoic acid

Authors: *L. LU[1,2,3], P. LU[1,2,3], C. M. ANDERSON[1,2,3];
[1]Univ. of Manitoba, Winnipeg, MB, Canada; [2]Kleysen Inst. for Advanced Med., Winnipeg, MB, Canada; [3]Hlth. Sci. Ctr., Winnipeg, MB, Canada

Abstract: Brain ischemia and reperfusion is associated with secondary neuronal death resulting from prolonged regional hypoperfusion, in part mediated by constriction of arterioles and capillaries. The mechanisms behind ischemic constriction are not clear but it was recently shown that brain slice capillaries constrict in response to simulated ischemia without reperfusion, in a manner dependent on pericyte contraction and death. We hypothesized that brain ischemia also constricts pre-capillary arterioles in a manner dependent on shifted balances of arachidonic acid (AA) metabolites.
Acute cortical slices were exposed to oxygen-glucose deprivation (OGD) and analyzed by two-photon microscopy to monitor arteriolar diameter and corresponding dynamic changes in intracellular Ca^{2+} of adjacent astrocytes, which are known to influence lumen diameter. OGD caused significant arteriolar constriction, starting at 10 min and reaching -15.8 ± 2.8% of baseline lumen diameter after 20 min (9 vessels). The cumulative constriction response, assessed as area under the time course lumen diameter curve from 10-20 min, was 4.3-fold greater than control. 20-hydroxyeicosatetraenoic acid (20-HETE) is the ω-hydroxylation product of AA and is a potent physiological and pathophysiological inducer of smooth muscle contraction. OGD significantly increased cortical slice 20-HETE content from 18.93 ± 2.37 to 36.59 ± 4.67 ng/mg protein at the 20 min time point, suggesting it could be a mediator of constriction. Consistent with this, bath application of the ω-hydroxylase inhibitor, HET0016 (1 μM), reduced the 20 min OGD-induced reduction in lumen diameter from -15.8 ± 2.8% to -9.4 ± 2.5%. OGD increased perivascular astrocytic Ca^{2+} levels by 1.6-fold of baseline at 10 min, commensurate with reduced arteriolar lumen diameter and increased 20-HETE production. We can't yet directly link astrocytic function with OGD-induced arteriolar constriction and AA. This will be an important component of ongoing experiments.

Disclosures: L. Lu: None. P. Lu: None. C.M. Anderson: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.02/Y2

Topic: C.07. Ischemia

Support: the National Nature Science Foundation of China (30971530)

the Program of Introducing Talents of Discipline to Universities from China (B14036)

Title: Protective effect of LBP on cortical neurons exposed to OGD by reducing glutamic excitotoxicity Protective effect of LBP on cortical neurons exposed to OGD by reducing glutamic excitotoxicity

Authors: *L. T. TING[1,2], Z. SHI[2], L. ZHU[2], L. XIA[2], X. HAN[2], K.-F. SO[3], Y. RUAN[2]; [1]Jinan Univ., Guangdong, China; [2]Jinan Univ., Guangzhou, China; [3]Hongkong Univ., Hongkong, China

Abstract: Lycium barbarum polysaccharides (LBP) is an extraction from Lycium barbarum fruit, which has been reported various biological activities such as anti-oxidation and anti-apoptosis. Glutamic excitotoxicity has been believed the major cause of ischemic neuronal death. Based on our recent results that 80-90% cortical neurons were protected by LBP when these neurons exposed to oxygen-glucose deprivation (OGD) in vitro, we hypothesized that LBP may play a protective effect against hypoxia/ischemia by reducing glutamic excitotoxicity. In the present study, we investigated changes in NMD receptor subunit NR2A and NR2B signaling pathways after OGD. The results showed that LBP could antagonize the increase in expression of NR2B, nNOS, Bad, CytC and cleaved caspase3, and inhibit the decrease in expression of NR2A, pAkt and p CREB. Also, LBP could block excessive calcium influx induced by administration of NMDA when neurons were exposed to OGD for 1 hour. Furthermore, NR2A inhibitor or NR2B co-agonist could attenuate the protective effects of LBP. Our results suggest that LBP can be as a candidate of Chinese medicine to be applied to treatment for the stroke patients in the future.

Disclosures: L.T. Ting: None. Z. Shi: None. L. Zhu: None. L. Xia: None. X. Han: None. K. So: None. Y. Ruan: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.03/Y3

Topic: C.07. Ischemia

Support: NIH Grant R01AA017413

NIH Grant R01MH077791

NIH Grant R01MH078823

NIH Grant R01MH101874

NIH Grant R21MH104506

Bantly Foundation

Washington University McDonnell Center for Cellular and Molecular Neurobiology postdoctoral fellowship

Title: 24S-hydroxycholesterol and 25-hydroxycholesterol differentially impact neuronal survival following oxygen-glucose deprivation in primary hippocampal neuronal culture

Authors: *M.-Y. SUN[1], A. TAYLOR[1], C. ZORUMSKI[1,2,3], S. MENNERICK[1,2,3];
[1]Psychiatry, [2]Neurosci., Washington Univ. in St. Louis, Saint Louis, MO; [3]Taylor Family Inst. for Innovative Psychiatric Res., Saint Louis, MO

Abstract: N-methyl-D-aspartate receptors (NMDARs), a major subtype of glutamate receptors mediating excitatory transmission throughout the CNS, participate in ischemia-induced neuronal death. Unfortunately, undesired side effects have limited the strategy of inhibiting/blocking NMDARs as therapy. Targeting endogenous positive allosteric modulators of NMDAR function may offer a strategy with fewer downsides. Here, we explored whether 24S-hydroxycholesterol (24S-HC), an endogenous positive NMDAR modulator characterized recently by our group, participates in NMDAR-mediated excitotoxicity following oxygen-glucose deprivation (OGD) in primary neuron cultures. 24S-HC is the major brain cholesterol metabolite produced exclusively in neurons near sites of glutamate transmission. By selectively potentiating NMDAR current, 24S-HC may participate in NMDAR-mediated excitotoxicity following energy failure, thus impacting recovery after stroke. In support of this hypothesis, our findings indicate that exogenous application of 24S-HC exacerbates NMDAR-dependent excitotoxicity in primary neuron culture following OGD, an ischemic-like challenge. Similarly, enhancement of endogenous 24S-HC synthesis reduced survival rate. On the other hand, reducing endogenous 24S-HC synthesis alleviated OGD-induced cell death. We found that 25-HC, another oxysterol that antagonizes 24S-HC potentiation, partially rescued OGD-mediated cell death in the presence or absence of exogenous 24S-HC application, and 25-HC exhibited NMDAR-dependent/24S-HC-dependent neuroprotection, as well as NMDAR-independent neuroprotection. Our findings suggest that both endogenous and exogenous 24S-HC exacerbate OGD-induced damage via NMDAR activation, while 25-HC is neuroprotective through both NMDAR-dependent and independent mechanisms.

Disclosures: **M. Sun:** None. **A. Taylor:** None. **C. Zorumski:** F. Consulting Fees (e.g., advisory boards); Sage Therapeutics. **S. Mennerick:** None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.04/Y4

Topic: C.07. Ischemia

Support: NS051288

HL007736

GM109089

Title: L-type voltage gated calcium channel blockers reduce the severity of spreading depolarizations in murine brain slices

Authors: *B. S. MEAD[1], B. E. LINDQUIST[1], K. M. REINHART[1], A. P. CARLSON[2], C. W. SHUTTLEWORTH[1];
[1]Neurosci., [2]Neurosurg., Univ. of New Mexico, Sch. of Med., Albuquerque, NM

Abstract: Spreading Depolarizations (SD) are coordinated waves of neuronal and glial depolarization that propagate slowly (2-5 mm/min) through brain tissue and are characterized by dramatic Ca^{2+} influx, increases in metabolic demand and changes in vascular supply. SDs have been implicated in the pathophysiological progression of ischemic injury following subarachnoid hemorrhage (SAH). L-type voltage gated calcium channel (L-VGCC) blockers have been shown to improve outcomes in SAH, but the cellular target(s) are poorly defined, and have commonly been assumed to be vascular. However, L-VGCCs are also expressed on neurons and glia, and thus may alter properties of SD. Therefore, we evaluated the effects of nicardipine, an L-VGCC antagonist, on properties of SD elicited by focal application of K^+ in normoxic conditions, or by global oxygen-glucose deprivation (OGD), simulating ischemia. Experiments were conducted in acute brain slices, a system which allows examination of parenchymal (neuronal and glial) responses independent of vascular perfusion. We evaluated SD incidence, latency, propagation rate, and direct current (DC) shift, together with intrinsic optical signals (IOS) in C57Bl/6 brain slices and fluorescent signals in slices from transgenic mice expressing a Ca^{2+} indicator (GCaMP). SD was readily initiated in the hippocampal CA1 region by KCl microinjection in the presence of nicardipine (10 μm), and the propagation rate was unchanged when compared to vehicle control (4.8 ± 0.5 vs 4.8 ± 0.4 mm/min, veh vs nicardipine, p=0.96, n=7,8). However, DC shift duration was shortened relative to within-slice controls (-17.0 ± 3.5 vs. -34.0 ± 6.4% change, veh vs nicardipine, p=0.02, n=8,9). Similarly, nicardipine decreased Ca^{2+} influx into neuronal cell bodies as measured by the duration of GCaMP fluorescence transients (45.0 ± 7.9 vs 21.0 ± 3.2 s, veh vs nicardipine, p=0.01, n= 7,6) and area under the curve (1337 ± 88 vs 2028 ± 256 AFU·s, veh vs nicardipine, p=0.02, n=7,6). In OGD, nicardipine did not affect SD

incidence or latency (11.5 ± 0.8 vs 10.9 ± 0.8 min, veh vs nicardipine, p=0.62, n=4,4) nor propagation rate (10.3 ± 2.8 vs. 15.0 ± 5.9 mm/min, veh vs nicardipine, p=0.50, n=4,4). Thus L-VGCCs can contribute to the duration of tissue depolarization and Ca^{2+} influx during SD in acute brain slices, in normoxic but not in ischemia-like conditions. These results support the possibility that clinical benefits of L-VGCC blockers may be in part due to direct effects on neuronal Ca^{2+} loading during SD, in addition to the more commonly assumed effects on vascular smooth muscle cells.

Disclosures: B.S. Mead: None. B.E. Lindquist: None. K.M. Reinhart: None. A.P. Carlson: None. C.W. Shuttleworth: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.05/Y5

Topic: C.07. Ischemia

Support: NIH grant NS073779

Title: Title: Increased intra-ischemic acidosis in recurrent hypoglycemia exposed rats may activate acid-sensing ion channels in vascular smooth muscle cells.

Authors: *V. SHUKLA, A. K. REHNI, K. R. DAVE;
NEUROLOGY, MILLER SCHOOL OF MEDICINE, UNIVERSITY OF MIAMI, Miami, FL

Abstract: More than 29 million Americans suffer from diabetes. Recurrent hypoglycemia (RH) is common in treated diabetics. Earlier we observed that prior exposure to RH increases cerebral ischemic damage in insulin treated diabetic (ITD) rats. We also observed pronounced intra-ischemic acidosis and post-ischemia hypoperfusion in RH-exposed ITD rats compared to the control group. The goal of the present study was to determine if increased intra-ischemic acidosis contributes to severe hypoperfusion in RH-exposed ITD rats. We hypothesized that increased intra-ischemic acidosis leads to activation of acid-sensing ion channels (ASICs) in vascular smooth muscle cells (VSMCs) contributing to pronounced hypoperfusion in RH-exposed ITD rats. This hypothesis was tested using VSMCs cell line A7r5. We determined contribution of ASICs in Store Operated Calcium Entry (SOCE) at different pH, and in presence and absence of inhibitors of ASIC-1 (PcTX-1) and ASIC-3 (APETx2). Freshly grown A7r5 cells were loaded with Ca^{2+} sensitive fluorescent indicator Fluo-4AM in presence of pluronic acid in calcium medium (135 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11.5 mM glucose and 11.6 mM Hepes, pH=7.3) and incubated for 40 minutes at 37^{o}C and then for an additional 20

minutes at room temperature. We measured SOCE at three different pH (7.4, 6.5 and 6.0). Fluo-4AM loaded cells were super fused with Ca^{2+} free medium with respective pH (135 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 11.5 mM glucose and 11.6 mM Hepes, 50 μM diltiazem, and 10 μM cyclopiazonic acid). The changes in Fluo-4 AM florescence from the baseline, as an indicator of SOCE induced increase in [Ca2+]i, was determined upon repletion of extracellular Ca^{2+} (1.5 mM) in presence of diltiazem and cyclopiazonic acid for 30 min. The experiment was performed in presence of PcTX-1, APETx2, or respective vehicle controls. As expected, we observed pH dependent SOCE in A7r5 cells. We also observed that lower pH-induced increase in intracellular Ca^{2+}: an indicator of SOCE was inhibited by APETx2. The intracellular Ca^{2+} influx was reduced significantly ($p<0.05$) to 40 % at pH 6.0 in presence of APETx2 but no significant difference was observed at pH 7.0 and pH 6.5 compared to respective vehicle control. Also, no significant difference in intracellular Ca^{2+} influx was observed at any pH (7.4, 6.5 and 6.0) in presence of PcTX-1 when compared to respective vehicle control. We conclude that activation of ASIC-3 *via* increased intra-ischemic acidosis may contribute to increased cerebral ischemic damage in RH-exposed ITD rats.

Disclosures: V. Shukla: None. A.K. Rehni: None. K.R. Dave: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.06/Y6

Topic: C.07. Ischemia

Support: HL007736

NS051288

GM109089

Title: Influence of tissue metabolic status on Ca^{2+} and glutamate accumulation during spreading depolarization in brain slices

Authors: *K. M. REINHART, C. W. SHUTTLEWORTH, R. A. MORTON;
Dept. of Neurosciences, Univ. of New Mexico, Sch. of Med., Albuquerque, NM

Abstract: Spreading depolarization (SD) is a slowly propagating (~2-4 mm/min) wave of neuronal and glial depolarization, which occurs with high incidence in the stroke brain. SD is closely linked to secondary injury expansion that is likely mediated by substantial glutamate release and excitotoxic Ca^{2+} influx through N-methyl-D-aspartate receptors (NMDARs). We

utilized electrophysiological and fluorescence recordings in brain slices to test the hypothesis that progressive metabolic compromise enhances glutamate and Ca^{2+} accumulation during SD. Transients in healthy preparations were compared to responses in slices with intermediate or profound metabolic compromise. Extracellular glutamate levels were detected using a glutamate-sensing fluorescent reporter (iGluSnFR). Virus containing the iGluSnFR construct was delivered *in vivo* via stereotaxic injection into the hippocampus of C57Bl/6 mice, and brain slices were prepared ~2-4 weeks post injection. Neuronal Ca^{2+} transients were assessed in brain slices from mice expressing the intracellular Ca^{2+}-sensitive fluorescent protein (GCaMP5G). Under healthy conditions, SD initiated by KCl microinjection resulted in large glutamate transients that returned to baseline levels within ~20s (n=8). In these conditions, Ca^{2+} transients persisted longer, but still fully recovered within ~55s (n=10). Intermediate metabolic compromise was achieved by reducing O_2 and glucose availability. Under these conditions, the majority of slices did not recover from SD generated by KCl microinjection (~14% survival; n=7). Here, glutamate transients were prolonged, but returned to baseline levels after SD. In contrast, Ca^{2+} transients were markedly extended and remained ~35% above baseline levels 2 minutes post SD (n=5). Under conditions of complete metabolic depletion (oxygen and glucose deprivation; OGD) SDs were generated spontaneously, with glutamate and Ca^{2+} transients both sharply increasing at the onset of SD, and were irrecoverable throughout the duration of recordings (~10 min). We next evaluated the effect of the NMDAR antagonist ketamine (30μM) on intracellular Ca^{2+} loading. In healthy slices, this concentration of ketamine did not block SD, but reduced Ca^{2+} transient duration (P=0.01; n=4). In intermediately compromised conditions, ketamine prevented tissue death (6/6) and attenuated Ca^{2+} dysregulation after SD ($P<0.05$). These results provide evidence that slice metabolic status influences both glutamate and Ca^{2+} accumulation during SD. Furthermore, our findings with ketamine support the clinical utility of this drug as an intervention that can reduce deleterious consequences of SD.

Disclosures: K.M. Reinhart: None. C.W. Shuttleworth: None. R.A. Morton: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.07/Y7

Topic: C.07. Ischemia

Support: AHA 14BGIA18440006

NIH Grant NS080851

NIH GRANT HD077260

CCTSI CNS-Pilot Basic Research Independent Award

NIH GRANT HHSN272202000046C

Title: Function of perivascular stromal cells in stroke injury

Authors: K. K. KELLY[1], A. MACPHERSON[1], F. STRNAD[2], M. KANE[3], P. S. HERSON[2], *J. SIEGENTHALER[1];
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Abstract: Stroke is a leading cause of death in the United States and costs $34 billion yearly. Current treatments for stroke are few and require application soon after the onset of symptoms. The development of novel therapies with broader application times that treat acute and chronic stages of stroke is an active area research. Recent work has shown that a little understood cell type termed perivascular stromal cells (PSC) is a major component of the cellular milieu of the stroke lesion, appearing within days of ischemia and persisting long after the initial injury. In the uninjured brain, PSCs are infrequent and occupy the perivascular niche around large diameter blood vessels. PSCs are delineated by Collagen 1a1 (Col1a1) expression and the *Col1a1-GFP* transgenic mouse line has been an instrumental tool to describe PSC post-injury fibrosis. Following injury, PSCs undergo significant cellular transformation, substantially expand their numbers and play a major part in post-injury fibrotic scar formation. The signal(s) that activate PSCs following injury has not been identified and potential functions for PSCs beyond fibrosis have not been explored. Our research characterizing perivascular cell development and vascular post-injury response has identified a separate, non-fibrotic function of PSCs following ischemic stroke. We have found that PSCs express Raldh1 and Raldh2, enzymes capable of synthesizing bioactive Retinoic Acid (RA), a neuro-protective hormone. Moreover, we have a developed a PSC cell culture platform to aid in our efforts to understand PSC activation and function following stroke injury. Our over-arching *hypothesis* is that PSCs are activated by signals in the stroke environment and, once activated, secrete protective signals like RA that are 'sensed' by neurons, glial, vascular and inflammatory cells in and around the stroke lesion. The goal of our ongoing experiments is to create a model of how PSCs fit into the milieu of cell-cell interactions within the stroke environment and knowledge of molecular pathways stimulated during PSC activation. This will provide a framework for future studies testing PSC activation and function in preclinical models of stroke.

Disclosures: K.K. Kelly: None. A. Macpherson: None. F. Strnad: None. M. Kane: None. P.S. Herson: None. J. Siegenthaler: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.08/Y8

Topic: C.07. Ischemia

Support: Heart and Stroke Foundation of Canada

Saskatchewan Health Research Foundation

Natural Sciences and Engineering Research Council of Canada

Canada Foundation for Innovation

Title: Systemic administration of adenosine A1 receptor agonist produces hippocampal-dependent spatial memory deficits: role of microglia activation

Authors: *F. S. CAYABYAB[1], J. STOCKWELL[1], X. QIN[1], O. FRIESEN[1], Z. MING[1], Z. CHEN[1], W. WALZ[2];
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Abstract: Previously, we reported that hypoxia/reperfusion injury and in vivo focal cortical ischemia models both lead to adenosine A1 receptor (A1R)-dependent hippocampal neuronal damage, which was partially mediated by clathrin-induced internalization of GluA2-containing AMPA receptors (AMPARs). In the present study, we investigated whether microglia activation by A1R stimulation contributes to hippocampal neuronal damage and learning deficits in stroke-like conditions. Intraperitoneal injections of the A1R agonist CPA (5mg/kg) for 2-7 days produced hippocampal neurodegeneration, which was prevented by the GluA2 endocytosis inhibitor Tat-GluA2-3Y peptide, the A1R antagonist DPCPX (5mg/kg), or the general inhibitor of microglia activation minocycline (45mg/kg). Similarly this A1R-dependent neurodegeneration was also observed in pial vessel disruption (PVD) stroke model. Tat-GluA2-3Y peptide, DPCPX and minocycline all reduced the CPA-mediated GluA2- and GluA1-containing AMPAR internalization, implicating microglia activation in the hippocampal neurodegeneration. Chronic CPA injections or PVD also produced deficits in chemically induced long term potentiation (evoked by forskolin and rolipram), which was prevented by Tat-GluA2-3Y, DPCPX and minocycline. Increased microglia activation, as reflected by increased CD-11 and CD-68 staining in the hippocampus after CPA treatment, was prevented by DPCPX or minocycline. Rats treated with CPA alone or PVD alone showed significant hippocampal-dependent spatial memory deficits, as assessed by Y-maze assays, which was not observed by prior treatments of animals with Tat-GluA2-3Y peptide, DPCPX, or minocycline. These results indicate that A1R stimulation of microglia in stroke-like conditions contributes to hippocampal neurodegeneration

and hippocampal-dependent spatial memory deficits. Whether peripheral effects of A1R stimulation include activation and subsequent infiltration of peripheral immune cells to the brain is currently under investigation.

Disclosures: F.S. Cayabyab: None. J. Stockwell: None. X. Qin: None. O. Friesen: None. Z. Ming: None. Z. Chen: None. W. Walz: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.09/Y9

Topic: C.07. Ischemia

Support: AHA Predoctoral Fellowship 15PRE25080088 (MKT)

NIH/NIAID Grant 1P01AI089556 - 01A1 (AMB)

NIH/NIA Grant R01AG033570 (OL)

Title: Hypoxia-induced changes in neural stem cell and microglia characteristics

Authors: *M. K. TOBIN[1], A. M. BARTHOLOMEW[2], O. LAZAROV[1];
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Abstract: Despite the proliferative burst of neural stem cells (NSC), neurogenic mechanisms fail to repair damaged brain tissue following ischemic stroke. The mechanisms leading to this failure are largely unknown but loss of trophic support as well as contribution of post-ischemia inflammation have been posited as important mediators of the failure of endogenous repair mechanisms. To address these issues, we examined the effect of hypoxia on NSC proliferation. We found that 24 hours after oxygen-glucose deprivation (OGD) there is a significant increase in NSC proliferation; however, this increase falls back to normal levels beyond 24 hours. Further, mRNA expression of GDNF, IGF-1, BDNF, NT-3, and NGF are all significantly increased after 24 hours of OGD with levels of GDNF, IGF-1, and BDNF declining to below basal levels at 48 hours. These data could account for the initial proliferative burst followed by the decline after 48 hours. Next, because of their ability to interconvert microglia from an inflammatory to a regenerative phenotype, we utilized mesenchymal stem cells (MSC) to investigate whether the pro-inflammatory phenotype of microglia following hypoxia can be changed. For this purpose, microglia were grown in conditioned media from both naïve MSCs (nMSC) as well as interferon-γ (IFN-γ)-activated MSCs (aMSCγ). Interestingly, microglia treated by aMSCγ conditioned media exhibited a reduction in expression of the pro-inflammatory cytokines IL-1β,

IL-6, and TNF-α up to 48 hours in culture and had increased expression of the pro-regenerative cytokines IL-10 and IL-4 up to 7 days in culture. Additionally, MSCs secrete numerous neurotrophic factors suggesting that they could be directly beneficial to the NSCs as well. Interestingly, when co-cultured with aMSCγ for 24 hours, there is a significant increase in NSC mRNA expression of GDNF with trending increases in BDNF, NT-3, and NGF, demonstrating that MSCs could help sustain the neurotrophic loss experienced by NSCs following hypoxia. Taken together, these results suggest that neural progenitor cells may be beneficial for neuronal survival and repair following an ischemic event, and that MSCs may have the capacity to modulate both neurogenesis and post-ischemia inflammation to enhance brain repair.

Disclosures: M.K. Tobin: None. A.M. Bartholomew: None. O. Lazarov: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.10/Y10

Topic: C.07. Ischemia

Support: R01 NS037459

Title: Changes in excitation-inhibition balance after focal cortical ischemia

Authors: *N. V. POVYSHEVA[1], M. E. ROSE[2], F. ZHANG[2], S. H. GRAHAM[2], G. BARRIONUEVO[1];
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Abstract: Cerebral ischemia produces devastating changes in neurons that include persistent depolarization of neurons, spreading waves of neuronal depression, and eventually leads to neuronal death in the ischemic core. Neurons in the penumbra, while being able to survive the initial insult, sustain chronic pathological changes that are still not quite understood. In particular, it is not clear how the balance between excitation and inhibition (E/I) is affected (Clarkson et al., 2010; Wang, 2003). Here we address pathophysiological changes of the E/I balance in neocortical neurons produced by the middle cerebral artery occlusion (MCAO) in mice. MCAO and sham surgeries were performed as previously described (Poloyac et al., 2006), and physiological properties of penumbral neurons were assessed 7 days later. Whole-cell recordings were made from pyramidal neurons in layer 2-3 of the mouse neocortex located in the close proximity to the ischemic core in the MCAO and in the similar location in the sham mice. During recordings neurons were filled with fluorescent dye for morphological analysis.

Comparative analysis of intrinsic membrane properties revealed lower excitability of the MCAO (n=8) as compared to the sham neurons (n=14). The former demonstrated more negative resting membrane potential (-72±1 mV vs. -67±2 mV, $p<0.01$), less negative spike threshold (-35±1 mV vs. -39±1 mV, $p<0.05$), lower firing frequency (19±1 Hz vs. 25±2 Hz, $p<0.01$) and lower input resistance (87±7 MΩ vs. 130±14 MΩ , $p<0.01$). Spontaneous excitatory postsynaptic currents (sEPSCs) recorded at the holding potential -70 mV in the presence of gabazine (10 μM) had lower frequency in the MCAO (n=8) as compared to the sham neurons (n=14) (2.3±0.8 Hz vs. 5.8±2.5 Hz, $p<0.05$), as well as the mean current estimated as average charge transfer*frequency of events (257±80 pA vs. 642±243 pA, $p<0.05$). To directly estimate the E/I ratio, neurons were held at -55 mV, a membrane potential that allowed detection of both excitatory and inhibitory events. E/I ratio, estimated as mean excitatory current/mean inhibitory current, was lower in MCAO (n=4) than in control mice (n=8) (1.9±0.4 vs. 10.6±4.0, $p<0.05$). Morphological analysis did not reveal any differences between the MCAO and sham neurons. Thus, our data indicate that the E/I balance in penumbra is shifted away from excitation.

Disclosures: N.V. Povysheva: None. M.E. Rose: None. F. Zhang: None. S.H. Graham: None. G. Barrionuevo: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.11/Y11

Topic: C.07. Ischemia

Support: NIH grant NS081629

Title: Intracellular zinc increase is required for initial increase of mitochondrial ROS during hypoxia

Authors: K. SLEPCHENKO[1], Q. LU[2], H. HARAGOPAL[3], *Y. V. LI[1];
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Abstract: Reactive oxygen species (ROS) have been linked to pathology of ischemia, of special interest are ROS produced by mitochondria. Zinc accumulates during ischemia in neurons and also have been implied in recent years as major players of hypoxic damage. To determine the temporal relationship between these two important events, we have employed fluorescent imaging of live cells during chemical hypoxia with oxygen scavenger sodium dithionite. The zinc increase (detected by FluoZin-3, AM) was observed within 60 seconds of induction of hypoxia and lasted about 2 minutes, then the zinc transient sharply decreased, resembling a

calcium wave, This transient wave of intracellular zinc surge resemble to the well documented calcium wave. Accumulation of mitochondrial ROS (detected by MitoSOX Red) was observed within 4.5 minutes of hypoxia and continued to increase for the duration of observation (10 minutes). When zinc was chelated with TPEN during the hypoxia, the increase in mitochondrial ROS was not significant, we suggest that zinc wave is required for the initial mitochondrial ROS increase. In addition, the exogenous application of zinc induced mitochondrial ROS in normoxic conditions. Inhibition of NADPH oxidase with apocynin, during application of exogenous zinc, showed significant reduction in zinc induced mitochondrial ROS. We showed that initial zinc increase during hypoxia (zinc wave) precedes increase in mitochondrial ROS and zinc wave is required for initial increase in ROS. We proposed that NADPH oxidase may be a major contributor to zinc induced mitochondrial ROS accumulation.

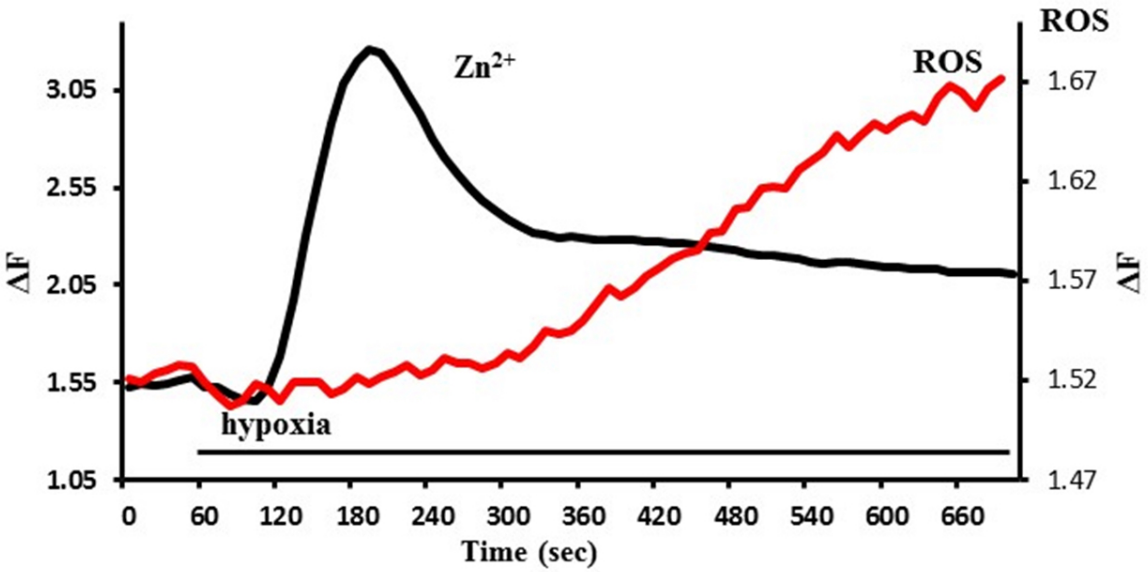

Figure legend: Two representative traces of zinc and ROS accumulation showing the relationship between zinc and the ROS transients during hypoxia. Zinc concentration is on the left y-axis, and ROS concentration is on the right y-axis.

Disclosures: K. Slepchenko: None. Q. Lu: None. H. Haragopal: None. Y.V. Li: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.12/Y12

Topic: C.07. Ischemia

Support: GAUK 618216

GACR 13-02154S

GACR P304/12/G069

Title: Proliferation and differentiation of NG2-glia following different types of brain disorders

Authors: *D. KIRDAJOVA[1,2], P. HONSA[1,3], M. ANDEROVA[1,3];
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Abstract: NG2-glia, a fourth major glial cell population, are present in the adult central nervous system and display distinct morphology, antigens and functions from other mature glial cell types. Recently, many studies have shown that these cells are multipotent in vitro and they also display wide differentiation potential under pathological conditions in vivo, where they give rise predominantly to reactive astrocytes.
The aim of this study was to identify the rate of proliferation and differentiation after different types of brain disorders, such as global and focal cerebral ischemia (GCI, FCI), stab wound (SW) and demyelination (DEMY). We used transgenic Cspg4-cre/CAG-tdTomato mice, which after administration of tamoxifen express red fluorescent protein (tdTomato) in NG2-glia and cells derived therefrom. Proliferation and differentiation potential of tdTomato positive ($tdTomato^+$) cells in sham-operated mice (controls) and those after injury were determined by immunohistochemistry - e.g. proliferating cell nuclear antigen (PCNA), glial fibrillary acidic protein (GFAP). FCI was induced by middle cerebral artery occlusion, GCI by carotid occlusion with hypotension, SW by sagittal cortical cut and DEMY by feeding mice with copper chelator cuprizone. To determine the phenotype of $tdTomato^+$ cells, the coronal brain slices were used from controls and mice 3 and 7 days after FCI, GCI, SW or DEMY. The percentage of cells that were double positive for tdTomato and PCNA or GFAP was estimated in the cortex (FCI, SW), in the hippocampus (GCI) or in the corpus callosum (DEMY).
We have shown that NG2-glia increase their proliferation rate after all four types of brain disorders as after ischemia, cortical injury as well as after demyelination. In case of acute injuries (FCI, GCI, SW) the highest proliferation rate was observed three days after the insults and even seven days after injury the proliferation rate was increased compared to controls. In case of

DEMY the proliferation increases seven days after withdrawal of cuprizone diet. In accordance with previous studies, differentiation of NG2-glia into astrocytes was almost absent in uninjured nervous tissue, however, the type of injury significantly influenced NG2-glia differentiation towards astrocytes. After FCI, 32.7±2.6% of tdTomato^{+} cells expressed markers of reactive astrocytes, while after SW or GCI this number was only 2.8±1.2% and 3.3±0.5%, respectively. Taken together, increased proliferation of NG2-glia is the typical feature after all types of pathological conditions, while differentiation into astrocytes depends on the type of injury.

Disclosures: D. Kirdajova: None. P. Honsa: None. M. Anderova: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.13/Y13

Topic: C.07. Ischemia

Support: NIH grant NS083544

Title: Knocking down SIRT3 preserved mitochondrial function and prevented brain injury after stroke

Authors: *T. I. GUDZ[1], S. A. NOVGORODOV[1], C. L. RILEY[1], J. A. KEFFLER[1], J. YU[1,2], W. B. MACKLIN[2];
[1]Dept Neurosci., Med. Univ. South Carolina, Charleston, SC; [2]Univ. of Colorado, Aurora, CO

Abstract: Experimental evidence supports the role of mitochondrial ceramide accumulation as a cause of mitochondrial dysfunction and brain injury after stroke. We report that SIRT3 regulates mitochondrial ceramide biosynthesis via deacetylation of ceramide synthase (CerS) 1, 2 and 6. Reciprocal immunoprecipitation experiments revealed that CerS1, CerS2 and CerS6, but not CerS4, are associated with SIRT3 in cerebral mitochondria. Furthermore, CerS1, 2 and 6 are hyper-acetylated in the mitochondria of SIRT3-null mice and SIRT3 directly deacetylates the ceramide synthases in a NAD^{+}-dependent manner that increases enzyme activity. Investigation of SIRT3 role in mitochondrial response to brain ischemia/reperfusion (IR) showed that SIRT3-mediated deacetylation of ceramide synthases increased enzyme activity and ceramide accumulation after IR. Functional studies demonstrated that absence of SIRT3 rescued the IR-induced blockade of the electron-transport chain at the level of Complex III, decreased ROS generation and protein carbonyls in mitochondria. Importantly, Sirt3 gene ablation protected the brain from IR injury. These data support the hypothesis that IR activates SIRT3, resulting in the deacetylation of ceramide synthases and the elevation of ceramide which could inhibit Complex

III, leading to increased ROS generation and brain injury. The results of these studies highlight a novel mechanism of SIRT3 involvement in modulating mitochondrial ceramide biosynthesis and suggest a critical role of SIRT3 in promoting mitochondrial dysfunction and brain injury after stroke.

Disclosures: T.I. Gudz: None. S.A. Novgorodov: None. C.L. Riley: None. J.A. Keffler: None. J. Yu: None. W.B. Macklin: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.14/Y14

Topic: C.07. Ischemia

Support: NIH Grant NS065219

Title: Differential vulnerability of CA1 vs CA3 pyramidal neurons after ischemia: possible relationship to sources of Zn^{2+} accumulation and its entry into and prolonged effects on mitochondria

Authors: Y. MEDVEDEVA, *J. H. WEISS;
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Abstract: Excitotoxic mechanisms contribute to the degeneration of hippocampal pyramidal neurons (HPNs) after recurrent seizures and brain ischemia. However, susceptibility differs, with CA1 neurons preferentially degenerating after global ischemia, and CA3 neurons after limbic seizures. Whereas most studies address contributions of excitotoxic Ca^{2+} entry, it is apparent that Zn^{2+} also contributes, reflecting accumulation in neurons either after pre-synaptic release and entry through post-synaptic channels or upon mobilization from intracellular Zn^{2+} binding proteins like metallothionein-III (MT-III). Using hippocampal slices to study acute oxygen glucose deprivation (OGD) triggered neurodegeneration, we find evidence for early contributions of both excitotoxic Ca^{2+} entry and Zn^{2+} accumulation in both CA1 and CA3, as supported by the ability of Zn^{2+} chelators or Ca^{2+} entry blockers to delay pyramidal neuronal death in both regions. However, use of knockout animals lacking vesicular Zn^{2+} transporter, ZnT-3 or MT-III revealed substantial differences between these neurons. The Zn^{2+} contribution to acute OGD induced death of CA3 neurons appeared to depend substantially upon pre-synaptic Zn^{2+} release and its permeation through Ca^{2+} permeable AMPA channels, which are also highly Zn^{2+} permeable. In contrast, mobilization of Zn^{2+} from MT-III appeared to contribute importantly to the death of CA1 neurons. To assess consequences of the cytosolic Zn^{2+}

accumulation, we employed OGD exposures slightly shorter than those causing acute neuronal death; under these conditions, cytosolic Zn^{2+} rises persisted for ~10-30 min after OGD, followed by recovery over ~40-60 min. Furthermore, the recovery appeared to be accompanied by mitochondrial Zn^{2+} accumulation, largely via the mitochondrial Ca^{2+} uniporter, in CA1 but not in CA3 neurons. This late Zn^{2+} accumulation was substantially diminished in MT-III knockout mice, suggesting that it depended upon Zn^{2+} mobilization from intracellular stores bound to this protein. These data are consistent with observations of prominent mitochondrial dysfunction as a critical early event in the delayed degeneration of CA1 neurons after ischemia, and with the idea that mitochondrial Zn^{2+} accumulation in the early reperfusion period, resulting largely from mobilization of MT-III bound pools, may be a key and potentially targetable trigger of the events leading to the delayed injury.

Disclosures: Y. Medvedeva: None. J.H. Weiss: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.15/Y15

Topic: C.07. Ischemia

Support: Heart and Stroke Foundation of Canada

Saskatchewan Health Research Foundation

Natural Sciences and Engineering Research Council of Canada

Canada Foundation for Innovation

Title: Systemic administration of adenosine A1 receptor agonist increases leukocyte specific protein-1 (LSP-1) expression in hippocampus: role in hippocampal neurodegeneration

Authors: *J. STOCKWELL[1], L. HAO[2], R. TENEYCKE[1], S. NOSIB[1], O. FRIESEN[1], L. LIU[2], F. S. CAYABYAB, S7N 5E5[3];
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Abstract: The leukocyte specific protein-1 (LSP-1) is a critical Ca^{2+}- and F-actin-binding protein that was first identified in hematopoietic immune cells, including B and T lymphocytes, monocytes, macrophages, and neutrophils. LSP-1 is also a substrate of p38 MAPK and PKC, and contributes to tissue inflammation and vascular permeability. Recently, we reported that LSP-1 was expressed not only in neutrophils but also in endothelial cells that line the cremasteric

vasculature, and contributes to neutrophil transmigration and tissue inflammation. However, the distribution and role of LSP-1 in the brain have not yet been investigated. Since we previously showed that adenosine A1 receptor (A1R) leads to p38 MAPK activation and neurodegeneration of rat hippocampus, we sought to characterize the cellular distribution of LSP-1 before and after A1R stimulation with the A1R agonist CPA. Systemic administration of CPA (5mg/kg) in rats increased LSP-1 expression in NeuN-positive CA1 pyramidal neurons and also in NeuN-negative cells in the stratum radiatum and stratum oriens. CPA also increased colocalization of LSP-1 with CD-11 and CD-68-positive cells (macrophage/microglia markers) and with GFAP-positive cells (astrocyte marker). Together, these imaging results indicate that LSP-1 is also expressed in hippocampal neurons, astrocytes and the monocyte-derived resident immune cells, the microglia. We also recently showed that CPA intraperitoneal injections increased microglia activation, which contributed to hippocampal neurodegeneration. We therefore hypothesized that expression of LSP-1 in the brain could regulate hippocampal-dependent learning behavior. Compared to wild type mice, LSP-1 knockout mice with or without systemically administered CPA produced similar levels of hippocampal-dependent memory deficits in the Y-maze test. Together, these results show for the first time that LSP-1 is normally expressed in CNS neurons, astrocytes and microglia, to regulate inflammatory response, hippocampal neurodegeneration, and learning and memory.

Disclosures: J. Stockwell: None. L. Hao: None. R. Teneycke: None. S. Nosib: None. O. Friesen: None. L. Liu: None. F.S. Cayabyab: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.16/Y16

Topic: C.07. Ischemia

Support: PRESTO, JST

KAKENHI (15H03019)

Title: Cell division of hypoxic-ischemic cortical neurons by overriding mitotic safeguards

Authors: *I. AJIOKA[1,2], M. OSHIKAWA[1];
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Abstract: Neuronal differentiation and cell cycle exit are tightly coordinated, even in pathological situations. When pathological neurons re-enter the cell cycle and progress through the S phase, they undergo cell death instead of division. However, the mechanisms of mitotic safeguard are mostly unknown. The Retinoblastoma protein family (Rb, p107, p130) plays a central role in preventing cells from entering the S phase. When Rb family expression is lost in neuronal progenitor cells, the subsequent coordination of cell-cycle exit and neuronal differentiation is lost, and neurons can divide in some cases such as retinoblastoma (Ajioka et al., *Cell* 2007). We recently developed a technique for conditionally inactivating all of the Rb family members in mouse cortical progenitors, either before or immediately after cell-cycle exit, by electroporation with Cre-expressing plasmids containing a ubiquitous pCAG promoter or a neuron-specific pMAP2 promoter (Oshikawa et al., *Development* 2013). When the Rb family is inactivated using pCAG-Cre, immature neurons generated from the pCAG-induced Rb-TKO (*Rb−/−; p107−/−; p130−/−*) progenitors divide. In contrast, the pMAP2-induced Rb-TKO immature neurons enter the S phase, but undergo cell death. Thus, once progenitor daughter cells exit the cell-cycle and initiate neuronal differentiation, they are prevented from undergoing cell division, and maintain mitotic resistance even after acute Rb family inactivation. These findings led us to hypothesize that hypoxic-ischemic neurons in the S phase undergo cell death by activating mitotic safeguards. In this presentation, we will show and discuss such mitotic safeguards. We will also demonstrate the cell division of dying hypoxic cortical neurons in the S phase by overriding mitotic safeguards. Our results may represent a novel strategy for treating neurological disorders.

Disclosures: I. Ajioka: None. M. Oshikawa: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.17/Y17

Topic: C.07. Ischemia

Support: Sinshemier Foundation P60134007

Title: Upstream modulation of Insulin/IGF signaling-mediated neuroprotection in a nematode model of excitotoxicity

Authors: *A. CHOWDHURY[1,2,4], S. PRASHAD[3], I. MANO[2];
[1]City Col. CUNY, New York, NY; [2]Physiol Pharm & Neurosci, The CUNY Sch. of Med., [3]Biochem., City College, The City Univ. of New York, New York, NY; [4]Psychology, The Grad. Ctr. of The City Univ. of New York, New York, NY

Abstract: Glutamate (Glu) excitotoxicity is the leading cause of neurodegeneration following ischemic stroke and an important contributing factor in neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's. Diminished function or reduced expression of Glu transporters in these conditions causes dysregulation and accumulation of Glu in the synaptic region, leading to excitotoxic Glu signaling; activating neurodegenerative pathways. Recent studies suggest that the GluR-triggered neurodegenerative processes is partially counterbalanced by pro-survival cascades. Investigation into neuroprotective pathways, partially active under excitotoxicity, will allow us to target pathways and proteins to counterbalance the neurodegenerative pathways overly active under these circumstances. The Insulin/IGF Signaling (IIS) cascade, which regulates FoxO/DAF-16 (a transcription factor known to regulate animal longevity and cell stress resistance), may play a role in maintaining neuroprotective balance. We study excitotoxicity in *C. elegans*, allowing us to follow GluR-triggered neuronal necrosis in a more simplified and less redundant system where core mechanisms are conserved, allowing to decipher important signaling pathways homologous to those found in mammals. The IIS cascade is one such pathway highly conserved in *C. elegans* and mammals, making nematodes an ideal organism for our research. Our lab created a nematode excitotoxicity model where a deletion of a Glu transporter gene (*glt-3*) in a sensitized background results in high levels of GluR-dependent necrotic cell death that can be visualized in live worms as vacuole-like structures. Using our excitotoxic strain, we have recently shown that inhibition of the IIS pathway (i.e., increased FoxO/DAF-16 nuclear activity) *reduces* necrotic neurodegeneration under excitotoxicity. Furthermore, we found that the Cytohesin/GRP-1 complex is an important upstream regulator of IIS signaling in excitotoxicity. We now investigate the possibility of further upstream regulation of IIS/FoxO-mediated neuroprotection. We also investigate how IIS pathway and the upstream regulator affect localization of FoxO/DAF-16 to further solidify the neuroprotective pathway regulated under excitotoxicity. We expect that if these experiments are successful, we will be able to point at a new mechanism regulating IIS signaling and susceptibility to excitotoxicity. The results from our studies might further be carried over to mammalian research and suggest novel directions in therapeutic drug targets to promote neuroprotection under excitotoxicity.

Disclosures: A. Chowdhury: None. S. Prashad: None. I. Mano: None.

Poster

231. Ischemia: Cellular Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 231.18/Y18

Topic: C.07. Ischemia

Support: Heart and Stroke Canada

MS Society of Canada

Brain repair centre Dalhousie

Micheal J Fox foundation

RADIANT

Title: The mitochondrial calcium uniporter is essential for hypoxic preconditioning but not ischemic brain injury

Authors: *M. J. NICHOLS[1], P. A. ELUSTONDO[2], A. THIRUMARAN[3], E. V. PAVLOV[4], G. S. ROBERTSON[5];
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Abstract: Hypoxic preconditioning (HPC) activates calcium (Ca^{2+}) signaling pathways that confer profound resistance to ischemic brain damage. Mitochondria are strategically positioned to sense and respond rapidly to elevations in cytosolic Ca^{2+} concentrations that regulate neuronal cell death and survival. Mitochondrial Ca^{2+} uptake into cardiac myocytes by the mitochondrial Ca^{2+} uniporter (MCU) has recently been shown to play a pivotal role in regulating susceptibility to ischemic heart injury. In view of the considerable implications of these findings for ischemic brain damage, we have examined the effects of global MCU (G-MCU) deficiency on HPC in a model of hypoxic-ischemic (HI) brain injury. Both Ca^{2+} uptake and Ca^{2+}-induced permeability transition pore activity were inhibited in forebrain mitochondria isolated from G-MCU nulls. Despite evidence that these effects should be protective, G-MCU null mice were not resistant to HI-induced brain damage and sensorimotor deficits. Cortical neurons derived from G-MCU nulls were also not protected from *in vitro* ischemic cell death produced by oxygen glucose deprivation (OGD). Oxygen consumption rates were comparable for cortical neuron cultures derived from G-MCU null and wild-type (WT) mice but spare respiratory capacity was reduced in G-MCU deficient neurons. This was accompanied by increased glycolysis that may compensate for impaired ATP production by oxidative phosphorylation in G-MCU neurons. Although G-MCU deficiency did not alter HI brain injury, the protective effects of HPC against HI-induced brain injury and sensorimotor impairments were completed blocked by G-MCU ablation. G-MCU deficiency produced exactly the same induction patterns for genes encoding MT apoptosis-regulators (BCL-XL, BAX), sodium (Na^{+})/Ca^{2+} exchangers-1, -2, and -3 and the plasma membrane Ca^{2+} ATPase-2 (PMCA2) in the hippocampus as did HPC in WT mice. Apart from a modest increase in hippocampal expression of PMCA-2 (50%), HPC failed to further elevate mRNA levels for these genes. G-MCU deficiency therefore engaged the same protective mechanisms as HPC that oppose impairments in MT function and cytosolic Ca^{2+} handling implicated in ischemic brain damage. Such adaptations likely compensate for impaired Ca^{2+} handling in G-MCU nulls. This conclusion is consistent with evidence that activity-induced preconditioning protects cultured neurons from excitotoxicity by transcriptional repression of the

MCU. However, saturation of these protective mechanisms may also have impaired HPC in G-MCU nulls.

Disclosures: M.J. Nichols: None. P.A. Elustondo: None. A. Thirumaran: None. E.V. Pavlov: None. G.S. Robertson: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.01/Z1

Topic: C.09. Brain Injury and Trauma

Title: Examination of conditioned suppression in rodents after frontal tbi with an intervention of magnesium

Authors: *J. YOUNG[1], M. PRICE[2], E. JACOBS[2], M. HOANE[1];
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Abstract: Conditioned suppression is a respondent conditioning procedure that leads to a conditioned emotional response. Abnormal emotional responses, such as heightened or reduced fear, are symptoms after traumatic brain injury (TBI) that can affect the daily activities of a person. This procedure in conjunction with a magnesium treatment after TBI has yet to have been examined. Magnesium is a multimodal treatment that can decrease apoptosis, decrease breakdown of the blood brain barrier, and lessen brain edema after a TBI, which can affect the recovery of a patient. This study will explore, magnesium's outcome on fear expression after frontal TBI. Conditioned suppression will take place in a standard two-lever operant chamber, which has been adapted to emanate sound as well as to generate an electric shock. A single subject design will be utilized in order to focus on the evaluation of conditioned suppression fear in rodents after TBI rather than on a comparison of group differences. 10 Sprague-Dawley rats about 3 months of age will be divided into groups; MAG/TBI, VEH/TBI, and Sham. Rats will first be trained to lever press on a variable interval (VI) schedule of 30 seconds for multiple 30 minute sessions. The injury induction will be a severe bilateral frontal injury, after which they will receive 2 mmol/kg injections of magnesium chloride (MAG) or a saline solution (VEH). Injections will be given i.p. and start four hours after injury, then at 24 hours, and again at 72 hours. After a week recovery, rats will be reintroduced to lever pressing on a VI of 30 seconds. Fear conditioning training will take place over two 30 minute sessions, during which rats will be given four 30 second tones that will co-terminate with an electric shock. Electric shock duration will be 0.5 seconds at 0.5 mA. Conditioned inhibition of lever

pressing for food will be the measure of fear for this study. To examine conditioned suppression, a tone will be presented at 4 different time points during 30 minutes. Presentation of the tone will last for 30 seconds. This phase will occur for ten days with one session occurring each day. It is hypothesized that rats who receive the magnesium treatment after TBI will exhibit less conditioned inhibition compared to injured rats who will receive the vehicle treatment. Sham animals will not show conditioned inhibition and will quickly extinguish the behavior. It is expected that injured rats will take longer to extinguish or to not extinguish the behavior.

Disclosures: J. Young: None. M. Price: None. E. Jacobs: None. M. Hoane: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.02/Z2

Topic: C.09. Brain Injury and Trauma

Title: Exogenous addition of sphingomyelin restores impairments in hippocampal synaptic transmission and plasticity in a mouse model of traumatic brain injury

Authors: *K. WHITNEY[1], L. BUITRAGO-SOTO[1], J. IQBAL[2], M. HUSSAIN[2], H. MORENO[1], P. J. BERGOLD[1];
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Abstract: The closed head injury (CHI) mouse model of traumatic brain injury (TBI) produces a heterogeneous gray and white matter injury. Experimental TBI produces a persistent increase in hippocampal sphingomyelin (SM) levels (Abdullah, et al). We therefore measured SM in multiple brain regions to ask whether SM levels change soon after injury. Two days after injury, multiple sphingolipid species increased in cortex and hippocampus proximal to the injury site, but decreased at cerebellum which is distal to the injury. Hippocampal SM had the greatest increase which may reflect either disrupted sphingolipid transport (efflux) or metabolism. CHI also impairs long term potentiation (LTP) at the CA3-CA1 synapse. We examined whether altered SM levels in hippocampus are, in part, responsible for impaired LTP. Unexpectedly, impaired LTP was partially restored by exogenous addition of SM, but not by an abundant phospholipid, phosphatidylcholine. Spontaneous excitatory activity induced by the GABA-A antagonist picrotoxin was further increased by exogenous SM in the subiculum, dentate gyrus and medial entorhinal cortex in slices from injured animals. These effects of SM were not seen in hippocampal slices isolated from uninjured animals. Our data suggests that CHI induces alterations in SM metabolism or transport that likely impair excitatory synaptic transmission and

plasticity. The deficits in SM metabolism or transport contribute to electrophysiological and, perhaps, behavioral deficits seen after CHI.
Abdullah, et al*., Lipidomic analyses identify injury-specific phospholipid changes 3 months after traumatic brain injury*. The FASEB Journal, (2014). 28(12): p. 5311-5321.

Disclosures: K. Whitney: None. L. Buitrago-Soto: None. J. Iqbal: None. M. Hussain: None. H. Moreno: None. P.J. Bergold: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.03/Z3

Topic: C.09. Brain Injury and Trauma

Title: Choice impulsivity is increased following mild, closed head injury in rats

Authors: *K. M. MARTENS, C. WINSTANLEY, C. WELLINGTON, C. VONDER HAAR; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: *Background:* Traumatic brain injury (TBI) is associated with numerous deleterious consequences, including chronic changes in cognitive function. Persons with brain injury frequently show sub-optimal or even deleterious choice behavior, resulting in considerable financial, social, and work-related strain. Despite the obvious problems for human patients, many animal studies have neglected the study of decision making following TBI. *Methods:* The current study followed up previous work exploring shifts in impulsive choice behavior using the delay discounting paradigm following open-skull TBI. In the current study, rats were trained on the delay discounting task for 35 sessions. Once choice behavior had stabilized, they were given a closed head injury or sham procedure using the CHIMERA system, a non-surgical closed head impact acceleration model that produces primarily diffuse axonal injury as a result of unconstrained head movement after impact. Rats were then re-tested for a period of two weeks followed by a second CHIMERA injury. This pattern continued for a total of four injuries in eight weeks, with behavioral testing between each. *Results:* CHIMERA injury increased impulsive choice in injured rats. The effect was initially small and affected very few rats. However, after subsequent injuries, there were larger increases in impulsivity and more rats showed changes in choice. *Discussion:* In the current study, we show that choice impulsivity is increased in rats following closed head injury. This resembles reports in the clinical literature regarding impulse control problems in human patients. By examining decision making in

animals following a TBI, we are able to more closely mimic the human condition, which could lead to novel and/or improved treatments for brain injury.

Disclosures: K.M. Martens: None. C. Winstanley: None. C. Wellington: None. C. Vonder Haar: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.04/Z4

Topic: C.09. Brain Injury and Trauma

Support: NSERC Grant 249853

Agusti Pedro i Pons Foundation

Title: Effects of repeated mild traumatic brain injury on hippocampal synaptic plasticity

Authors: *C. PINAR-CABEZA[1], C. J. FONTAINE[1], B. R. CHRISTIE[1,2];
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Abstract: Traumatic brain injury (TBI) is the leading cause of disability in individuals under 45 years of age, with concussion, or mild TBI (mTBI), accounting for up to 75% of all brain injuries occurring annually in the US. Growing evidence indicates that repeated concussions may result in cumulative and long-term behavioural symptoms, neuropathological changes and neurodegeneration. The juvenile brain may be particularly vulnerable to mTBI as it is in a period of robust synaptic reorganization and myelination. In these studies we have focused on the hippocampus, a brain structure that can be affected by mTBI, producing some of the cognitive and affective deficits that are characteristic of mTBI clinical presentation.
Several models have been developed to mimic the clinical consequences of repeated mild TBI, however, most of these models use anesthesia or surgical procedures to induce the injury, which might confound the interpretation of these studies. To examine this issue in the juvenile brain, we assessed changes in hippocampal synaptic plasticity using an awake closed-head mTBI model. Following 4 closed-head mTBI in Long-Evans rats (25-28 days of age) synaptic plasticity of field excitatory post-synaptic potentials (fEPSPs) was assessed using in vitro electrophysiology at either one day, three days, five days or seven days post-injury in the dentate gyrus (DG) region of the hippocampus. Our results indicate that repeated mTBI can impact bidirectional synaptic plasticity in the hippocampus of juvenile animals.

Disclosures: C. Pinar-Cabeza: None. C.J. Fontaine: None. B.R. Christie: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.05/Z5

Topic: C.09. Brain Injury and Trauma

Support: NIH-NS40125

NIH-NS060672

VAI01RX001127

NIH-1-F32NS090748

Title: Functional deficits are attenuated in human alpha synuclein BAC-transgenic rats after traumatic brain injury

Authors: *C. DIXON[1], Z. M. TAPP[2], S. W. CARLSON[2];
[1]Neurosurg., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) can produce synaptic protein loss that may contribute to functional deficits. We previously reported that a scaffold protein necessary for the assembly of synaptic SNARE proteins, cysteine string protein alpha (CSPα), is reduced after experimental TBI (Carlson, et al., J. Neurotrauma, 2016). Ruiz, et al., (Neuropharmacology, 2014) has shown that a genetic knock-down of CSPa produces impaired synaptic SNARE protein assembly that can be reversed by upregulation of alpha synuclein (αSyn). While αSyn has been speculated to have some linkage between TBI and chronic TBI-associated Parkinsonism, we posit an acute role in preserving the machinery of SNARE-mediated vesicle trafficking after TBI. This functional outcome experiment determined the effects of TBI in transgenic rats that carry the wild type (WT) human αSyn and have elevated αSyn levels in young adults (Nuber, et al., Brain, 2013). Twenty three adult (3-4 mo) male Sprague Dawley rats were prepared for controlled cortical impact (CCI) (4 m/sec, 2.8 mm deformation) or sham. Rats were divided into three groups: CCI in BAC human αSyn transgenic rats (Taconic) (n=7), CCI in WT rats (n=8), or Sham WT rats (n=8). Functional outcomes were tested via Beam Balance Task and Beam Walking Test (days 1-5), Morris water maze (MWM) acquisition (days 10-14) followed by a probe test. In the Beam balance task the transgenic rats have significantly longer latencies that the WT rats after CCI ($p=0.001$). For the Beam walking test the transgenic rats have significantly shorter latencies that the WT rats after CCI ($p>0.0001$). For the MWM test the transgenic rats

have significantly shorter swim latencies than the WT rats after CCI ($p>0.01$). There were differences in the probe test. Transgenic rats with human αSyn are less sensitive to CCI vs. WT rats on tests of motor and cognitive performance. αSyn may be a novel target for acute post-traumatic dysfunction.

Disclosures: C. Dixon: None. Z.M. Tapp: None. S.W. Carlson: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.06/Z6

Topic: C.09. Brain Injury and Trauma

Support: SUNY Research Foundation

Title: Unique neurocognitive and affective impairments in mouse models f TBI, PTSD and co-morbid TBI-PTSD

Authors: *A. FESHARAKI[1], J. MIYAUCHI[2], S.-A. E. TSIRKA[3], P. BERGOLD[4];
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Abstract: Traumatic brain injury (TBI) is frequently co-morbid with post-traumatic disorder (PTSD). Both TBI and PTSD produce a long-lasting inflammation in the brain. Key questions are whether neuroinflammation differs between TBI, PTSD and co-morbid TBI and PTSD and does this neuroinflammation contribute to cognitive and affective impairment. C57Bl/6mice (28g) received either closed head injury (CHI), a model of TBI; chronic variable stress (CVS), a model of PTSD; or CHI followed by CVS, a model of co-morbid TBI/PTSD. CHI consisted of a single strike to the skull over the parietal lobe. CVS consisted of four days of variable chronic stress consisting of cold-water swim, transient restraint, cage tilt, wet cage, intermittent white noise, and food deprivation. Seven days after sham-CHI or CHI, the mildly aversive Barnes Maze (BM) and more aversive Active Place Avoidance (APA) assayed cognition and memory. Elevate Plus Maze (EPM) assayed basal anxiety and Acoustic Startle Response (ASR) assayed fear potentiated anxiety. CORT levels were assessed after injury and at the end of behavioral testing. Assay of additional parameters of neuroinflammation in plasma and brain tissue are in progress. Mice in the CHI, CVS, and CHI/CVS groups had similarly acquisition of BM and APA as sham-CHI, sham-CVS mice, suggesting no differences in cognition. Mice in the CHI group had a significantly shorter time to 1st entrance than the other 3 groups. These data suggest a memory deficit in the CHI group that was not when mice received both CHI and CVS. This

suggested that CHI/CVS mice had a heightened ability to recall aversive stimuli as compared to CHI mice. The CVS and CHI/CVS group showed SIGNIFICANTLY higher basal anxiety as assessed by less time and fewer entrances into the open arm of the EPM. On ASR, CHI mice had significantly more freezing while CVS and CHI/CVS mice trended toward exaggerated startle amplitude. CORT levels in the CHI and CVS groups trended to be lower than sham-CHI, sham-CVS but not in the CHI/CVS groups. These data suggest that CHI, CVS and CHI/CVS are present a unique spectra of behavioral and endocrine outcomes. Studies in progress will determine if this is accompanied by unique patterns of neuroinflammation.

Disclosures: A. Fesharaki: None. J. Miyauchi: None. S.E. Tsirka: None. P. Bergold: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.07/Z7

Topic: C.09. Brain Injury and Trauma

Support: VA Rehabilitation R&D Grant #I01RX001144

Gordon Project/C.S.C./U.W.S.F. Fellowships

Title: A novel mouse comorbid model of traumatic brain injury and fear conditioning with cognitive, behavioral and neuropathological impact

Authors: *G. B. KAPLAN[1], S. C. HEINRICHS[2], K. A. LEITE-MORRIS[1], X. ZENG[3], L. WU[3], D. T. ARENA[2], O. H. NGUYEN[4], Y. D. TENG[3];
[1]Psychiatry and Pharmacol., VA Boston Healthcare System/Boston Univ. Sch. Med., Boston, MA; [2]Res., VA Boston Healthcare Syst., Boston, MA; [3]SCI/Neurosurgery and PMR, VA Boston Healthcare System/Harvard Med. Sch., Boston, MA; [4]Neurosci., Boston Univ/VA Boston Healthcare Syst., Boston, MA

Abstract: Mild traumatic brain injury (mTBI) and posttraumatic stress disorder (PTSD) were modeled in male C57Bl/6 mice in order to determine the co-morbid effects of mTBI and PTSD on learning and other behaviors and neuroinflammation and neurodegeneration. Mice with mTBI induced by fluid percussion injury (FPI) at one of three intensity levels (1.0, 1.5 or 2.0 atmospheres) were subsequently fear conditioned and assessed for spatial learning performance over two post-operative recovery weeks. Following completion of behavioral testing, brains were harvested to quantify inflammation and neuropathology in naive control mice as well as craniotomy sham surgery and FPI exposed groups. Neurological severity scoring revealed no

deficits in balance, movement, or physical vigor in the immediately after surgery or FPI exposure. Similarly, acquisition/expression of fear conditioning and general exploratory/locomotor behavior did not vary as a function of treatment group over the two week testing period. In contrast, spatial learning acquisition in the Morris Water Maze task was significantly slowed and attenuated in FPI mice relative to controls during 4 daily trials and the degree of impairment was enhanced by increasing injury severity. Immunocytochemical staining of pan-T cell marker CD3, activated macrophage/microglia marker CD68, and pro-inflammatory marker TNF-alpha or IL-1beta showed that there were infiltrating T cells and activated macrophage/microglia present in parietal and subcortical brain regions as a consequence of mTBI. Evidence from this mouse model of FPI with co-occurring fear conditioning reveals a short-term cognitive impairment accompanied by neuroinflammatory and degenerative neural changes in cortical and hippocampal regions. Future studies will examine these behavioral and neural changes at later term time points post-injury. Supported by a VA Merit Award to GBK and Gordon Project/C.S.C./U.W.S.F. fellowships to XZ and LW.

Disclosures: G.B. Kaplan: None. S.C. Heinrichs: None. K.A. Leite-Morris: None. X. Zeng: None. L. Wu: None. D.T. Arena: None. O.H. Nguyen: None. Y.D. Teng: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.08/Z8

Topic: C.09. Brain Injury and Trauma

Support: T32HL07713

R37HD059288

R01NS069629

Title: Effect of mild TBI on specific cognitive dysfunction with *In vivo* hippocampal activity

Authors: *R. PATERNO[1], H. METHENY[2], G. XIONG[2], A. S. COHEN[2];
[1]Children Hosp. of Philadelphia, Philadelphia, PA; [2]Children's Hosp. of Philadelphia, 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 mechanism(s) for TBI-induced cognitive dysfunction remain unclear. However, many lines of research have implicated the hippocampal dysfunction in the pathophysiology of traumatic brain injury. In

order to investigate the effects of traumatic brain injury on specific cognitive dysfunction involving the hippocampus, we used a lateral fluid percussion injury animal model and recorded *in vivo* electrophysiological activity in the hippocampi of mice. Specifically, we simultaneously measured local field potentials in areas CA1 and CA3 of the hippocampus during a spatial recognition task in TBI and Sham animals. In vivo electrophysiological recordings showed increased theta activity in both CA1 and CA3 during exploration versus resting states in both TBI and in Sham animals. Furthermore, we found that the overall broadband power in area CA1 and CA3 was decreased in TBI animals compared to Sham control group during exploration of a new object compared to resting activity. Also, we found differential activity in CA1 versus CA3 in a complex relationship depending on behavior performance between TBI animal group compared to the Sham control group. These findings indicated for the first time that both area CA1 and CA3 of the hippocampus are impaired after TBI and further explores the neural basis underlying cognitive dysfunction.

Disclosures: R. Paterno: None. H. Metheny: None. G. Xiong: None. A.S. Cohen: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.09/Z9

Topic: C.09. Brain Injury and Trauma

Support: Veterans Affairs Rehabilitation Research & Development, Career Development Award #IK2-RX001479

CURE Taking Flight Award

Codman Resident Neurotrauma Research Award

Title: Disrupted entrainment of CA1 hippocampal neurons to hippocampal theta after traumatic brain injury

Authors: *P. KOCH[1], R. RUSSO[1], M. WEBER[1], D. H. SMITH[1], V. E. JOHNSON[1], J. A. WOLF[1,2];
[1]Dept. of Neurosurgery, Ctr. for Brain Injury and Repair, Univ. of Pennsylvania, Philadelphia, PA; [2]Corporal Michael J. Crescenz VA Med. Ctr., Philadelphia, PA

Abstract: Network level mechanisms of persistent memory impairment after TBI are poorly understood. Studies using the lateral fluid percussion injury (FPI) model of TBI in rodents have reported reduced theta power in the hippocampus (HC) coupled with memory impairment after

injury, although power reductions may recover. Reciprocal connections between HC and medial septum (MS) are important contributors to HC theta. Presumed disruption of this network is implicated in spatial memory deficits after FPI. Limited recovery of such deficits has been reported with fornix and MS stimulation at theta frequencies. However, precisely how MS-HC interactions are disrupted after TBI, and how this may influence memory-relevant HC processing is unknown. Male Sprague-Dawley rats (n=21, 350-425g) were trained on the Morris Water Maze (MWM, test of spatial memory) and then underwent FPI (n=11, 2.01-2.38 atm), or sham injury (n=10). MWM testing on post-injury day (PID) 2 revealed a significant reduction in memory performance (p=0.005). Rats (n=5 per group) underwent simultaneous MS and HC recordings under isoflurane anesthesia on PID 7. HC recordings were performed with a laminar 32 channel probe (NeuroNexus) spanning HC CA1 from str. oriens to str. radiatum, while MS recordings were performed with high impedence tetrodes (Thomas Rec). There was no difference in theta power (4-10 Hz) between sham and injured rats, nor in recording time dominated by theta. Overall firing rates of CA1 neurons were not significantly different between sham (n=117) and injured (n=86). The majority of CA1 neurons were significantly entrained to HC theta in both sham (71.8%) and injured (83.7%) groups. Further, approximately one third of recorded MS neurons were synchronized to HC theta in both groups. However, while the distribution of preferred phases of the theta cycle among entrained sham CA1 neurons showed a wide distribution with a peak at ~0.7 rad, the distribution of preferred phases among injured CA1 neurons revealed 2 sharp peaks at ~0 rad and 2.45 rad. Persistence of abnormal and possibly exaggerated CA1 theta entrainment, but normal CA1 firing rates, normal theta power, and persistent MS-HC synchronization at PID 7 could be due in part to a corrective increase in the "gain" of CA1 neurons in response to a loss of afferent input due to axonal injury. This could result in a stereotyped, hyperexcitable HC response and failure to appropriately coordinate inputs under conditions of high demand such as memory performance. Aberrant entrainment of CA1 neurons may underlie aspects of memory disruption following TBI as encoding of memory is presumed to require appropriate entrainment to the theta oscillation.

Disclosures: P. Koch: None. R. Russo: None. M. Weber: None. D.H. Smith: None. V.E. Johnson: None. J.A. Wolf: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.10/Z10

Topic: C.09. Brain Injury and Trauma

Support: SVSU faculty led grant

Title: Pre-enriched housing impacts progesterone's effect on functional recovery in male rats after traumatic brain injury.

Authors: *Z. BOWERS, N. SANISLO, N. ALAM, M. SEARLES, J. SMITH;
Saginaw Valley State Univ., University Center, MI

Abstract: In the United States 1.7 million people receive a TBI annually and it is estimated there are 5.3 million people in the U.S. living with long term disabilities. Currently, there are no clinical treatments that are effective in alleviating the functional deficits of TBI. Progesterone (PROG), a neurosteroid with pleiotropic effects has been shown to be beneficial in multiple brain injury models. The purpose of this study was to investigate the neurologically protective effect of progesterone following traumatic brain injury in animals reared in Enriched Environments (EE). The current study used 27 male Long-Evans rats purchased at post-natal day 25 and reared to maturity in EE. After 91 days, 18 subjects received a bilateral controlled cortical impact (CCI) placed over the medial frontal cortex to produce a moderately severe injury. Rats were administered intraperitoneal injection of either 10mg/kg PROG or vehicle injections (peanut oil) 4h post-injury and every 12h for 72h following the initial injection. Seven days post-injury the rats were tested on several behavioral tasks including the open field test, Barnes maze, Morris water maze, rotor-rod task, elevated plus maze, and forced swim task. Results from behavioral testing suggest that overall the intact animals performed significantly better than injured animals. Contrary to the established literature we found an intermediate effect of PROG when animals were tested on the MWM and rotor rod tasks, where intact animals performed significantly better than untreated group. An interesting additional finding using Elevated plus maze (EPM) to assess anxiety-like behaviors, overall intact animals spent significantly greater time in the closed arms than the injured groups. Analysis of the behavioral data from this study has shown that enriched housing before injury can impact behavior tasks in intact animals as well as functional recovery in injured animals when in combination with PROG. Future research should explore potential mechanisms related to pre-enriched housing that may influence recovery from TBI.

Disclosures: Z. Bowers: None. N. Sanislo: None. N. Alam: None. M. Searles: None. J. Smith: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.11/Z11

Topic: C.09. Brain Injury and Trauma

Title: A la-icpms time course analysis of changes in cerebral metals following a controlled cortical impact in young and aged mice.

Authors: *S. D. PORTBURY[1], D. HARE[1], C. SGAMBELLONI[1], D. P. BISHOP[2], P. A. DOBLE[2], D. FINKELSTEIN[1], P. A. ADLARD[1];
[1]Neurodegeneration, Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia; [2]Elemental Bio-imaging, Univ. of Technol. Sydney, Sydney, Australia

Abstract: Traumatic brain injury (TBI) is complicated by the prominent involvement of the biological transition metals iron (Fe), copper (Cu) and zinc (Zn). Non-heme bound Fe has been demonstrated to be abnormally elevated immediately after TBI, and the toxic liberation of Zn after brain injury has also been confirmed in many studies. Similarly, Cu has been shown to be deficient following brain trauma. Taken together, these alterations in brain metal levels are likely to contribute to the hallmark pathologies that occur within the brain post-injury (such as cell death and diffuse axonal injury) and also to both the acute and chronic neuropsychological sequelae (such as depression, anxiety and cognitive loss) that are prominent in brain-injured patients. In order to determine whether the reported increased susceptibility to adverse TBI-related outcomes in the elderly population may involve a differential modulation of metals across age (metals are known to be critical in the pathogenesis of a number of neurodegenerative disorders and other brain dysfunction), we have surveyed Fe, Zn and Cu levels using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICPMS). The ipsilateral hemisphere in 3 and 24 month old mice subject to a controlled cortical impact was compared to contralateral hemisphere and regional metal analyses performed. Significant spatial elevations of Fe, Zn and Cu were detected immediately and up to 28 days after TBI in both age groups. Significantly, the elderly group exhibited an appreciably enhanced and persistent elevation of all metals in every region surveyed, potentially contributing to the accelerated onset of neurodegeneration observed in the aged population subsequent to brain injury. These data point to the potential importance of maintaining metal ion homeostasis across the time course of injury in both young, and particularly older, TBI casualties in order to ameliorate the associated behavioural deficits that are potentially mediated by these alterations in metal levels.

Disclosures: S.D. Portbury: None. D. Hare: None. C. Sgambelloni: None. D.P. Bishop: None. P.A. Doble: None. D. Finkelstein: None. P.A. Adlard: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.12/Z12

Topic: C.09. Brain Injury and Trauma

Title: Manipulating cognitive reserve: pre-injury environmental conditions influence the severity of concussion symptomology and pathophysiology.

Authors: *R. M. MYCHASIUK[1], H. HEHAR[2], I. MA[2], K. YU[2], K. YEATES[2];
[1]Neurosci., [2]Psychology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Neuroplasticity, the brain's propensity to change in response to experience, is the key process involved in maintaining cognitive reserve. An individual's cognitive reserve is believed to be a 'summation' of all positive and negative changes in neuroplasticity, whereby positive experiences increase reserve and negative experiences reduce the plastic abilities of the brain. As post-concussion symptomologies develop when the brain is not able to adequately repair and compensate for injury-induced dysfunction, premorbid differences in cognitive reserve likely contribute to individual heterogeneity in concussion susceptibility. To examine this, rats received a single concussion while maturing in one of 3 different environmental conditions that manipulated cognitive reserve; a high stress, high fat diet condition (NEG); an enriching, socially and physically stimulating condition (POS), and a standard condition (STD). Rats underwent a behavioural test battery shown to assess post-concussion syndrome (PCS), and brain tissue was harvested at sacrifice to investigate epigenetic and genetic markers of plasticity, injury, and repair. The study found that manipulating pre-injury conditions increased heterogeneity in PCS whereby POS and NEG experiences differentially influenced outcomes. For example, POS environmental conditions eliminated cognitive impairments in the novel context mismatch task that were identified in control males following a concussion. Conversely, the NEG environmental condition exacerbated the anxiolytic effect identified in control rats with a TBI, significantly reducing anxiety in the elevated plus maze, while the POS environmental condition increased anxiety in the same task post-injury. In addition, examination of *BDNF, FGF-2, GFAP* and *Tau,* from the prefrontal cortex, hippocampus, and nucleus accumbens, demonstrate similar changes whereby manipulation of premorbid cognitive reserve influenced the brain's pathological response to the injury. This study indicates that POS environmental conditions do not always improve PCS outcomes just as NEG conditions do not always exacerbate deficits; rather manipulation of pre-injury reserve appears to increase variability in symptomology, contributing to the heterogeneity in individual susceptibility to PCS.

Disclosures: R.M. Mychasiuk: None. H. Hehar: None. I. Ma: None. K. Yu: None. K. Yeates: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.13/Z13

Topic: C.09. Brain Injury and Trauma

Title: Affective outcomes following traumatic brain injury in mice

Authors: *J. POPOVITZ, H. ADWANIKAR;
Johns Hopkins Univ., Baltimore, MD

Abstract: The molecular and signaling mechanisms that mediate affective disorders following traumatic brain injury (TBI) are poorly understood. Here, we investigate the behavioral, neuroanatomical and molecular changes after a controlled cortical impact injury in mice. Specifically, we track anxiety-like behaviors, as well as spontaneous behavior exhibited in a familiar context, for a period of eight weeks following injury. Assessments include metrics from the open field test, elevated zero maze test (EZM), elevated plus maze test (EPM) and home-cage behaviors. We observe changes in measures of anxiety with no deficits in general motor behavior. Following this, we perform immunostaining and volumetric measurements in the amygdala, an area that is considered to be important in the control of affective behaviors. Results will begin to address the mechanisms linking TBI and affective outcomes. *Key-words*: traumatic brain injury, anxiety behaviors, amygdala, controlled cortical impact

Disclosures: J. Popovitz: None. H. Adwanikar: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.14/Z14

Topic: C.09. Brain Injury and Trauma

Support: CIHR

Title: Frontal traumatic brain injury alters risky choice on a rodent analog of the Iowa gambling task

Authors: *C. VONDER HAAR, C. A. WINSTANLEY;
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Traumatic brain injury is associated with numerous long-term deficits and increased risk for psychiatric comorbidities. Some of the most difficult and chronic problems are those associated with cognitive functioning. Despite this, animal models of TBI have historically not addressed cognitive functions such as decision-making. In the current study, rats were trained on a rodent version of the Iowa Gambling Task, a method for assessing decision-making in humans. Rats make choices across four options, each associated with a specific risk/reward profile which provides either sugar pellets, or a time-out from responding. After stable baseline behavior was obtained, rats received either a moderate controlled cortical impact bilaterally over the frontal cortex (depth: 1.7 mm @ 2 m/s) or sham procedures. After one week of recovery, rats were re-assessed on the rodent gambling task for six weeks. Brain injury chronically affected choice behavior. Initially, brain-injured rats avoided the high-risk, high-reward options in favor of the option with the lowest probability of punishment. Over the course of several weeks, choice behavior largely normalized with the exception of a reduction of choice for the option with the largest punishment. This study is one of the first to examine a choice behavior with more than two choices in brain-injured animals. Surprisingly, the injured animals did not replicate the typical shift towards increased risky choice that is seen in human TBI populations. This may be due to procedural differences - specifically the fact that humans are almost exclusively tested in acquisition, while the current study examined animals already at steady-state performance. Additionally, this data suggests that injured rats are more averse to punishment, which has rarely been examined in TBI studies. Further studies will be required to determine whether acquisition testing replicates what is observed in human patients and to what degree the aversion to punishment observed in the current study generalizes.

Disclosures: C. Vonder Haar: None. C.A. Winstanley: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.15/AA1

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant 5R01HD055813

Title: Plasticity of retinal ganglion cell axon terminals in dorsal lateral geniculate nucleus following traumatic brain injury

Authors: *V. C. PATEL, C. W. D. JURGENS, T. E. KRAHE, J. T. POVLISHOCK;
Neuroanatomy and Neurosci., Virginia Commonwealth Univ., Richmond, VA

Abstract: Diffuse axonal injury (DAI) is a consistent feature of traumatic brain injury (TBI) and is strongly associated with morbidity. Little is known however, regarding the downstream consequences of DAI. Previous publications by our lab demonstrated that even mild TBI can induce DAI within the optic nerve, resulting in diffuse and scattered axonal disconnection, with the caveat that a large fraction of axons within the optic nerve remained intact despite this diffuse injury. In the current study, we sought to evaluate if the axon terminals of these intact retinal projections redistribute or undergo adaptive reorganization in their downstream target, the dorsal lateral geniculate nucleus (dLGN), following injury. We utilized anterograde tracing, fluorescent immunohistochemistry (FIHC), and epifluorescent, confocal, and electron microscopy to study retinogeniculate axon terminals in dLGN over a 20 day post-injury period. Using 9-12 week old C57BL/6J mice, we performed mild central fluid percussion injury (cFPI) with the use of sham controls. On post-injury days 1, 7, and 17, a subset of mice underwent anterograde axon terminal labeling through the use of recombinant cholera toxin subunit B (CTB) conjugated to different Alexa dyes for each eye followed by perfusion with fixative 72 hrs later. All other mice underwent perfusion at 4, 10, and 20 days following sham surgery or injury with coronal sections processed for VGLUT2, a selective marker for retinogeniculate axon terminals within dLGN. Through this approach, we found within dLGN scattered loss of both CTB and VGLUT2 positive axon terminals at 4 day post-injury, with significant increases in both VGLUT2 and CTB positive axon terminals compared to sham, 10 days, and 20 days post-injury ($p < 0.05$). These quantitative evaluations were supported by parallel EM studies which confirmed extensive axon terminal loss and Wallerian debris followed by axon terminal recovery. Through the CTB studies, we observed that the axon terminal sprouting retained the organization of ipsilateral and contralateral eye distributions within dLGN. We also observed within the contralateral eye zone of dLGN that the core vs shell appeared particularly vulnerable to axon terminal loss following cFPI. In addition, the core underwent axon terminal repopulation over the period of 20 days post-injury, although this recovery did not reach sham levels. In conclusion, axon terminals of intact retinogeniculate projections are capable of adaptive reorganization in dLGN of adult mice following TBI-induced DAI. Future efforts will include electrophysiologic studies of functional relevance of our observed structural plasticity.

Disclosures: V.C. Patel: None. C.W.D. Jurgens: None. T.E. Krahe: None. J.T. Povlishock: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.16/AA2

Topic: C.09. Brain Injury and Trauma

Title: Changes in rodent emotionality following repeated subconcussive head rotational accelerations

Authors: *B. D. STEMPER[1], N. LETKO[2], A. SHAH[2], R. CHIARIELLO[2], A. GLAVASKI[2], M. MCCREA[2], F. PINTAR[2];
[1]Med. Col. of Wisconsin Dept. of Neurosurg., Milwaukee, WI; [2]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Understanding the effect of subconcussive head impact exposure on concussive injury tolerance and cognitive/emotional changes in humans is complicated by a number of factors, including concussion history and variation in head impact characteristics (number/severity). In general, significant subconcussive head impact exposure is thought to decrease biomechanical tolerance for concussion and was associated with changes in cognitive performance, even in the absence of concussion, although the strength of those conclusions is tempered by individual variability. A biomechanically accurate preclinical model of subconcussive exposure can delineate mechanisms of cumulative damage and decreased concussive tolerance without the confounding variables present in humans. Accordingly, this study developed a rat model of subconcussive head impact exposure using the MCW Rotational Injury model. Anesthetized rats received 25 (n=40) or 50 (n=30) exposures to subconcussive head rotational acceleration (SCHRA) (167+/-12 krad/s^2). Acceleration magnitude was defined in our prior studies as head rotational acceleration that did not produce remarkable differences from shams following single exposure. A separate cohort of sham rats (n=48) were exposed to the entire experimental protocol, including anesthesia, without exposure to head rotational acceleration. SCHRA and sham procedures were performed over a 5-day period (5 or 10 per day). Rats were then exposed to behavioral testing during days 3-7 (acute) or days 24-28 (chronic) following the final SCHRA, consisting of the elevated plus and Morris water maze assessments. Separate cohorts were used for each assessment period. Exposure to 25 or 50 SCHRA resulted in no significant cognitive deficits at acute or chronic time points. However, significant changes in emotionality were evident. Rats receiving 25 and 50 SCHRA demonstrated significantly increased ($p<0.005$) activity during the acute phase measured as total arm changes and distance traveled, and a trend of increased activity ($p<0.1$) during the chronic phase. In terms of emotionality, rats receiving SCHRA demonstrated significantly increased ($p<0.01$) open arm time per entry (OATPE) acutely and decreased open arm time per entry during the chronic phase. Changes in emotionality were dose dependent, with acutely increasing and chronically decreasing OATPE

from controls to the 25-exposure group to the 50-exposure group. This study has produced a preclinical model of subconcussive head impact exposure in the rat using realistic and scalable biomechanics that has produced significant and dose-dependent behavioral changes following 25 and 50 exposures.

Disclosures: **B.D. Stemper:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin, Zablocki VA Medical Center. **N. Letko:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin. **A. Shah:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin. **R. Chiariello:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin. **A. Glavaski:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin. **M. McCrea:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin. **F. Pintar:** A. Employment/Salary (full or part-time): Department of Neurosurgery, Medical College of Wisconsin.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.17/AA3

Topic: C.09. Brain Injury and Trauma

Support: Dean of Faculty, Hamilton College

Title: Learning and memory deficits in a *Drosophila* model of traumatic brain injury

Authors: H. ZUCKER[1], J. ROBBINS[1], S. WU[2], *H. K. LEHMAN[3];
[1]Neurosci. Program, [2]Biochem. Program, [3]Biol. Dept., Hamilton Col., Clinton, NY

Abstract: Traumatic brain injury (TBI) is characterized by the loss of normal brain function following an external, injurious force that may lead to a host of adverse long-term behavioral effects, including impaired cognition and memory, sensorimotor deficits, mood disorders, and in extreme cases, neurodegenerative diseases like chronic traumatic encephalopathy. The mechanisms that underlie the myriad of pathologies, however, remain illusive. The fruit fly, *Drosophila melanogaster*, has proved to be a useful model system to study various human neurodegenerative disorders, and indeed, fly models of TBI have been recently developed (Katzenberger et al., 2013; Barekat et al., 2016). Our present study investigated learning and memory deficits in this TBI model. Specifically, wild-type flies were subjected to mechanical TBI, trained under a classical conditioning paradigm with an olfactory conditioned stimulus

(CS), and tested for motor skills, learning and memory behavior, and anatomical changes. Brain-injured flies performed normally on motor skill tasks, but significantly worse than control flies on the olfactory learning task, suggesting they had an impairment in learning and memory attributable to TBI. Furthermore, we observed morphological correlates to TBI and learning impairments. These behavioral and physical attributes of brain-injured Drosophila align with observations of human TBI and thus further validate the efficacy of this TBI model. Therefore, the present work may enable future studies focused on TBI pathophysiology, symptomatology, and treatment.

Disclosures: H. Zucker: None. J. Robbins: None. S. Wu: None. H.K. Lehman: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.18/AA4

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS077675

Title: Parvalbumin interneuron axonal damage after mild traumatic brain injury (mTBI): structural evidence implicating GABAergic input loss in neocortical circuit disruption

Authors: *M. VASCAK, J. POVLISHOCK;
Dept. of Anat. and Neurobio., Virginia Commonwealth University, MCV Campus, Richmond, VA

Abstract: A major feature of human mTBI is the presence of diffuse axonal injury (DAI) in the absence of any gross focal pathology. Historically, DAI has been studied in white matter, where long-distance projections are susceptible to the forces of injury. Recent clinical and experimental evidence however, suggest the involvement of inhibitory neurons within neocortical gray matter. The current investigation sought to determine if mTBI alters locally projecting axons from neocortical GABAergic interneurons. We evaluated parvalbumin (PV)-expressing interneurons, which control pyramidal neuron output and regulate neocortical excitatory-inhibitory balance. PV-cre mice were crossed with the Ai9 cre-dependent reporter line (PV-cre;Ai9) to genetically label PV interneurons with tdTomato. PV-cre;Ai9 mice subjected to either sham or mild central fluid percussion injury were sacrificed after 3 or 24 h and then prepared for confocal and ultrastructural analyses. Importantly, this model of mTBI induces DAI without any overt gross pathology. By 3 h post-mTBI, evidence of DAI was found in tdTomato+ perisomatic axonal swellings observed throughout the somatosensory cortex. Continuous with their soma of origin,

the proximal swellings were associated with their distal detached segments revealing pathology consistent with axonal degeneration. Ultrastructural analysis corroborated these findings. PV interneuron DAI was further confirmed via the colocalization of tdTomato+ axonal swellings with APP, an established marker of DAI. These tdTomato+/APP+ axonal swellings also colocalized with the GABA-synthesizing enzyme GAD67, which undergoes fast axonal transport. Quantitative analysis of GAD67/APP colocalization indicated that GAD67 is a sensitive and specific marker of DAI. Lastly, the majority of these GAD67+/APP+ swellings colocalized with tdTomato. This same tdTomato+ perisomatic axotomized population showed activation of c-Jun, a known regulator of axon regeneration. By 24 h post-mTBI, a 5-fold increase in phospho-c-Jun+/tdTomato+ cells occurred, corresponding to an overall injury burden of approximately 10% of total tdTomato+ cells. These observations reveal for the first time that locally projecting GABAergic interneurons are susceptible to DAI, with the majority of the injured population represented by PV interneurons. We further have characterized a novel marker of DAI specific to neocortical GABAergic interneurons. Together, our study has major implications for circuit disruption and unbalanced excitation-inhibition after mTBI, providing investigators with a new tool to explore a previously unrecognized gray matter neuropathology.

Disclosures: M. Vascak: None. J. Povlishock: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.19/AA5

Topic: C.09. Brain Injury and Trauma

Support: VA CDA #IK2-RX001479

VA MERIT #B1097-I

Title: Effects of TBI on the limbic system in a differential fear conditioning model of PTSD

Authors: C. D. ADAM[1,2], R. J. RUSSO[1], M. T. WEBER[1], V. E. JOHNSON[1], *J. A. WOLF[1,2]; [1]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [2]Corporal Michael J. Crescenz VA Med. Ctr., Philadelphia, PA

Abstract: Traumatic Brain Injury (TBI) is considered the signature injury of recent US military conflicts, with approximately 15% of deployed service members experiencing single or multiple TBIs. Post-Traumatic Stress Disorder (PTSD) is a frequent comorbidity with TBI, with almost 35% of TBI-exposed Veterans reporting qualifying symptoms associated with their service in

theater. Previous animal studies have shown increased acquisition and decreased extinction of conditioned fear following TBI. Studies have also shown differential roles of the hippocampus (HC), basolateral amygdala (BLA), basomedial amygdala (BMA), and medial prefrontal cortex (mPFC) in the acquisition and extinction of conditioned fear; however, the effects of TBI on these regions leading to the observed behavioral changes are unknown. This study sought to characterize behavior following TBI using a differential fear conditioning model of PTSD in rats, and investigate structural and functional changes within and between the mPFC, BLA, BMA, and HC that may underlie these changes. This study used lateral fluid percussion to induce a mild to moderate (1.8-2.0 atm) TBI in rats. Animals were tested in the Morris Water Maze 2 days post injury in order to demonstrate HC dependent memory deficits. Differential fear conditioning began 13 days post injury and consisted of 2 days of training followed by a day of contextual extinction then 2 days of cued extinction. During training, auditory stimuli consisting of 50ms white noise or pure-tone sound pips were delivered at 1Hz for 30 seconds and either co-terminated with a mild (0.6mA, 1 sec) shock (CS+) or were unpaired (CS-). Each day of training, rats were presented with 5 CS+ and 5 CS- in a random order. During contextual extinction rats were placed in the same context as in training, but no auditory stimuli were presented and no shocks were delivered. During each day of cued extinction, rats were placed in a novel context and were presented with the same CS+ and CS- auditory cues (five of each in a random order); however, no foot shocks were delivered. Freezing was used as the behavioral measure during all phases of differential fear conditioning. We found that injured rats had higher freezing to the CS+ during both days of training, indicating they acquired the conditioned response faster than sham animals. We also found that injured rats had lower freezing during contextual extinction, indicating they were unable to associate their context with the aversive stimulus. Electrophysiological recordings and neuropathology are currently being utilized to investigate structure/function relationships underlying the neurophysiological basis of these TBI induced changes.

Disclosures: C.D. Adam: None. R.J. Russo: None. M.T. Weber: None. V.E. Johnson: None. J.A. Wolf: None.

Poster

232. Traumatic Brain Injury: Plasticity and Behavioral Deficits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 232.20/AA6

Topic: C.09. Brain Injury and Trauma

Title: Increased aggression and social contact following traumatic brain injury in male zebra finches.

Authors: *K. A. DUNCAN, E. KELLMAN;
Biol., Vassar Col., Poughkeepsie, NY

Abstract: Traumatic brain injury (TBI) is associated with a wide range of both physiological and behavioral changes. The behavioral changes may include memory and attention deficits as well as emotional disorders such as anxiety, depression, aggression, impulse control, and decreased social cognition. While mechanisms underlying the physiological changes are better known, the mechanisms behind the behavioral changes are poorly understood. Following injury in the zebra finch brain, an upregulation of steroidogenic enzymes (aromatase; estrogen synthase) and receptors (androgen and estrogen) help to promote neurogenesis and neuronal survival. However, what role if any these increased steroidal products have on behavior has yet to be determined. Thus, the goal of this study was to determine the effect of injury on social behavior in male zebra finches. Behavioral testing (stress, social contact, and colony behaviors) was conducted at three times during the study (pre-injury, 1 week post-injury, and approximately 2 months post-injury). Following TBI, a significant increase in social contact and aggression suggests that social behavior is altered following injury. This effect was still present 2 months following injury. Interestingly, active coping stress was not significantly different following TBI. These data suggest that injury does alter behavior by promoting a shift from more affiliative behaviors to aggressive behaviors.

Disclosures: K.A. Duncan: None. E. Kellman: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.01/AA7

Topic: D.02. Somatosensation: Pain

Support: KAKEN Grant 16K20653

KAKEN Grant 26861799

Miyata Research Grant in E

Title: Effects of TRPV1 receptor antagonism on orthodontic force induced pain

Authors: *N. HASEGAWA[1], A. SASAKI[1], T. TSUCHIYA[1], N. SUDA[1], H. SAKAGAMI[2], K. ADACHI[2];
[1]Orthodontology, [2]Parmacology, Meikai Univ. Sch. of Dent., Sakado, Japan

Abstract: Recently, we have reported that the jaw-opening reflex (JOR) excitability is increased in 1-3 days and is decreased in 7 days after orthodontic force application in rats. Because this temporal alteration of the JOR excitability is similarly observed in the clinical reports for the orthodontic pain, this animal model may allow us to investigate the novel approach to treatment of orthodontic pain. The aim of this study was comparison of analgesic effect of common analgesics (aspirin and acetaminophen) and TRPV1 antagonist on the orthodontic pain in rats. In this model, general anesthetized rats were applied continuous orthodontic force by Ti-Ni coil spring to only right maxillary first molar. Aspirin (25, 50 and 100 mg/kg/day), acetaminophen (100 mg/kg/day), A-889425 (1.25, 2.5, 5.0 μmol/kg/day) and vehicle (CMC) were repetitively administrated (i.p., 3 times/day) from immediately after orthodontic force application. In one day, rats were generally anesthetized and electrical current (200 μs) was applied to bilateral maxillary first molar to compare the threshold for inducing JOR and those physiological features (latency, duration and AUC of anterior digastric muscle activity) between left and right sides. The maxillary tooth arch of each animal was impressed by silicone impression paste before application of coil spring and after JOR investigation to obtain study casts. The amount of tooth movement were obtained by measuring the study cast with vernier caliper. In vehicle treated animals, the JOR threshold in right side was significantly ($P < 0.05$) lower compared with that of left side. In comparison with vehicle, aspirin (100 mg/kg) and A-889425 (5.0 μmol/kg) significantly ($P < 0.05$) increased the right side JOR threshold. On the other hand, acetaminophen failed to increase the right side JOR threshold. Loading of continuous orthodontic force induced medial movement of the first molar and any treatments did not alter of the tooth movement. These data suggest that both NSAIDs and TRPV1 receptor antagonist, but not acetaminophen, are able to reduce orthodontic pain, however, both agents are known as mediators of osteoclast differentiation. Further studies to investigate the effects of prolongation of drug, in particular A-889425, treatment period on morphological alterations (e.g., tooth movement and expression of mature osteoclasts) and physiological features are required.

Disclosures: N. Hasegawa: None. A. Sasaki: None. T. Tsuchiya: None. N. Suda: None. H. Sakagami: None. K. Adachi: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.02/AA8

Topic: D.02. Somatosensation: Pain

Support: Spanish Ministry of Economy and Competitiveness Grant BFU2012-39092-C02-1

Spanish Ministry of Economy and Competitiveness Grant SAF2015-66275-C2-1-R

Title: New tools to interrogate molecular mechanisms in pain transduction.

Authors: M. ARTERO[1], F. TABERNER[2], G. FERNÁNDEZ-BALLESTER[1], *A. V. FERRER-MONTIEL[3];
[1]Inst. of Mol. and Cell. Biology, UMH, Elche, Spain; [2]Inst. of Pharmacology, Heidelberg Univ., Heidelberg, Germany; [3]Univ. Miguel Hernandez, Elche, Spain

Abstract: Disentangling the mechanisms driving pain sensation is a notable challenge in neurobiology. Pain is the result of a complex processing of biochemical and molecular signals at different levels of the peripheral and central nervous systems. The high heterogeneity of peripheral nociceptors represents a large handicap to understand the cellular and molecular mechanisms implicated in nociceptor sensitization by pro-algesic agents. Thus, there is a need to develop new tools that help to identify specific subpopulations of neurons and to investigate their role in pain transduction. Members of the family of Transient Receptor Potential channels (TRP) are key players in the transduction of nociceptive stimuli in somatosensory neurons. We have designed a knock-in mouse model, which expresses the yellow fusion protein (YFP) bound to N-terminal of TRPV1 (YFP-TRPV1), aimed at labelling cells that express the thermoTRP channel. After confirming the knock-in model through genetic analysis, our preliminary results point out to a tissue, cellular and subcellular distribution of the YFP-TRPV1 similar to wild type. Likewise, channel functionality and pharmacology was not affected. The implications of our preliminary results will be discussed in the context of the potential application of the knock-in model to investigate the molecular and cellular mechanisms underlying algesic sensitization of nociceptors.

Disclosures: M. Artero: None. F. Taberner: None. G. Fernández-Ballester: None. A.V. Ferrer-Montiel: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.03/AA9

Topic: D.02. Somatosensation: Pain

Support: NS055159

Title: Inhibition of TRPM3 ion channels by G-protein beta-gamma subunits

Authors: *T. ROHACS, D. BADHEKA, Y. YUDIN, I. BORBIRO;
Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Transient Receptor Potential Melastatin 3 (TRPM3) channels are activated by heat, and chemical activators such as pregnenolone sulphate and CIM0216. Here we show that activation of receptors coupled to heterotrimeric Gi proteins inhibits TRPM3 currents in a mammalian expression system. This inhibition was alleviated by co-expression of proteins that act as sinks for the βγ subunits of heterotrimeric G-proteins (Gβγ). Co-expression of Gβ1γ2, but not a constitutively active mutant of Gαo inhibited TRPM3 channel activity induced by pregnenolone sulphate. TRPM3 currents were also inhibited by purified Gβγ proteins applied to excised inside out patches, indicating a direct effect. Baclofen and somatostatin, agonists of Gi coupled receptors, inhibited Ca^{2+} signals induced by pregnenolone sulphate and CIM0216 in dorsal root ganglion (DRG) neurons. The GABAB receptor agonist baclofen also inhibited CIM0216-induced currents in DRG neurons, and nocifensive responses elicited by this TRPM3 agonist *in vivo*. Our data show that Gi-coupled receptors regulate TRPM3 channels via direct inhibition by Gβγ.

Disclosures: T. Rohacs: None. D. Badheka: None. Y. Yudin: None. I. Borbiro: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.04/AA10

Topic: D.02. Somatosensation: Pain

Support: NIH RO1 grant NS87988

NIH RO1 grant DE17794

NIH RO1 grant DE22743

NIH RO1 grant MH098114

NIH RO1 grant MH104326

Title: SHANK3 regulates TRPV1 signaling in mouse and human primary sensory neurons

Authors: *Y. KIM[1], Q. HAN[1], X. WANG[2], D. LIU[1], Y. ZHANG[1], T. BERTA[1], Y.-H. JIANG[2], R.-R. JI[1];
[1]Anesthesiol., [2]Pediatrics, Duke Univ. Med. Ctr., Durham, NC

Abstract: SHANK3 is a postsynaptic scaffold protein in the central nervous system. *SHANK3* mutation/deletion is implicated in autism spectrum disorder (ASD). *SHANK3* maps to the critical chromosome region of 22q13.3. Clinical and genomic evaluation of 201 patients with Phelan-McDermid syndrome, also known as 22q13 deletion syndrome which includes the deletion of entire *SHANK3* gene, showed a decrease in pain sensitivity in 77% of patients and this problem sustains with increased age. However, the molecular mechanisms of pain dysregulation in ASD are unclear. We found SHANK3 expression in primary sensory neurons of mouse and human dorsal root ganglia (DRG). SHANK3 is heavily co-localized with TRPV1 in DRG neurons. Capsaicin-induced inward currents in DRG neurons, as well as capsaicin-induced spontaneous pain are substantially reduced in *Shank3* knockout mice. Finally, knockdown of *SHNAK3* expression in human DRG neurons also abrogates TRPV1 function. Our findings reveal a novel peripheral mechanism of SHANK3, which may underlie pain deficits via functional downregulation of TRPV1 in *SHANK3*-related autism.
This project was supported in part by NIH RO1 grants NS87988, DE17794, and DE22743 to R.R.J and MH098114 and MH104326 to Y.H.J.

Disclosures: Y. Kim: None. Q. Han: None. X. Wang: None. D. Liu: None. Y. Zhang: None. T. Berta: None. Y. Jiang: None. R. Ji: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.05/AA11

Topic: D.02. Somatosensation: Pain

Support: Jerusalem Brain Community, Postdoctoral Scholarship

ISF Grants 1721/12 and 1368/12

CIG Grant FP7-CIG-321899

Title: Tyrosine residue at 511 position in the TRPV1 tightly fasten vanilloids to delay channel deactivation

Authors: *R. KUMAR, A. PRIEL;
The Hebrew Univ., Jerusalem, Israel

Abstract: **Background:** Capsaicin is a naturally occurring vanilloid that cause a hot, pungent sensation. It is an agonist to the TRPV1 receptor, a well characterized ion channel expressed on primary afferent neurons to sense both pain and heat. TRPV1 is a polymodal activated cation

channel acting as a key sensor of pain. The past few years have witnessed a tremendous progress in the structural elucidation of TRPV1. Recent developments in high-resolution cryo-EM have paved the way towards structural visualization of TRPV1 in both resting and ligand-bound states. The highly conserved tyrosine in position 511 (Y511) of the rat TRPV1 (rTRPV1) was the first residue to be identified as a necessary participant in vanilloid-mediated receptor activation, and the receptor cryo-EM structures point to a close proximity of this residue to the agonist. Furthermore, rotation of this residue and its steric function during vanilloid binding was proposed, still the usefulness of its rotation in vanilloid sensitivity and channel activation has not been fully examined.
Objective: We aim to understand the mechanism through which rTRPV1 Y511 residue participates in vanilloid-evoked receptor activation.
Methods: We generated an array of rTRPV1 mutants, containing the whole spectrum of Y511 substitutions, and tested their response to the exo-vanilloid capsaicin and the endo-vanilloid N-Arachidonoyl dopamine (NADA) by calcium imaging and electrophysiology.
Results: Our analysis indicates that, Y511 substitutions to only aromatic amino acids retained rTRPV1 sensitivity to vanilloids, although ligand binding was impaired. Interestingly, while activation rate was unaffected in rTRPV1 mutants carrying Tryptophan or Phenylalanine, the ligand washout rate was significantly faster than wild type.
Conclusion: Our findings indicate that, the Y511 residue in rTRPV1 enhances agonist sensitivity and channel activation by entrapping vanilloids in the binding pocket. Thus, the Y511 residue is required for vanilloid-evoked TRPV1 activation and pain sensation by stabilizing ligand-receptor interaction.

Disclosures: R. Kumar: None. A. Priel: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.06/AA12

Topic: D.02. Somatosensation: Pain

Support: McGill University 8510

Title: New mechanisms of EP1 and EP4 receptors in enhancing the functionality of TRPV1 in dorsal root ganglia (DRG) neurons

Authors: *S. M. JAFFAL;
McGill Univ., Montreal, QC, Canada

Abstract: Transient Receptor Potential Vanilloid 1 (TRPV1) is a non-selective cation channel that is modulated by inflammatory mediators (e.g., prostaglandin E2, PGE2) in the pain pathway through mediating the cell surface insertion of the channel and/or enhancing its activity. The experiments in this study were conducted using DRG neurons from male Sprague Dawley rats, different inhibitors, agonists [50 μM 17PGE2 -an agonist for EP1- or 50 μM PGE1OH -an agonist for EP4] & cobalt (Co^{+2}) assay. The results show that the pre-treatment of DRG neurons with an inhibitor of extracellular calcium (100 μM BAPTA) strongly inhibited the enhancement of capsaicin induced Co^{+2} influx by the 2 agonists. Similar effect was obtained when different types of the voltage dependent calcium channels (VDCCs) were inhibited using a cocktail of inhibitors (10 μM Amlodipine, 1 μM ω-Conotoxin & 1 nM SNX-482) in an indication that VDCCs are activated as a source of calcium entry in this pathway. In contrast, the mechanisms of the 2 receptors differed significantly with respect to the involvement of the synaptosomal-associated protein 25kDa (SNAP25 protein) which has a crucial role in vesicle fusion. SNAP25 inhibitor reduced the enhancement of capsaicin induced Co^{+2} influx by EP1, but not EP4. Accordingly, the results suggest that TRPV1 insertion to the surface of DRGs is a prerequisite for EP1 in the enhancement of TRPV1 functionality and that EP1 follow the same route, in this context, of other inflammatory mediators (NGF, ATP and IGF-1) as mentioned in previous studies. The involvement of SNAP25 in this pathway/tissue is in an agreement with my results (presented at the Canadian Association of Neuroscience meeting, CAN2015; poster # 2-D-109) that suggest the switch in the type of the t-SNARE protein during dmPGE2 induced trafficking of TRPV1 from the DRGs to the periphery [SNAP25 was the t-SNARE involved in this trafficking in DRG neurons while syntaxin-1 was the t-SNARE involved in the periphery: sciatic nerve and skin of the hindpaw of the rats]. Finally, my data show- in agreement with the literature- that the pre-treatment of the DRGs with 10 μM PKCε-V1-2 inhibitor or 50 μM H89 inhibitor abolished the augmentation of capsaicin induced Co^{+2} influx by EP1 and EP4 respectively indicating that the effect of EP1 involves protein kinase C epsilon (PKCε) while the effect of EP4 requires protein kinase A (PKA).
In conclusion, the current and previous results shed the light on the differential mechanisms of EP1/EP4 recptors in mediating the functionality and surface availability of TRPV1 in DRG neurons and can be of therapeutic value.
Budget number 8510 from Faculty of Medicine at McGill University to Sahar Jaffal

Disclosures: S.M. Jaffal: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.07/AA13

Topic: D.02. Somatosensation: Pain

Support: Maryland Industrial Partnership grant #5403

Title: Calcium-dependent ablation of trpv1-lineage axonal terminals by capsaicin

Authors: S. WANG[1], S. WANG[1], J. JOSEPH[1], J. RO[1], F. WEI[1], J. CAMPBELL[2], *M.-K. CHUNG[1];
[1]Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD; [2]the Centrexion, Baltimore, MD

Abstract: Capsaicin, the principal pungent ingredient of spicy peppers, activates the transient receptor potential vanilloid 1 (TRPV1) expressed on the cell surface membrane of nociceptors. Although capsaicin acutely induces intense burning pain by activating TRPV1, it has also been used as an analgesic due to enduring effects on nociceptive terminals. Topical capsaicin is FDA approved for treating post-herpetic neuralgia, and injection of capsaicin into painful tissues also shows analgesic efficacy. Peripherally administered vanilloids induce ablation of nociceptive terminals limited to the treated area without ablation of the cell body. Although Ca^{2+}- and mitochondria-dependent capsaicin-induced cell death has been shown, mechanisms underlying capsaicin-induced ablation of nociceptive terminals are less known. By combination of TRPV1-lineage reporter mice and multicompartmental culture, we established a method to monitor capsaicin-induced changes of TRPV1-lineage afferent terminals in real-time, limited to afferent terminals without affecting soma. We found that Ca^{2+} influx through TRPV1 is necessary and sufficient for capsaicin-induced ablation of nociceptive terminals. Notably, cyclosporin A or TRP19622 (inhibitors of the mitochondria transition pore) and phenyl alpha-tert-butyl nitrone or antioxidant supplement (scavenger of reactive oxygen species), did not attenuate capsaicin-induced ablation. Ablation of TRPV1-lineage nociceptive terminals in mouse hindpaw skin following the local injection of capsaicin was sustained for more than a month and reversed after two months. This ablation in vivo was substantially prevented by the chelation of Ca^{2+} but was not affected by cyclosporin A. These results suggest that TRPV1/Ca^{2+}-dependent signaling plays dominant roles in capsaicin-induced ablation of nociceptive terminals *in vitro* and *in vivo*. This study should lead to a better understanding of the molecular mechanisms and further improvement of capsaicin-induced analgesia.

Disclosures: **S. Wang:** None. **S. Wang:** None. **J. Joseph:** None. **J. Ro:** None. **F. Wei:** None. **J. Campbell:** A. Employment/Salary (full or part-time): James Campbell is an employee of the Centrexion. **M. Chung:** 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; Man-Kyo Chung conducts a research contract between the Centrexion and the University of Maryland.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.08/AA14

Topic: D.02. Somatosensation: Pain

Support: NS072432

NS092851

Title: The complement system component C5a produces thermal and mechanical hyperalgesia via macrophage-to-nociceptor signaling that requires TRPV1

Authors: *C. WARWICK[1], L. P. SHUTOV[1], T. M. WOODRUFF[2], A. J. SHEPHERD[3], D. P. MOHAPATRA[3], Y. M. USACHEV[1];
[1]Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Sch. of Biomed. Sci., Univ. of Queensland, St. Lucia, Australia; [3]Washington Univ. Pain Ctr., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: The complement system is a principal component of innate immunity. Recent studies have highlighted the importance of C5a and other components of the complement system in inflammatory and neuropathic pain, although the underlying mechanisms are largely unknown. In particular, it is unclear how the complement system communicates with nociceptors and which ion channels and receptors are involved. Here we propose that the complement fragment C5a triggers macrophage-to-neuron signaling which involves TRPV1 sensitization and ultimately leads to thermal and mechanical sensitization. The inflammatory thermal and mechanical hyperalgesia induced by intraplantar injection of complete Freund's adjuvant (CFA) was accompanied by upregulation of C5a in the hindpaw and was markedly reduced by C5a receptor (C5aR1) knock-out or treatment with the C5aR1 antagonist PMX53. Administration of C5a into the mouse hindpaw produced mechanical and thermal hyperalgesia in a dose-dependent manner with C5aR1 KO mice showing no hyperalgesia. Immunohistochemistry of mouse plantar skin revealed that C5aR1 was expressed primarily in resident skin macrophages. Additionally, C5a evoked strong Ca^{2+} mobilization in cultured macrophages dependent upon C5aR1 activation of Gβγ-phospholipase Cβ signaling and Ca^{2+} mobilization from ER calcium stores. Drug-induced macrophage depletion in transgenic macrophage Fas-induced apoptosis (MAFIA) mice abolished C5a-dependent thermal and mechanical hyperalgesia. Examination of inflammatory mediators following C5a injection revealed a rapid upregulation of numerous factors including NGF, a mediator known to sensitize TRPV1. Preinjection of an NGF-neutralizing antibody or Trk inhibitor GNF-5837 prevented C5a-induced thermal hyperalgesia. Notably, NGF-induced thermal hyperalgesia was unaffected by macrophage depletion. Interestingly, both thermal and mechanical hyperalgesia produced by C5a were absent in TRPV1 knock-out mice, and were blocked by coadministration of TRPV1 antagonist AMG9810. As TRPV1 is a noxious heat

sensor and is unlikely to directly mediate mechanical sensation, these data suggest that C5a produces heat hyperalgesia by sensitizing TRPV1 to heat stimuli while C5a-induced mechanical hyperalgesia is potentially dependent upon neurogenic inflammation originating from TRPV1 containing fibers. The potential for C5a induced neurogenic inflammation is currently being tested. Collectively, our findings highlight the importance of macrophage-to-neuron signaling in pain processing and identify C5a, NGF, and TRPV1 as key players in this cross-cellular communication.

Disclosures: C. Warwick: None. L.P. Shutov: None. T.M. Woodruff: None. A.J. Shepherd: None. D.P. Mohapatra: None. Y.M. Usachev: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.09/AA15

Topic: D.02. Somatosensation: Pain

Title: Alleviating neuropathic pain by selective expression of trpv1 interfering peptide aptamer in primary sensory neurons

Authors: *H. YU, Z. LIU, F. WANG, H. XIANG, G. FISCHER, B. PAN, Q. H. HOGAN; Anesthesiol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The transient receptor potential channel vanilloid type 1 (TRPV1), a non-selective cation channel highly expressed in the primary sensory neurons, is an important molecular sensor and mediator of pain signals. Interfering peptide aptamers (iPA) are designed as short fragments of endogenous protein encompassing a domain with high affinity and specificity in binding to cognate sites of target protein, whereby iPA can function as a decoy peptide to specifically inhibit endogenous protein function by interfering with associated protein-protein interaction. Several studies have identified TRPV1-specific iPAs that exert dominant-negative effects on TRPV1 functions by interfering with subunit organization and/or desensitizing TRPV1 binding to interaction proteins. In this study, we constructed an adeno-associated viral (AAV6) vector expressing a recombinant fluorescent TRPV1iPA, using an iPA derived from a 38mer tetrameric assembly domain (TAD) ranging the residues 735-772 of rat TRPV1 and fused to the C-terminus of enhanced green fluorescent protein (EGFP). Functional analysis of primary sensory neurons expressing EGFP-TRPV1iPA (EGFP-TAD), using the Ca^{2+}-sensitive dye fura-2 to measure intracellular [Ca^{2+}] and patch-clamp recording of TRPV1 channel activation, revealed decreased functional TRPV1 channel activity in response to capsaicin (a potent and selective TRPV1 agonist), compared to control neurons from naïve rats or expressing EGFP alone. To examine the

potential for inhibiting development of neuropathic pain, AAV-EGFP-TAD (AAV-TAD) was injected into lumbar (L) 4 and L5 DRGs of rats with neuropathic pain following tibial nerve injury (TNI). Results showed that AAV-mediated selective expression of EGFP-TAD in L4/L5 DRG neuron somata and their peripheral and central axonal projections is effective in persistent attenuation (8 weeks) of TNI-induced neuropathic pain including hypersensitivity to heat and mechanical stimulation. These observations indicate that AAV-encoded analgesic iPAs for selective inhibition of TRPV1 activity in the primary sensory neurons may have potentials for development as a gene therapy strategy to treat neuropathic pain.

Disclosures: H. Yu: None. Z. Liu: None. F. Wang: None. H. Xiang: None. G. Fischer: None. B. Pan: None. Q.H. Hogan: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.10/AA16

Topic: D.02. Somatosensation: Pain

Support: Rustaveli Science Foundation of Georgia

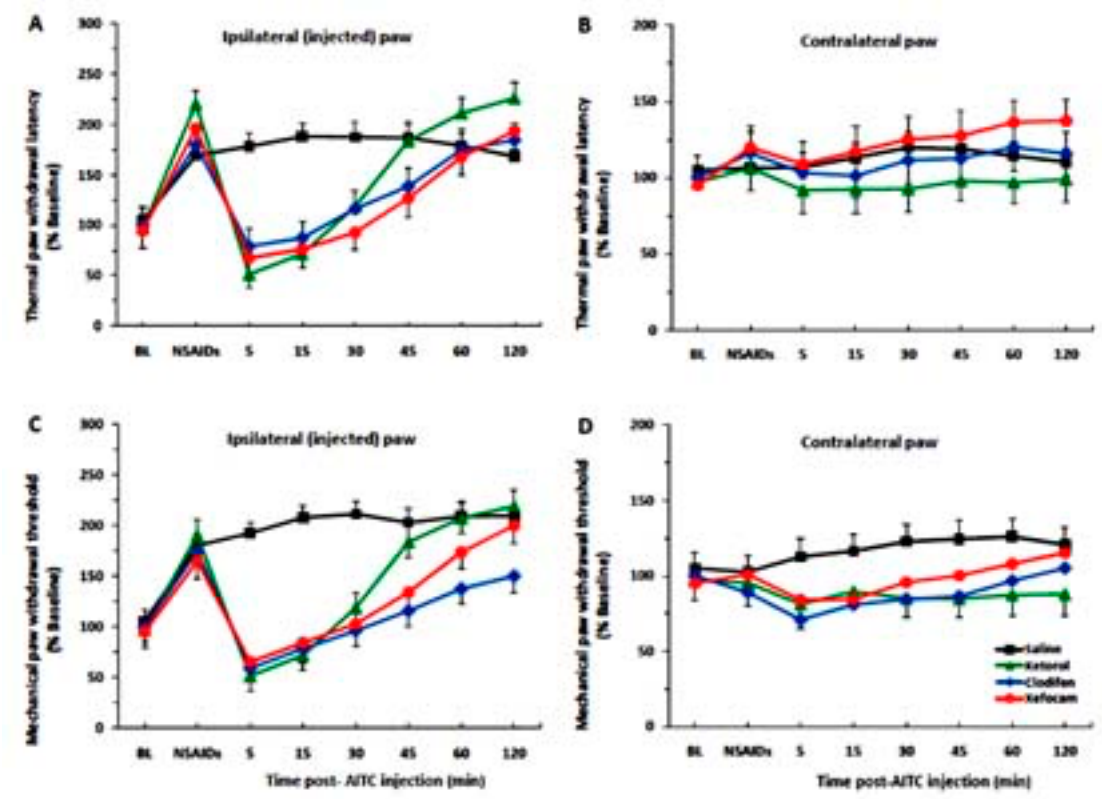
Title: NSAIDs attenuate hyperalgesia induced by TRP channelagonists

Authors: *M. G. TSAGARELI, I. NOZADZE, N. TSIKLAURI, G. GURTSKAIA;
Ivane Beritashvili Exptl. Biomedicine Ctr., Tbilisi, Georgia

Abstract: Transient receptor potential (TRP) cation channels have been extensively investigated as targets for analgesic drug discovery. Because some non-steroidal anti-inflammatory drugs (NSAIDs) are structural analogs of prostaglandins and NSAIDs attenuate heat nociception and mechanical allodynia in models of inflammatory and neuropathic pain, we examined three widely used NSAIDs (diclofenac, ketorolac, and xefocam) on the activation of TRPA1 and TRPV1 channels using thermal paw withdrawal (Hargreaves) and mechanical paw withdrawal (von Frey) tests in male rats. Thermal withdrawal latencies and mechanical thresholds for both hind paws were obtained with 5, 15, 30, 45, 60, and 120 min intraplantar post-injection of TRPA1 agonists, allyl isothiocyanate (AITC) (a natural compound of mustard oil) and cinnamaldehyde (CA), and TRPV1 agonist capsaicin or vehicle. Twenty minutes prior to the start of the experiment with TRP agonists, diclofenac, ketorolac or xefocam were pre-injected in the same hind paw and animals were examined by these two tests. After pretreatment of all three NSAIDs in the ipsilateral (injected) hindpaw that produced strong antinociceptive effects, AITC, CA, and capsaicin caused significant decreases in latency of the thermal withdrawal reflex

compared with vehicle or the contralateral hindpaw. The same findings were observed for the paw withdrawal threshold. In approximately 30 min the effects of CA, AITC, and capsaicin returned to baseline. The data are different from our previous evidence, where TRPA1 agonists AITC and CA and TRPV1 agonist capsaicin produced hyperalgesia for nearly 2 h and resulted in facilitation of these withdrawal reflexes (Tsagareli et al., Neurophysiol., 2013, 45:329-39; Nozadze et al., Behav. Pharmacol., 2016, 27:29-36). Our results, thus, showing that NSAIDs suppress thermal and mechanical hyperalgesia following TRP activation could presumably due to inactivation or desensitization of TRPA1 and TRPV1 channels by NSAIDs.

Acknowledgement. Supported by the grant from Shota Rustaveli National Science Foundation of Georgia (#31/40).



Disclosures: M.G. Tsagareli: None. I. Nozadze: None. N. Tsiklauri: None. G. Gurtskaia: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.11/AA17

Topic: D.02. Somatosensation: Pain

Support: TWU Research Enhancement Grant

TWU Chancellor's Research Fellowship

Title: 17-beta-Estradiol enhances serotonergic neuromodulation of the transient receptor potential vanilloid 1 channel-expressing subpopulation of trigeminal sensory neurons

Authors: *S. LULLA, R. H. BESHER, W. L. BENTON, S. ANANTH, D. L. AVERITT; Biol., Texas Woman's Univ., Denton, TX

Abstract: Many pain conditions occur predominantly in women making pain a major women's health issue. The peripheral activity of the monoamine neurotransmitter serotonin or 5-Hydroxytryptamine (5HT) is implicated in many pain conditions more prevalent in women. 5HT can elicit pain via nociceptors that express the transient receptor potential vanilloid 1 channel (TRPV1), activated by noxious heat and capsaicin and results in the release of calcitonin gene-related peptide (CGRP). 5HT potentiates capsaicin-evoked calcium influx and CGRP release from male nociceptors. This pain mechanism may be under hormonal modulation as 5HT potentiates capsaicin-evoked CGRP release from female human nociceptors during the luteal phase of the menstrual cycle. In support, we recently reported that peripheral 5HT evokes greater pain behaviors in female rats during stages of the estrous cycle when gonadal hormones are fluctuating. Yet it remains unclear whether estrogen enhances or attenuates this serotonergic pain mechanism. We hypothesized that 17-β-estradiol (E2) enhances the potentiating effect of 5HT on the TRPV1-expressing population of cultured trigeminal sensory neurons. Sprague-Dawley female rats (~200g) were ovariectomized and rapidly decapitated 2 weeks later following hormone dissipation. Trigeminal ganglia (n=3 rats/6 ganglia per plate; performed in duplicate per condition) were extracted, dissociated with trypsin and collagenase, and neurons were grown on 24 well poly-D-lysine coated plates. Cultures were maintained for 5 days with media containing nerve growth factor. Cells were then washed and treated with HBSS for 15 minutes. Fractions were collected as basal release and cells were then pretreated with E2 (50 nM) or vehicle prior to treatment with E2 + 5HT (100 μM), E2 only, 5HT only, or vehicle followed by stimulation with capsaicin (50 nM). CGRP release was measured per fraction by ELISA and reported as percent basal release. Here we report that capsaicin excited and 5HT inhibited CGRP release from trigeminal nociceptors, while E2 alone had no effect. When trigeminal nociceptors were treated with both 5HT and E2, capsaicin-evoked CGRP release was significantly increased. These data indicate that estrogen may enhance the pain-provoking effects of 5HT on trigeminal nociceptors, which may provide one mechanism underlying the greater prevalence of pain disorders in women. Our current studies are aimed at analyzing the underlying anatomical substrate for E2 and 5HT activity on trigeminal nociceptors by co-labeling 5HT receptor mRNA, TRPV1 protein, and E2 receptors localized on female trigeminal sensory neurons.

Disclosures: S. Lulla: None. R.H. Besher: None. W.L. Benton: None. S. Ananth: None. D.L. Averitt: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.12/AA18

Topic: D.02. Somatosensation: Pain

Title: Behavioral characterization of CRISPR generated TRPA1 knockout rats

Authors: R. M. REESE, *S. D. SHIELDS, M. DOURADO, X. WU, E. SUTO, W. P. LEE, A. BALESTRINI, L. RIOL BLANCO, D. HACKOS;
Genentech, South San Francisco, CA

Abstract: The ion channel TRPA1 detects noxious chemical and environmental irritants as well as endogenous inflammatory factors, is present in primary nociceptors, and has been proposed to contribute to inflammatory pain, chemotherapy-induced peripheral neuropathy (CIPN), and asthma. To investigate the involvement of TRPA1 in these disorders and its potential as a therapeutic target, we generated a line of rats that harbors a null mutation in the gene encoding TRPA1 (TRPA1 KO rats) using CRISPR technology and characterized them in a battery of behavioral tests of neurological and sensory function and pain models. TRPA1 mRNA was undetectable in dorsal root ganglion of KO rats by RT-PCR and loss of functional TRPA1 channels was confirmed by calcium imaging. Neither male nor female TRPA1 KO rats showed significant difference from littermate controls in locomotor activity and neurological function exams; baseline sensitivity to noxious heat, mechanical, and cold stimulation was also normal. Additionally, TRPA1 KO rats showed no significant protection from mechanical and heat hypersensitivity in two separate models of inflammatory pain, Bradykinin and Complete Freund's Adjuvant. In two models of neuropathic pain, chronic constriction injury and Bortezomib-induced CIPN, no significant difference was detected in mechanical hypersensitivity between KO rats and controls. Notably, TRPA1 KO rats do not display nocifensive behavior after intraplantar injection of allylisothiocyanate (AITC), a TRPA1 agonist, but no difference from wildtype rats in display of nocifensive behavior after intraplantar injection of capsaicin, a TRPV1 agonist. Inhibition of AITC-induced behavior can be recapitulated pharmacologically with potent small molecule inhibitors, and we have developed this test as a reliable TRPA1 target engagement assay. TRPA1 is also essential for multiple mechanisms of action in asthma, including sensory neuron-triggered airway hyperresponsiveness and smooth muscle contraction. When challenged in the ovalbumin model of asthma TRPA1 KO rats have a striking reduction in eosinophils and neutrophils present in bronchoalveolar fluid compared to littermate controls. Thus, TRPA1 is an attractive target for the treatment of asthma and possibly certain types of pain. To the best of our knowledge, this is the first reported characterization of TRPA1 gene deletion in the rat.

Disclosures: **R.M. Reese:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **S.D. Shields:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **M. Dourado:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **X. Wu:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **E. Suto:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **W.P. Lee:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **A. Balestrini:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **L. Riol Blanco:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche. **D. Hackos:** A. Employment/Salary (full or part-time): Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roche.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.13/BB1

Topic: D.02. Somatosensation: Pain

Support: Kaete Klausner Scholarship

ISF Grant 1721/12

ISF Grant 1368/12

CIG Grant FP7-CIG-321899

Title: The pain receptor TRPV1 displays agonist-dependent activation stoichiometry

Authors: *A. HAZAN, R. KUMAR, H. MATZNER, A. PRIEL;
Pharmacol., The Hebrew Univ. of Jerusalem, Hadssah Ein Kerem, Israel

Abstract: The nociceptive pathway faces the unique challenge of detecting innumerable amounts of noxious stimuli descending onto a limited number of receptors. This creates polymodality, the ability of a single receptor to detect multiple different agonists, which can be attributed to the multiple binding sites all along the receptor sequence. One notable polymodal receptor, Transient Receptor Potential, Vanilloid 1 (TRPV1), was originally described as the heat and capsaicin detector. Comprised of four identical subunits that organize into a non-selective cationic permeable channel, this receptor is now known to have a variety of binding sites responsible for detecting their respective agonists such as protons, capsaicin, inflammatory mediators such as anandamide, peptide toxins and others. Although its physiological role as a chemosensor has been described in detail, TRPV1 stoichiometry of activation by its different ligands remains unknown. Here, we reconstructed the TRPV1 channel as a tetrameric concatemer harboring a set numbers of mutated binding sites in either the proton or vanilloid binding sites. In doing so, we were able to apply patch-clamp recordings in order to determine the stoichiometry for TRPV1 activation through the vanilloid binding site and the outer-pore domain by capsaicin and protons, respectively. We showed that, while a single capsaicin-bound subunit was sufficient to achieve a maximal open-channel lifetime, all four proton-binding sites were required. Thus, our results demonstrate a distinct stoichiometry of TRPV1 activation through two of its different agonist-binding domains.

Disclosures: A. Hazan: None. R. Kumar: None. H. Matzner: None. A. Priel: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.14/BB2

Topic: D.02. Somatosensation: Pain

Support: JSPS KAKENHI 23700470

JSPS KAKENHI 15K08673

Title: Citral enhances spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons by activating TRPA1 channels

Authors: L. ZHU, *T. FUJITA, C.-Y. JIANG, C. WANG, T. YU, R. HIRAO, R. SUZUKI, N. MAGORI, E. KUMAMOTO;
Saga Med. Sch., Saga, Japan

Abstract: Transient receptor potential (TRP) channels are involved in modulating nociceptive transmission in spinal dorsal horn lamina II (substantia gelatinosa; SG) neurons that play a pivotal role in regulating the transmission from the periphery. We have previously reported that the TRP channels are activated by various plant-derived chemicals such as capsaicin, allyl isothiocyanate, resiniferatoxin, piperine, eugenol, zingerone, carvacrol, thymol, (-)-carvone, (+)-carvone, 1,4-cineole and 1,8-cineole, resulting in an increase in the spontaneous release of L-glutamate onto SG neurons in spinal cord slices. Citral, which is abundantly contained in lemongrass, has various actions including spasmolysis, anti-inflammation, nerve conduction inhibition, antinociception and the activation of TRP vanilloid-1 (TRPV1), TRP vanilloid-3 (TRPV3), TRP ankyrin-1 (TRPA1) and TRP melastatin-8 (TRPM8) channels expressed in heterologous cells. It has not been examined yet how citral affects synaptic transmission. It is unknown what types of TRP channel in native neurons are activated by citral. With a focus on TRP activation, we examined the effect of citral on glutamatergic spontaneous excitatory transmission at a holding potential of -70 mV by applying the whole-cell patch-clamp technique to SG neurons of adult rat spinal cord slices. Citral superfused for 3 min increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) in a concentration-dependent manner (half-maximal effective concentration, EC_{50} = 0.58 mM) with a small increase in its amplitude. The sEPSC frequency increase produced by citral was repeated at a time interval of 30 min, albeit this action recovered with a slow time course after washout. The presynaptic effect of citral was inhibited by a TRPA1 antagonist HC-030031 (50 μM) but not by a voltage-gated Na^+-channel blocker tetrodotoxin (0.5 μM), a TRPV1 antagonist capsazepine (10 μM) and a TRPM8 antagonist BCTC (3 μM). In conclusion, as with many plant-derived chemicals, citral increased the spontaneous release of L-glutamate onto SG neurons by activating TRPA1 channels. Their efficacy sequence for the TRPA1 activation was thymol (EC_{50} = 0.18 mM) > citral (0.58 mM) ≥ carvacrol (0.69 mM) ≥ (+)-carvone (0.72 mM) > 1,8-cineole (3.2 mM) ≥ eugenol (3.8 mM). This result may serve to know the property of TRPA1 channels located in the central terminal of primary-afferent neuron in the SG. The citral activity as revealed in the present study could contribute to at least a part of its modulatory action on nociceptive transmission in the SG.

Disclosures: L. Zhu: None. T. Fujita: None. C. Jiang: None. C. Wang: None. T. Yu: None. R. Hirao: None. R. Suzuki: None. N. Magori: None. E. Kumamoto: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.15/BB3

Topic: D.02. Somatosensation: Pain

Support: JSPS grant 16K21691

Title: Identification of the molecular target of crotamiton, an anti-itch agent

Authors: *H. KITTAKA[1], M. TOMINAGA[1,2];
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Abstract: The sensation of itch is defined as "an unpleasant cutaneous sensation provoking a desire to scratch". For most people, itch sensation is not lethal, but it is clear that long-lasting itch can be felt torturous and decreases their quality of life. Crotamiton (*N*-ethyl-*o*-crotonotoluidide) was initially found as a scabicide and has been used as an anti-itch agent for human for more than 50 years. Regardless of the long use of crotamton, the mechanism of how crotamton acts as an itch relief remains unknown. We first compared chemical structure of crotamiton with other chemicals composing commercially available anti-itch ointments. As a result, we found that crotamiton is not like steroids, and it doesn't have antihistaminic, local anesthetic and anti-inflammatory effects. In addition, the chemical structure is not like natural compounds such as menthol and camphor, which are commonly used in ointments.
Therefore, we hypothesized that crotamiton could have effects on other targets including transient receptor potential (TRP) channels expressed in the peripheral sensory neurons and the skin. We focused on and screened TRPV (vanilloid) 1, TRPV2, TRPV3, TRPV4, TRPA (ankyrin) 1 and TRPM (melastatin) 8 because they are frequently reported to be involved in the itch sensation, and the involvement in crotamiton's action is not clear. We first examined if crotamiton activates or inhibits these TRP channels in a heterologous expression system using human embryonic kidney 293T cells. We found that crotamiton activated none of the TRP channels and strongly inhibited TRPV4 channel, suggesting that TRPV4 expressed in the peripheral sensory neurons and/or the skin is involved in itch sensation.
We also investigated the detailed mechanism about how crotamiton inhibits TRPV4, focusing on the washout currents from its inhibition, in which extremely large TRPV4 currents were observed. Comparing single-channel open probability and current amplitudes of TRPV4, increases in the both parameters were found to contribute to the washout currents. Because the change in current amplitudes suggested pore dilation of TRPV4, we examined the possibility with experiments of cation replacement and those observing shift of reversal potentials. We found that both larger cation influx and shift of reversal potentials upon crotamiton application to

the cells expressing TRPV4, suggesting the pore dilation of TRPV4 in its disinhibition state. From these results, we identified the molecular target of crotamiton, and moreover, we revealed pore dilation of TRPV4 upon washout of crotamiton. Our clarification of crotamiton effects on TRPV4 would be useful for the future understanding of itch sensation.

Disclosures: H. Kittaka: None. M. Tominaga: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.16/BB4

Topic: D.02. Somatosensation: Pain

Support: RO1NS087033

Title: Store-operated calcium channels are involved in the function of TRPV1 in dorsal root ganglion neurons

Authors: *D. WEI, H. HU;
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Abstract: Store-operated Ca^{2+} channels (SOCs) are highly Ca^{2+} selective cation channels that mediate Ca^{2+} entry in many different cell types. Our previous studies have shown that YM-58483 (a SOC inhibitor) attenuates thermal and capsaicin (an agonist of transient receptor potential vanilloid type 1, TRPV1)-induced pain. Additionally, it has been indicated that the function of SOCs in sensory neurons is enhanced under painful nerve injury, suggesting that SOCs may be involved in peripheral sensitization. However, it remains elusive how SOCs impact peripheral sensitization and influence the pain process. Here we demonstrate that the SOC family is expressed in dorsal root ganglia (DRG) neurons, and that both STIM1 and STIM2 are important in mediating SOC entry (SOCE) in mouse DRG neurons. While Orai1 is the only subunit mediating SOCE in most cell types, including cortical and spinal cord neurons, we strikingly found that Orai3 is also responsible for SOCE in DRG neurons. Interestingly, depletion of intracellular Ca^{2+} stores-induced SOCE was more robust in small diameter DRG neurons (< 34μm) and TRPV1 positive DRG neurons. TRPV1 is a non-selective cation channel considered to act as a transducer and molecular integrator of nociceptive stimuli in the periphery. It has been shown that activation of TRPV1 causes Ca^{2+} release from the ER in cell lines expressing TRPV1 and native rodent DRG neurons. To determine whether TRPV1-induced Ca^{2+} release from the ER can activate endogenous SOCs, we examined the effects of two SOC inhibitors, YM-58483 and Synta66, on capsaicin-induced Ca^{2+} influx. Our data show that YM-

58483 and Synta66 partially decreased the sustained Ca^{2+} entry, but not Ca^{2+} release. To confirm that SOCs are involved in the function of TRPV1, we tested TRPV1-induced Ca^{2+} responses in DRG neurons from Orai1-/- mice. We found that capsaicin-induced Ca^{2+} influx was significantly reduced in DRG neurons from Orai1-/- mice when compared to those from WT mice. More importantly, capsaicin-induced nociception was significantly attenuated in Orai1 -/- mice. Taken together, our novel findings reveal that activation of TRPV1 leads to SOC entry, suggesting SOCs may play an important role in the transmission and modulation of pain.

Disclosures: **D. Wei:** A. Employment/Salary (full or part-time): Drexel University College of Medicine. **H. Hu:** A. Employment/Salary (full or part-time): Drexel University College of Medicine.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.17/BB5

Topic: B.04. Ion Channels

Support: FONDECYT 11140731

PAI 79140059

Title: Expression and distribution of TRPM4 channels in mouse prefrontal cortex

Authors: *E. LEIVA-SALCEDO[1], C. VILLEGAS[2], H. VALENZUELA[2], D. RIQUELME[1]; [2]Dept. de Biologia, [1]Univ. de Santiago, Santiago, Chile

Abstract: TRPM4 is non-selective cation channel activated by intracellular Ca^{2+}, but impermeable to divalent cations. TRPM4 participates in several physiological and pathophysiological processes, such as neuronal bursting in preBötzinger nucleus, afterdepolarization currents in Purkinje neurons, dendritic depolarization in CA1 pyramidal neurons and ischemic neuronal death. TRPM4 is assembled as a tetramer with 6 transmembrane regions with the N and C-terminal in the intracellular side, with several sites of post-translational modification. Their sensitivity to Ca^{2+} is given by calmodulin through its 5 calmodulin binding sites. TRPM4 is expressed in numerous brain areas like mitral neurons in accessory olfactory bulb, in the hypothalamus in a subset of neurons in the supraoptic and paraventricular nuclei. Recently, *in situ* hybridization experiments demonstrated the expression of TRPM4 in the *stratum pyramidale* of hippocampal CA1 area. Moreover, the expression of TRPM4 prefrontal cortex has not been completely characterized and only their expression layer 5 has been reported.

Despite of this information, the detailed TRPM4 expression in the prefrontal cortex or the type of neurons expressing the channels or their distribution in neuronal subcompartments (axon, dendrites, and soma) has not been fully described. In this study we performed a immunohistochemical characterization of TRPM4 expression and different neuronal markers (Neurogranin, MAP2, GAD67, Ankyrin G, NeuN) in the medial prefrontal cortex of adult mice (4-8 weeks). We found that TRPM4 is widely expressed through all cortical layers of the prefrontal cortex, moreover, TRPM4 expression is located mainly in the somatodendritic region of the principal neurons. The expression pattern of TRPM4 in prefrontal regions could bring insight in the properties of the neurons and how they can contribute to different neuronal responses through the modulation of the intracellular Ca^{2+} and then to the neuronal excitability.

Disclosures: E. Leiva-Salcedo: None. C. Villegas: None. H. Valenzuela: None. D. Riquelme: None.

Poster

233. Transient Receptor Potential Channels: Regulation and Function

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 233.18/BB6

Topic: B.04. Ion Channels

Support: NIH MH082881

NSF #IIA-1355466

Title: Group I metabotropic glutamate receptors increase excitability of layer III entorhinal pyramidal neurons and changes in excitability involve the activation of a TRPC4/5-like current

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Abstract: The entorhinal cortex (EC) mediates the majority of the cortical input to the hippocampus and is a critical structure for both limbic function and memory. Axons from layer III EC pyramidal neurons form the temporoammonic pathway, which synapses onto region CA1, and these EC neurons are modulated by metabotropic receptors for glutamate. We chose to focus on the neuromodulatory role of group I receptors on these EC neurons. Bath application of the group I agonist DHPG (10 μM) resulted in a significant increase in the action potential firing frequency of layer III EC pyramidal neurons. The calculated EC_{50} for this response was 1.74 μM. Pretreatment and continuous bath application of either the mGluR1 antagonist LY367385 or mGluR5 antagonist MPEP significantly reduced, but did not block, DHPG-induced increased AP

firing. Only in the presence of both antagonists was DHPG-induced increased action potential firing blocked, suggesting the functional presence of both receptors in the EC. Western blot analysis of EC lysates confirmed protein expression for both group I receptors in the EC. In addition to increased action potential firing, DHPG-induced increased excitability was also evidenced by a significant depolarization of the resting membrane potential by about ~4.5 mV and the generation of an inward current of about ~-25 pA. We investigated the ionic mechanism mediating this increased subthreshold excitability by measuring changes in holding current at -60 mV under various ion substitution conditions. Replacement of intracellular K^+ with Cs^+ failed to significantly reduce DHPG-induced inward currents. Reduction of extracellular Na^+ but not Ca^{2+} significantly reduced DHPG-induced currents, suggesting a non-selective cation channel (NSCC) is involved. Consistent with involvement of NSCC, inward currents were sensitive to flufenamic acid, extracellular but not intracellular 2-APB, and were potentiated by extracellular La^{3+} and Gd^{3+}. The selective TRPC4/5 blocker ML204 significantly reduced DHPG-induced inward currents. These currents were significantly reduced in the presence of PLC inhibitors U73122 or edelfosine but remained unaffected by the PKC inhibitor GF109203X. These results suggest that DHPG acting via group I receptors increased excitability of EC neurons and that PLC but not PKC was required for the activation of a TRPC4/5 like current, which may underlie the DHPG-induced increased excitability.

Disclosures: N.I. Cilz: None. B. Hu: None. S. Lei: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.01/BB7

Topic: D.02. Somatosensation: Pain

Title: Peripheral nerve injury-induced synaptic rearrangement in the spinal dorsal horn revealed by cross-correlation analysis

Authors: T. ASAKAWA[1], Y. TAKEMURA[1], T. FUJIWARA[2], K. AKAGAWA[2], *Y. HORI[1]; [1]Physiol. and Biol. Information, Dokkyo Univ. Sch. Med., Tochigi, Japan; [2]Cell Physiol., Kyorin Univ. Sch. Med., Tokyo, Japan

Abstract: Syntaxin 1A (STX1A) is a membrane protein that is involved in exocytosis and membrane trafficking. It has been reported that the knockdown of STX1A results in axonal elongation and sprouting of cultured central neurons, including spinal dorsal horn neurons. Additionally, we have previously shown that partial sciatic nerve ligation (PSNL) decreased the expression of STX1A and increased the number of synapses on neurons in the spinal dorsal horn

(DH). Based on these observations, it is suggested that PSNL-induced downregulation of STX1A results in rearrangement of the synaptic connection between neurons in the DH. In order to examine this hypothesis, we evaluated the effects of PSNL on the functional connectivity between neurons in the DH by means of cross-correlation analysis. Furthermore, comparison was made between STX1A knockout (KO) mice and wild-type (WT) littermate control mice. Experiments were performed on 6-7-week-old mice. PSNL was performed under halothane anesthesia and the development of mechanical allodynia was accessed by von Frey filaments. Under ketamine/xylazine anesthesia, the lumbar spinal cord was removed and transverse slices (400 μm in thickness) were made. The slices were placed into a recording chamber with multi-electrode array (Multi Channel Systems, Germany). Electrodes were arranged 30-200 μm apart in an 8x8 pattern. The signals from the MEA electrodes were sampled at 25 kHz. Cross-correlograms between spontaneous spike trains of simultaneously recorded neurons in the DH were constructed off-line using DataView (Heitler, 2009) and Matlab R2010b. The observed pattern of cross-correlograms included a flat histogram (which indicates no synaptic connection between neurons), a histogram with a central peak (common excitatory synaptic input to neurons), a histogram with a lagged peak (monosynaptic excitatory interaction between neurons), and a histogram with a lagged trough (monosynaptic inhibitory interaction between neurons). The incidence of histograms with a central peak, lagged peak and lagged trough was increased in mice subjected to PSNL. Additionally, the effects of PSNL on the patterns of cross-correlograms were more prominent in STX1A KO mice compared to WT control mice. The present observations seem to indicate that neurons in the DH make excitatory and/or inhibitory synapses on the nearby neurons, and that synaptic connections among neurons in the DH might change significantly after peripheral nerve injury. Furthermore, such synaptic rearrangement is at least partly attributed to the function of STX1A.

Disclosures: T. Asakawa: None. Y. Takemura: None. T. Fujiwara: None. K. Akagawa: None. Y. Hori: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.02/BB8

Topic: D.02. Somatosensation: Pain

Support: Medical Research Council UK Grant MR/K022636/1

Title: Long-term effects of neonatal surgical injury in rats: sexual dimorphism in response to neonatal microglial inhibition

Authors: *O. MORIARTY[1,2], Y. TU[3], M. W. SALTER[3], S. BEGGS[1,2], S. M. WALKER[1,2]; [1]Univ. Col. London, London, United Kingdom; [2]UCL Inst. of Child Hlth., London, United Kingdom; [3]The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Adult incision-related hyperalgesia is enhanced following neonatal incision, with associated increased spinal microglial reactivity. As neuroimmune responses are sexually dimorphic in adult rodents, we investigated effects of microglial inhibition with minocycline at the time of neonatal injury, and evaluated sex-dependent effects on adult re-incision hyperalgesia. We also investigated the somatotopic extent of neonatal incision-induced hyperalgesia.

Male and female rat pups underwent left plantar hindpaw incision on postnatal day 3 (P3) with perioperative saline or minocycline (45mg/kg 30 min pre-incision, 22.5mg/kg 24h and 48h post incision). Following re-incision of the same hindpaw in adulthood, we evaluated spinal reflex sensitivity (thermal/mechanical withdrawal thresholds, EMG). Acute microglial reactivity (Iba1) and cell death were assessed in neonatal lumbar spinal cord. To investigate the anatomical extent of enhanced hyperalgesia following neonatal injury, male neonatal rat pups underwent incision of left or right hind or forepaws, or left thigh. As adults, all rats underwent left hindpaw incision, and spinal reflex sensitivity was evaluated. All procedures were carried out under licence during isoflurane-in-oxygen anaesthesia, and investigators were blinded to treatment groups.

Mechanical hyperalgesia was enhanced in rats with prior neonatal incision (hyperalgesic index, single incision + saline vs. repeat incision + saline: male 234 ± 15 vs. 435 ± 22, $p<0.001$; female 264 ± 9 vs. 378 ± 16, $p<0.001$). Neonatal minocycline attenuated this repeat incision-induced increase in male but not female rats (repeat incision + minocycline: male 286 ± 18, $p<0.01$ vs. repeat incision + saline; female 371 ± 33, not significant). The same pattern was evident for thermal hyperalgesia and EMG responses. Minocycline did not alter acute hyperalgesia following neonatal incision (P3-6), but attenuated the incision-induced increase in Iba1 in the medial ipsilateral lumbar dorsal horn only in male rats (P6). Neonatal incision increased spinal neuronal cell death in both sexes, and was not altered by minocycline treatment. Enhanced hyperalgesia following adult incision varied with location of neonatal incision, being greatest with neonatal incision at the same site, and least with neonatal incision of the contralateral forepaw.

Pain during critical neonatal periods is associated with long-term functional consequences. Our results extend previous findings of sexually dimorphic microglia-neuronal signalling. Neonatal minocycline treatment modulates adult injury response in male but not female rats.

Disclosures: O. Moriarty: None. Y. Tu: None. M.W. Salter: None. S. Beggs: None. S.M. Walker: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.03/BB9

Topic: D.02. Somatosensation: Pain

Support: JSPS KAKENHI Grant Number 16K20083

Title: Plasticity and circuitry of spinal pathways after peripheral nerve injury: An *In vivo* patch-clamp recording.

Authors: *M. KURABE, H. BABA, T. KOHNO;
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Abstract: Background: Plasticity of excitatory and inhibitory transmission in the spinal dorsal horn (SDH) is thought to be a key mechanism in neuropathic pain. Several studies have shown that these mechanisms are associated with nociceptor sensitization, ectopic firing of primary afferent and with changes in the molecular expression of ion channels, neurotransmitters and receptors in the nociceptive axons. Moreover, these changes may involve the dysregulation of the inhibitory neurons in the SDH and the descending modulatory pathways. Despite the expectation of synaptic plasticity in the SDH, *in vivo* patch-clamp studies to determine whether functional synaptic plasticity is involved in the SDH in neuropathic pain mechanisms have not been reported. We evaluated the plasticity by using *in vivo* patch-clamp technique in SDH neurons and recorded excitatory postsynaptic currents (EPSCs), inhibitory postsynaptic currents (IPSCs), and evoked EPSCs by noxious or innoxious stimuli in chronic constriction injury (CCI) rats.
Results: In the voltage clamp mode, both frequency and amplitude of spontaneous EPSCs from CCI rats significantly increased compared to naïve. In contrast, neither the frequency nor the amplitude of spontaneous IPSC from CCI rats changed compared to naïve. We next observed evoked EPSCs induced by direct peripheral noxious or innoxioius stimuli to rat hindlimb. We classified these neurons in three types; response to only noxious stimuli, response to only innoxious stimuli and response to both noxious and innoxious stimuli. In the CCI model, the neurons in response to only innoxious stimuli decreased and the neuron in response to both stimuli significantly increased.
Conclusions: In this study, we showed that enhancement of excitatory inputs and no change of inhibitory inputs to SDH in the CCI model. By using *in vivo* patch-clamp technique, we could compare their properties and demonstrate changes in properties of SDH neurons in the CCI model. Our results suggest that changes of property of SDH neurons contribute to behavioral hyperalgesia/allodynia after peripheral nerve injury.

Disclosures: M. Kurabe: None. H. Baba: None. T. Kohno: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.04/BB10

Topic: D.02. Somatosensation: Pain

Support: Craig H. Neilsen Foundation Grant 214980

NIH Grant 1U18EB021760

MiBrain Initiative Seed Fund (University of Michigan)

Title: Quantitative analysis of spatial distribution of cell bodies in feline dorsal root ganglia

Authors: *Z. J. SPERRY[1,2,3], A. K. OSTROWSKI[1,3], G. E. KULIK[1,3], T. M. BRUNS[1,2,3]; [2]Biointerfaces Inst., [3]Biomed. Engin., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: A neural interface with dorsal root ganglia (DRG) could be beneficial both for stimulating and recording peripheral neural activity. An ideal electrode interface for DRG should account for the specific spatial distribution of neural tissue. Although this distribution has previously been described qualitatively, no quantitative analysis has been performed. To accomplish this, we used light microscopy to image transverse cross-sections of feline sacral-level DRG stained with hematoxylin and eosin. These images were oriented dorsal-ventrally, and the locations of cell bodies manually labeled. Using a custom MATLAB program, the images were transformed to normalized polar coordinates so the cross sections could be directly compared. The normalized images were split into 10,000 annular sectors, and cell distribution was modeled as the proportion of each sector which consisted of cell body pixels. JMP statistical software was used to partition the sectors for greatest differences in both the radial and angular dimension. Angular dimension was defined from dorsal to ventral (0° to 180°), ignoring left-right orientation. The partition model suggested that the greatest proportion of cells was to be found in the outer 24% of the DRG, with a vertical angle of less than 81°, that is, mostly on the dorsal side. The mean proportion of cells in this region was found to be 36%, as compared to 18% for all sectors combined. ANOVA and Tukey HSD test confirmed that for the four regions produced by this split (i.e. inner-dorsal, outer-dorsal, inner-ventral, outer-ventral), the outer-dorsal region had a significantly higher proportion of cell bodies than all but the outer-ventral, with $p < 0.02$ (proportion was non-significantly higher for outer-dorsal than for outer-ventral, $p = 0.08$). These results demonstrate that the distribution of cell bodies in DRG is non-uniform and concentrated on the dorsal surface. The methodology for normalization is potentially useful for other quantitative anatomical studies involving somewhat round perimeters (ex. bone, vasculature, intracellular environments). Future work will involve analyzing additional feline

DRG from other spinal levels, progressing from 2D to 3D distributions, and analyzing human DRG.

Disclosures: Z.J. Sperry: None. A.K. Ostrowski: None. G.E. Kulik: None. T.M. Bruns: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.05/BB11

Topic: D.02. Somatosensation: Pain

Support: KAKEN 16K18885

Title: Activation of spinal dorsal horn astrocytes modulates the processing of tactile sensation

Authors: ***Y. KOHRO**[1], R. KATSURAGI[1], T. OKA[1], S. MUNETA[1], R. TASHIMA[1], H. TOZAKI-SAITOH[1], K. INOUE[2], M. TSUDA[1];
[1]Dept. of Life Innovation, Grad. Sch. of Pharmaceut. Sci., [2]Dept. of Mol. and Syst. Pharmacology, Grad. Sch. of Pharmaceut. Sci., Kyushu Univ., Fukuoka, Japan

Abstract: Spinal dorsal horn (SDH) astrocytes have an important role in several chronic diseases like neuropathic pain. Accumulating evidence indicates that SDH astrocytes become reactive states in chronic pain conditions, which is crucial for pathology. However, under normal conditions, little is known about how much SDH astrocytes actively influence sensory information processing *in vivo*. The aim of the present study was to establish the selective manipulation of activity of SDH astrocytes *in vivo* and to clarify their role in the processing of sensory information. Recently, several studies have shown that intracellular Ca^{2+} elevation is crucial factor for astrocytic activity change, we used Gq-DREADD technology for manipulating intracellular Ca^{2+} level *in vivo*. For selective introduction of Gq-DREADD into SDH astrocytes, we used *Gfap-Cre* mice in combination with our established microinjection technique of adeno-associated virus into the mouse SDH. We found that activation of Gq-DREADD expressed in astrocytes induced transient intracellular Ca^{2+} increases in acute spinal cord slices. Gq-DREADD stimulation *in vivo* induced transient tactile allodynia (pain hypersensitivity to innocuous stimuli) without producing spontaneous pain behaviors or affecting sensitivities to other noxious stimuli. Moreover, Gq-DREADD-induced tactile allodynia was suppressed by pre-treatment with antagonists for NMDA but not AMPA or P2 receptors. Furthermore, a pharmacological blockade of Aβ-fibers inhibited Gq-DREADD-induced tactile allodynia, and electrical stimulation of Aβ-fibers under Gq-DREADD activation increased the number of c-Fos-positive neuron in the superficial layers of SDH. Together, our findings imply that activation of SDH astrocytes under a

normal condition is sufficient to convert innocuous mechanical information to pain and propose a control of modality-specific sensory information processing by SDH astrocytes.

Disclosures: Y. Kohro: None. R. Katsuragi: None. T. Oka: None. S. Muneta: None. R. Tashima: None. H. Tozaki-Saitoh: None. K. Inoue: None. M. Tsuda: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.06/BB12

Topic: D.02. Somatosensation: Pain

Support: VA Merit Award I01-RX000378

VA Merit Award I01-RX001646

NIH Grant R01-DA033059

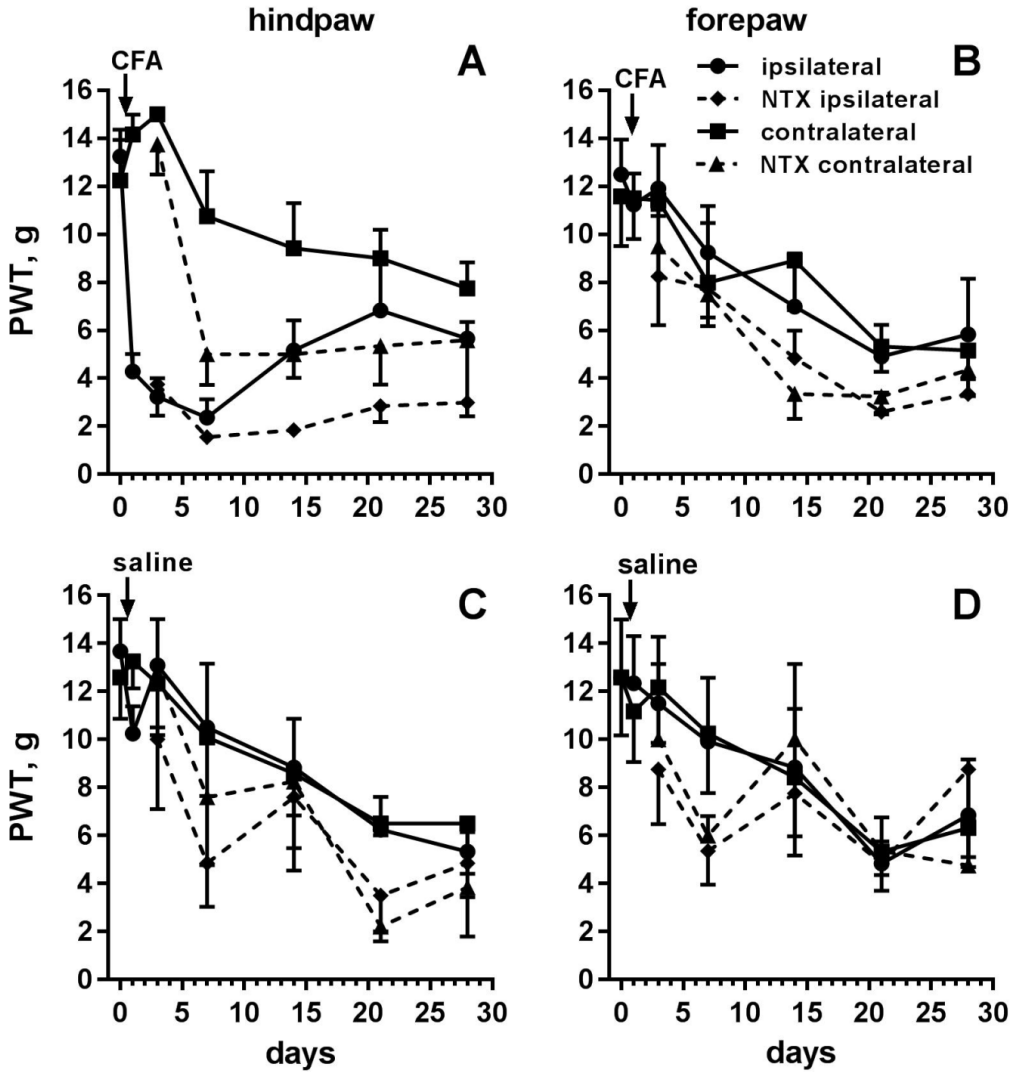
Title: Latent Sensitization: contralateral hyperalgesia and its suppression by mu-opioid receptors develop simultaneously and involve descending pathways

Authors: *J. G. MARVIZON[1], W. CHEN[1], W. M. WALWYN[2];
[1]Dept. of Med., UCLA / VA GLA HS, Los Angeles, CA; [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Latent Sensitization (LS) is new paradigm that proposes that chronic pain is a departure from normal pain transmission characterized by an enduring state of hyperalgesia that is suppressed by the continuous activation of opioid receptors. Although the injury that induces LS produces hyperalgesia ipsilaterally, blockade of mu-opioid receptor (MOR) constitutive activity by the inverse agonist naltrexone (NTX) reinstates the hyperalgesia bilaterally (Walwyn et al., 2016, J. Neurosci. 36:204). This suggests the involvement of descending pain modulatory pathways in both hyperalgesia and its suppression.
First, we determined when hyperalgesia appears in the contralateral hindpaw and whether it develops in the forepaws. Rats were injected with complete Freund's adjuvant (CFA, 50 µl; saline for controls) in one hindpaw and then were injected with NTX (1 mg/kg s.c.) on days 3, 7, 14, 21 and 28. At each of those days, responses of all four paws to von Frey filaments were measured before NTX and 15, 30, 60 and 120 min after NTX. Unexpectedly, the repeated injections of NTX caused progressive hyperalgesia in all paws of the CFA-injected and control rats. In the contralateral paw of the CFA-injected rats (panel A), NTX induced robust acute (at 15 min) hyperalgesia starting at day 7. In the ipsilateral paw, CFA induced hyperalgesia that

started subsiding at day 14 but was reinstated by NTX (panel A). Reinstatement by NTX was absent or small in the forepaws (panel B) of the CFA-injected rats or in any paw of the control rats (panels C, D). Therefore, in LS hyperalgesia and its suppression are bilateral but segmental, and appear in the first week after injury.

Second, we determined whether descending pain modulatory pathways are involved in LS. We used a cervical block of descending signals consisting of intrathecal lidocaine (10%, 1 µl) at the cervical level (C8-T1). Rats received 50 µl CFA in one hindpaw and von Frey responses were monitored until return to baseline. Intrathecal lidocaine, but not saline, reinstated hyperalgesia. Therefore, a descending pathway is involved in suppression of hyperalgesia in LS.



Disclosures: J.G. Marvizon: None. W. Chen: None. W.M. Walwyn: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.07/BB13

Topic: D.02. Somatosensation: Pain

Support: ARC CE140100003

Title: Does the allodynic spinal cord have a specific colour?

Authors: *M. R. HUTCHINSON[1,2], V. STAIKOPOULOS[1,2], M. GOSNELL[4,3], A. ANWAR[5,3], S. MUSTAFA[1,2], E. GOLDYS[5,3];
[1]Univ. Adelaide, Adelaide, Australia; [2]Australian Res. Council Ctr. of Excellence for Nanoscale BioPhotonics, Adelaide, Australia; [3]Australian Res. Council Ctr. of Excellence for Nanoscale BioPhotonics, Sydney, Australia; [4]Quantitative (Biotechnology) Pty Ltd, Sydney, Australia; [5]Macquarie Univ., Sydney, Australia

Abstract: The endogenous autofluorescence of biological samples, is generated by endogenous fluorescent molecules within the tissue. This background “colour” is usually the bane of immunofluorescence based image acquisition, as this can interfere with distinguishing the specific antibody tagged fluorescent signal. However, employing hyperspectral imaging of this endogenous tissue “colour” by using spectral and spatial imaging information, can provide novel target detection and classification at nanoscale level. For example, hyperspectral imaging can be used as label free method to resolve changes in endogenous fluorescent molecules such as flavins, bound and free NADH and retinoids that are involved in cell metabolism. Moreover, unguided phenotyping of spectral patterns can be employed to provide novel “fingerprinting” of specific disease states. Here we employed postmortem hyperspectral imaging of mouse brain and spinal cord in a graded model of chronic constriction injury induced allodynia to identify novel spectral features that changed proportional to the extent of allodynia. By using an endogenous source of contrast, subtle metabolic variations were detected within the somatosensory regions examined, making it possible to distinguish between animals from allodynic and non-allodynic groups. Data will be presented exploring the neuroanatomical location of the allodynia correlated features. The impact of sex on these features will also be explored.

Disclosures: **M.R. Hutchinson:** None. **V. Staikopoulos:** None. **M. Gosnell:** A. Employment/Salary (full or part-time): Quantitative (Biotechnology) Pty Ltd. **A. Anwar:** None. **S. Mustafa:** None. **E. Goldys:** None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.08/BB14

Topic: D.02. Somatosensation: Pain

Support: 5R01NS081707

Title: Flexible wireless NFC-powered optoelectronic devices for long term spinal and peripheral optogenetics

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Abstract: Optogenetic approaches have revolutionized the field of neuroscience by enabling temporal and spatial control of neuronal function. Recent technological advances have enabled implementation of optogenetics in freely moving, untethered animals. However, challenges in device durability, together with substantial expenses associated with implementation of these tools have limited their use, particularly for long-term implementation. Near-field wireless communication (NFC) technology offers unprecedented opportunities to deploy miniaturized implantable systems that offer long-term durability for spinal and peripheral applications. Here, we present durable wireless NFC-powered optoelectronics with robust, chronic stability for long-term operation for use in spinal and peripheral systems. Our devices support the use of various colors of μLEDs, allowing for wireless stimulation and inhibition of spinal and peripheral sensory circuits, with sustained operation in animal over periods of several weeks, and without the need for attachment to bony anchor points. Proof-of-concept experiments demonstrate robust operation in chronically implanted mice for the study of spinal and peripheral pain circuitry using excitatory and inhibitory opsins. The low-cost electronics required for control of the systems and durability of these wireless devices will allow broad application in future studies of spinal circuits, as well as various peripheral targets.

Disclosures: **M. Pullen-Colon:** None. **V.K. Samineni:** None. **A.D. Mickle:** None. **J. Yoon:** None. **Y. Jeong:** None. **J.G. Grajales-Reyes:** None. **J.P. Golden:** None. **K. McKenzie:** None. **G. Shin:** None. **J.A. Rogers:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroLux. **R.W. Gereau:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroLux.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.09/BB15

Topic: D.02. Somatosensation: Pain

Support: 631000

1043933

Title: Channel Rhodopsin assisted circuit mapping of calretinin neurons in the superficial dorsal horn

Authors: *K. M. SMITH[1], D. I. HUGHES[2], P. JOBLING[1], R. J. CALLISTER[1], C. V. DAYAS[1], B. A. GRAHAM[1];
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Abstract: The spinal dorsal horn (SDH) is a key region in the processing of sensory information, including nociception and pain. Within the SDH substantial neuron diversity makes identification and subsequent analysis of discrete neuron populations difficult. In the current study we have used channel rhodopsin-2 (ChR2) assisted circuit mapping to identify and study the connectivity of a population of dorsal horn interneurons expressing the calcium binding protein calretinin (CR). Despite CR being generally used as a marker of excitatory interneurons, we previously identified both excitatory (90%) and inhibitory (10%) populations of CR positive neurons (Smith et al, 2015). Transgenic mice (n=20) expressing ChR2 in CR positive neurons were anaesthetised (ketamine 100mg/kg) and parasagittal spinal cord slices were obtained (200μm). Targeted whole cell patch clamp recordings were made from identified CR positive, CR control and putative lamina I neurons. Local circuit connectivity was assessed in slices using brief photostimulation (1ms, 490nm) every 10 seconds. Photostimulation-evoked excitatory inputs (CNQX sensitive) were observed in 27/33 recordings from CR positive neurons and 16/18 CR negative neurons. Furthermore, photostimulation also evoked excitatory input to unidentified lamina I neurons (9/9). Under inhibitory recording conditions (CsCl-based internal) photstimulation evoked inhibitory input in 8/9 CR positive and 11/16 CR negative neurons. These connections were predominantly bicuculline sensitive suggesting GABAergic inhibition dominates in CR circuits. Finally, to assess the function of CR positive neurons in the intact nervous system we developed techniques to photostimulate CR neurons in freely behaving mice. Briefly, CRChR2 animals are anaesthetised and paraspinal musculature is retracted from the T12 and T13 vertebra. A fibre optic probe was secured between these vertebra and animals were allowed to recover for 1 week. Photostimulation (10s, 10mW @ 10Hz) of CRChR2 neurons evokes reliable and reproducible nocifensive behavioural responses directed to the ipsilateral hindpaw (n =10). This is consistent with our in vitro data showing CR neuron interconnectivity

and connections with laminae I. Together these results support an important role for CR positive interneurons in spinal nociceptive signalling. The high level of connectivity between excitatory CR neurons places them in an ideal position to amplify incoming sensory information before relay to higher brain regions.

Disclosures: K.M. Smith: None. D.I. Hughes: None. P. Jobling: None. R.J. Callister: None. C.V. Dayas: None. B.A. Graham: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.10/BB16

Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01NS086372

NIH Grant R01DE018025

NIH Grant P01 NS072040

Title: Genetic dissection of spinal circuits processing different forms of sensory modalities

Authors: *T. HUANG[1], B. DUAN[1], L. CHENG[1], Y. ZHANG[1], Y. ZHANG[1], M. GOULDING[2], Q. MA[1];
[1]Dana-Farber Cancer Institute, Harvard Med. Scho, Boston, MA; [2]Salk Inst., La Jolla, CA

Abstract: The goal of this study is to identify spinal neurons that process and transmit specific sensory modalities. In the past decade and half, our lab and other investigators have characterized the developmental origins of a large cohort of spinal excitatory and inhibitory neurons. Based on this knowledge and in collaboation with Dr. Martyn Goulding and his colleagues at the Salk Insitute, we have jointly developed an intersectional genetic strategy to mark and ablate these molecularly defined excitatory or inhibitory neurons. Subsequent behavioral and electrophysiological studies have identified excitatory neurons necessary for the transmission of different sensory modalities. Spinal neurons marked by the expression of the neuropeptide somatostatin (SOM) represent local excitatory interneurons enriched in laminae II/III. Mice with ablation of spinal SOM neurons fail to sense 1) light punctate stilumi evoked by von Frey filaments, 2) intense mechanical stimuli evoked by pinprick or pinch, or 3) itch evoked by lightest mechanical stimuli, without affecting thermal responses. Additionally, these mice fail to generate both filament-evoked static and brush-evoked dynamic forms of mechanical allodynia (pain by low theshold mechanical stimuli) induced by nerve lesions or inflammation.

Other molecularly defined spinal excitatory neurons are requried to transmit more specific forms of sensory information. Firstly, spinal neurons marked by calretin-Cre are requried to transmit light punctate stimuli, but dispensable for acute intense mechanical pain or chronic allodynia. Secondly, spinal neurons marked by Vglut3-Cre (a knock-in Cre that marks neurons not identifical to those marked by the transgenic *Vglut3-Cre* used by the Seal group) transmit both acute light punctate mechanical information and dynamic allodynia, but not acute intense mechanical pain or chronic static allodynia. Finally, our preliminary analyses have suggested the existence of another group of spinal excitatory neurons required selectively to sense acute intense mechanical pain. These studies suggest distinct spinal substrates for the transmission of different sensory modalities.

Disclosures: T. Huang: None. B. Duan: None. L. Cheng: None. Y. Zhang: None. Y. Zhang: None. M. Goulding: None. Q. Ma: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.11/BB17

Topic: D.02. Somatosensation: Pain

Title: NMDA receptors and large conductance calcium-activated potassium channels in enkephalinergic neurons in mouse spinal superficial dorsal horn

Authors: *E. KATO, Y. TAKEMURA, Y. HORI;
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Abstract: Enkephalins (Enks) are endogenous opioid pentapeptides derived from the preproenkephalin (Penk) precursor. Enks are involved in the modulation of nociceptive synaptic transmission in the central nervous system, including the superficial dorsal horn (SDH) of the spinal cord. We characterized Enk-containing neurons in the SDH of transgenic mice that expresses GFP fluorescence under the control of the *Penk* gene promoter (BMC Neurosci, 2011). The present experiments investigated the pharmacological properties of Enk-containing neurons in an attempt to further understand how the activity of Enk-containing neurons is regulated in the SDH.
The experiments were performed on 5- to 8-week-old male ICR mice. The spinal cord was removed under ketamine/xylazine anesthesia. Transverse slices of 350 μm in thickness were prepared at the lumbar enlargement. Tight-seal whole-cell recordings were obtained from neurons localized in the SDH under infrared differential interference contrast (IR-DIC) microscopy. L-glutamate (Glu, 200 μM) was dissolved in Kreb's solution and was applied by

pressure ejection through a glass pipette placed near the recorded neurons. All recordings were made in the presence of strychnine and bicuculline. After the electrophysiological experiments, the recorded neurons were aspirated into a collecting glass pipette. Then, single cell real-time RT-PCR was performed to analyze the expression profile of the *Penk* mRNA, the large-conductance calcium-activated potassium (BK) channel alpha subunit (*Kcnma1*) of mRNA, and the NMDA receptor subunits 1 (*Grin1*), 2A (*Grin2a*) and 2B (*Grin2b*) mRNAs in single SDH neurons.
Puff application of Glu evoked an inward current at a holding potential of -70 mV, which was abolished by bath application of CNQX (an antagonist against a non-MNDA receptor). When holding potential was depolarized, Glu evoked an outward current of long duration, which follows the inward current. Adding APV (an NMDA receptor antagonist) to the perfusate abolished the outward current. The outward current was also abolished by TEA (a potassium channel antagonist) and iberiotoxin (a selective BK channel antagonist). Single cell real-time RT-PCR analysis revealed that
single SDH neurons expressing the *Penk* mRNA also expressed the *Kcnma1*, *Grin1*, *Grin2a* and *Grin2b* mRNAs.
These results might suggest that calcium ion influx through NMDA receptors activates BK channels and hyperpolarizes Enk-containing SDH neurons, which might be involved in the regulation of the release of enkephalin in the SDH.

Disclosures: E. Kato: None. Y. Takemura: None. Y. Hori: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.12/BB18

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS080889

Title: PLA_2-prostaglandin signaling mediates spike timing-dependent LTP at primary afferent synapses onto spinal projection neurons

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Abstract: Recent evidence suggests that spike timing-dependent long-term potentiation (t-LTP) at primary afferent synapses onto spinal projection neurons involves an increase in the probability of glutamate release (P_r). Chelation of intracellular Ca^{2+} within projection neurons

prevented both the induction of t-LTP and the accompanying changes in P_r following highly correlated presynaptic and postsynaptic activity, suggesting that the release of a retrograde messenger contributes to t-LTP at these synapses. However, the nature of these retrograde signals remains unclear. The present study addressed this issue using in vitro patch clamp recordings from identified lamina I spino-parabrachial neurons in the adult mouse spinal cord. As reported previously, Pre → Post pairings at an interval of 10 ms (Δt = -10 ms) consistently produced t-LTP of monosynaptic, primary afferent-evoked EPSCs in ascending projection neurons. The application of the nitric oxide (NO) scavenger cPTIO during the pairing protocol failed to prevent the induction of t-LTP, arguing against a critical role for NO in regulating spike timing-dependent plasticity (STDP) at sensory synapses onto spinal projection neurons. In contrast, the administration of the phospholipase A_2 (PLA_2) inhibitor $AACOCF_3$ during the pairing protocol abolished the generation of t-LTP. Since it is known that PLA_2 can regulate synaptic efficacy via the downstream synthesis of prostaglandins such as PGE_2, and that PGE_2 can function as a retrograde messenger at CNS synapses, we next investigated a potential role for prostaglandin receptors in the generation of t-LTP in the superficial dorsal horn (SDH). Importantly, bath application of the selective EP2 receptor antagonist PF 04418948 also suppressed t-LTP at afferent synapses onto mature projection neurons. Collectively, these results demonstrate that PLA_2-prostaglandin signaling modulates activity-dependent synaptic plasticity in the key output neurons of the SDH network and thereby shapes the ascending flow of nociceptive information to the brain.

Disclosures: J. Li: None. M.L. Baccei: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.13/CC1

Topic: D.02. Somatosensation: Pain

Support: NIH Grant 1ZIADE000721-09

Title: A role for somatostatin in itch

Authors: J. HUANG[1], J. SOLINSKI[1], M. KRIEGBAUM[1], P.-Y. TSENG[1], *M. HOON[2]; [1]NIDCR, NIH, Bethesda, MD; [2]NIDCR, Bethesda, MD

Abstract: Itch is an unpleasant sensation that in chronic forms severely affects the quality of life of patients. However, the mechanisms which control itch sensation are still poorly understood. Here we examined the contribution of the neuropeptide somatostatin in modulating this sense in

mice. We find that somatostatin is expressed by a select population of DRG neurons and as has been reported previously is expressed by a large number of dorsal horn spinal cord neurons. The somatostatin positive DRG neurons exclusively co-express several molecular markers of itch and these neurons specifically innervate outer layers of the skin with free nerve fibers. These neurons also project afferent fibers to superficial lamina in the spinal cord. Chemogenetic and pharmacological investigation of the pathways that utilize somatostatin demonstrate that it activates specific targets in the itch sensory circuit in the spinal cord. Conditional elimination of somatostatin in the DRG, spinal cord, or both DRG and spinal cord establish that it is an important player in modulating pruritic responses.

Disclosures: J. Huang: None. J. Solinski: None. M. Kriegbaum: None. P. Tseng: None. M. Hoon: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.14/CC2

Topic: D.02. Somatosensation: Pain

Support: NHMRC Grant 631000

NHMRC Grant 1043933

Title: Characterisation of a novel population of spinal superficial dorsal horn neurons in the Thy1-GFP mice

Authors: *B. A. GRAHAM[1], K. M. SMITH[2], M. A. GRADWELL[1], R. J. CALLISTER[1], D. I. HUGHES[3], F. R. WALKER[1];
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Abstract: The superficial dorsal horn (SDH) of the spinal cord plays a critical role in processing and relaying sensory information from the periphery to higher brain centres. Signals carrying nociceptive, thermal, itch and light touch related information terminate in the SDH, and are processed by a heterogeneous population of neurons before they are relayed along the neuroaxis to finally shape perception. Several groups have undertaken the task of dissecting heterogeneity in this region by analyzing transgenic mice that express green fluorescent protein (GFP) in discrete SDH neuron subpopulations. In this study we report on a small population of SDH

neurons expressing GFP controlled by a neural-specific promoter for a protein referred to as Thy1. These mice have been used widely in neuroscience because they selectively label projection neurons in the neocortex, hippocampus and other brain regions. Previous work has reported that GFP positive neurons also exist in the SDH of Thy1GFP mouse (Porrero 2010 Brain Res 1345:59) and therefore we have undertaken an initial electrophysiological and morphological characterization of these neurons. Adult mice (n = 14, 2-6 months old, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated. Parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from targeted GFP+ neurons and unidentified GFP- neurons in the same region using a K-gluconate-based recording solution. GFP-expressing (Thy1+) neurons (n=28) had similar input resistances (397 ± 31 MΩ vs. 452 ± 57 MΩ) but more depolarized RMPs (-55 ± 1 mV vs. -60 ± 2 mV) than unidentified recordings (n=21). Action potential discharge patterns evoked by depolarisaing current steps (1s, 20pA increments) also differed between recordings. Tonic firing dominated in GFP+ recordings (22/26), with few neurons exhibiting initial busting (2/26) or delayed firing (2/26). Discharge was more variable in the unidentified sample with tonic firing (4/22), initial bursting (7/22), delayed firing (7/22), and single spiking (2/22) all represented in the sample. We also assessed the responsiveness of GFP+ neurons to somatostatin, which selectively evokes outward currents in some inhibitory SDH populations. All GFP+ neurons tested exhibited somatostatin responses (6/6), reinforcing the likelihood that Thy1+ SDH neurons are inhibitory. Finally, neurobiotin filled GFP+ neurons did not conform to the routinely described morphological classes described in the SDH. Together these findings suggest that Thy1+ neurons represent a novel inhibitory interneuron subclass in the SDH.

Disclosures: B.A. Graham: None. K.M. Smith: None. M.A. Gradwell: None. R.J. Callister: None. D.I. Hughes: None. F.R. Walker: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.15/CC3

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS080889

Title: Intrinsic excitability of spinal projection neurons is enhanced by a Na^+ leak conductance activated by substance P

Authors: *N. C. FORD, M. L. BACCEI;
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Abstract: Lamina I projection neurons (PNs) represent a major output of spinal nociceptive circuits, and serve as the conduit by which noxious stimuli detected in the periphery are transmitted to higher brain regions. While PNs exhibit high levels of spontaneous activity during the neonatal period, which may contribute to the proper formation of central pain networks, the underlying ionic mechanisms are poorly understood. Specifically, the voltage-independent (i.e. "leak") channels that regulate the intrinsic membrane excitability of this neuronal population, and how these channels respond to neuromodulators, remain unclear. The present study investigated the contribution of the non-selective Na^+ leak channel NALCN to the intrinsic excitability of identified spino-parabrachial (PB) neurons during early life. PNs were back-labeled by DiI injection into the PB at postnatal day 1 (P1). Whole-cell patch clamp recordings were obtained from spino-PB neurons using an in vitro, intact spinal cord preparation on P3-P5. Decreasing extracellular Na^+ levels to 15 mM evoked a positive shift in holding current and robust membrane hyperpolarization, thus demonstrating the presence of a Na^+-dependent leak conductance in immature PNs. Bath application of $GdCl_3$ (10-100 μM) prevented the effects of the low Na^+ bath on both the holding current and resting membrane potential (RMP), which is consistent with its ability to block the recently identified Na^+ leak channel NALCN. Importantly, the bath application of Gd^{3+} significantly hyperpolarized the RMP, decreased spontaneous activity, and increased rheobase levels in spino-PB neurons, suggesting that NALCN-like channels facilitate action potential discharge in neonatal PNs. In addition, NK1R activation by substance P (SP; 5 μM) significantly enhanced the NALCN conductance in a Gd^{3+}-sensitive manner. Finally, SP activation of NALCN channels was suppressed by the Src kinase inhibitor PP2 (30 μM) delivered via the patch electrode. We conclude that NALCN channels are functionally expressed in spino-PB projection neurons and are modulated by NK1R-dependent Src kinase signaling. The overall effect of this Na^+ leak conductance is to enhance the intrinsic excitability of spino-PB neurons and thereby increase the output of the spinal nociceptive network. As a result, age-related or neuropeptide-evoked changes in NALCN channel activity could profoundly alter the ascending flow of nociceptive transmission to the developing brain.

Disclosures: N.C. Ford: None. M.L. Baccei: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.16/CC4

Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 GM107054

Title: Subcellular distribution of Sigma-1 receptor in Dorsal Root Ganglion of rodents

Authors: *T. A. MAVLYUTOV[1], M. L. EPSTEIN[2], J. YANG[1];
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Abstract: Sigma-1 receptor (S1R) is a widely found signaling modulator and a promising target for treatment of a number of neurological diseases. Intrathecal administration of S1R specific antagonists has been shown to attenuate pain as shown by changes in behavior of rodents in both inflammatory and neuropathic pain models. Since primary afferents are the major component in the pain pathway, we examined the mouse and rat DRGs for the presence of the S1R. At both mRNA and protein levels, Western blots and qRT-PCR confirmed that the DRG contains significantly greater S1R expression in comparison to liver, spinal cord, or cortex. Using a custom-made highly specific antibody, we demonstrate the presence of a strong S1R immuno-fluorescence in all rat and mouse DRG neurons that colocalize with the NSE marker, but little or no signal in terminal neural processes, in GFAP-containing or glial satellite cells, or in dorsal horn neurons. Moreover, S1R was absent in afferent terminals in the skin and in the dorsal horn of the spinal cord. Using immuno-electron microscopy, we detected S1R in the nuclear envelope and endoplasmic reticulum of DRG neurons. In contrast to other cells, S1R is uniquely located directly at the plasma membrane of the DRG neurons. The presence of S1R in the nuclear envelope of all DRG neurons suggests a potential role of S1R as a regulator of neuronal nuclear activities and/or gene expression, a possibility which may provide insight towards new molecular targets for modulating nociception at the level of primary afferent neurons.

Disclosures: T.A. Mavlyutov: None. M.L. Epstein: None. J. Yang: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.17/CC5

Topic: D.02. Somatosensation: Pain

Support: FCOMP-01-0124-FEDER-029623 (PTDC/NEU-SCC/0347/2012)

János Bolyai Research Scholarship of the Hungarian Academy of Sciences and the Hungarian Brain Research Program (KTIA_NAP_ 13-2-2014-0005)

Title: Diverse firing properties and aβ, aδ and c afferent inputs of small local-circuit neurons in spinal lamina 1

Authors: *B. V. SAFRONOV[1], E. FERNANDES[2], L. L. LUZ[2], P. SZUCS[3];
[1]Neuronal Networks, [2]IBMC, Porto, Portugal; [3]Dept. of Physiol., MTA-DE-NAP B-Pain Control Res. Group, University of Debrecen, Hungary

Abstract: Spinal lamina I is a key element of the pain processing system which integrates primary afferent input and relays it to supraspinal areas. Intrinsic network of lamina I, to more than 90%, consists of interneurons whose roles in the signal processing are poorly understood. We used whole-cell patch-clamp recordings in an isolated spinal cord preparation with attached dorsal roots to examine morphological features and physiological properties of lamina I small interneurons (n=47). Successfully filled with biocytin neurons (n=17) had fusiform (n=10), flattened (n=4) and multipolar (n=3) somatodendritic morphology and their axons branched intensively terminating in laminae I-III. Small interneurons showed diverse firing patterns. In addition to standard tonic (n=16), adapting (n=7) and delayed firing (n=6), we also found interneurons generating intrinsic rhythmic discharges (n=6) and plateau potentials (n=10), the latter were suppressed by the L-type Ca^{2+}-channel blocker nifedipine. Small interneurons in lamina I received monosynaptic primary afferent inputs from Aδ and C fiber afferents, and some of them could generate bursts of spikes upon the root stimulation. In addition, interneurons (n=6) received monosynaptic inputs from the low-threshold Aβ afferents, which could be picked up by the ventrally protruding dendrites reaching lamina III. Small interneurons also received disynaptic inhibitory inputs driven by the Aβ, Aδ or C afferents. Thus, our results indicate that small lamina I interneurons show diverse firing properties, can generate rhythmic discharges and plateau potentials, and receive primary afferent inputs from Aβ, Aδ and C afferents. These properties are important for processing diverse modalities of nociceptive inputs in lamina I.

Disclosures: B.V. Safronov: None. E. Fernandes: None. L.L. Luz: None. P. Szucs: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.18/CC6

Topic: D.02. Somatosensation: Pain

Support: NHMRC Grant 631000

NHMRC Grant 1043933

Title: Postsynaptic and presynaptic inhibition revealed by optogenetic activation of spinal cord dorsal horn parvalbumin interneurons

Authors: *M. A. GRADWELL[1], R. J. CALLISTER[1], D. I. HUGHES[2], B. A. GRAHAM[1]; [1]Biomed. Sci. and Pharm., Univ. of Newcastle, Callaghan, Australia; [2]Vet. and Life Sci., Univ. of Glasgow, Glasgow, United Kingdom

Abstract: The dorsal horn (DH) of the spinal cord is essential for the appropriate encoding of sensory signals. The role of inhibitory interneurons in segregating nociceptive and tactile inputs within the spinal cord is critical to this sensory encoding. We have previously described a population of inhibitory parvalbumin-positive interneurons (PVINs) with functional properties and connectivity that would enable them to segregate tactile and nociceptive information (Hughes, 2012 J Physiol 16:3927). Furthermore, recent findings demonstrate PV^+INs are necessary for normal sensory processing and their loss produces allodynia (Petitjean, 2015 Cell rep 13:1246). The circuitry underlying these findings, however, remains unclear. To better understand the connectivity underlying the role of PV^+INs in sensory encoding we have taken an optogenetic approach, using transgenic mice that express Channelrhodopsin-2 in PV^+INs. Adult mice (2-12 months old, both sexes) were deeply anaesthetized with ketamine (100 mg/kg, i.p.) and decapitated. Parasagittal spinal cord slices were prepared from the lumbar cord. Whole cell patch clamp recordings were made from targeted PVINs and unidentified (PV-negative) neurons and slices photostimulated using brief whole-field illumination (488nm, 1ms). Synaptic responses and photocurrents were recorded using a range of pipette solutions including CsCl- and CsMSF-based internal (inhibitory responses), and K-gluc (excitatory responses). During inhibitory recordings, photostimulation evoked short latency mixed GABA/Glycine inhibitory postsynaptic currents (ie, bicuculline and strychnine sensitive) in 96% of PVINs and 74% of unidentified interneurons. Thus, PVINs are a highly interconnected network and provide postsynaptic inhibition to other DH populations. During excitatory recordings photostimulation evoked short latency excitatory postsynaptic currents (EPSCs - blocked by CNQX) in 54% of recordings (38/70), presumably arising from a small population of previously described excitatory PVINs. In other recordings (36%, 22/70), photostimulation evoked longer latency EPSCs that could be blocked by CNQX, but also bicuculline. This pharmacology suggests a polysynaptic circuit mediated by inhibitory PVINs. Under these conditions the observed EPSC likely results from primary afferent depolarisation, reflecting the role of PVINs in presynaptic inhibition. Together our findings indicate PV^+INs regulate a wide range of SDH circuits that regulate both incoming sensory signals and local interneurons. It is likely that modulation of PV^+IN function contributes to aberrant sensory experience in pathological pain states.

Disclosures: M.A. Gradwell: None. R.J. Callister: None. D.I. Hughes: None. B.A. Graham: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.19/CC7

Topic: D.02. Somatosensation: Pain

Support: CONACyT grant 50900

FI-DGR programme of AGAUR ECO/1551/2012

byTEC2011-29106-C02-02

The Barcelona Supercomputing Center (BSC-CNS) and CINVESTAV agreement

Sistema Nacional de investigadores-CONACyT, Mexico

Title: Markovian analysis of spontaneous spinal cord dorsum potentials reveals non random dynamic adaptive changes in the configuration of the functional connectivity between segmental populations of dorsal horn neurons in response to acute nociceptive stimulation.

Authors: *P. RUDOMIN[1,2], M. MARTIN[3], E. CONTRERAS-HERNANDEZ[1], J. BEJAR[3], G. ESPOSITO[3], D. CHAVEZ[1], U. CORTES[3,4], S. GLUSMAN[1];
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Abstract: We used a Markovian approach to examine the changes produced by the intradermic injection of capsaicin on the *state* of functional connectivity between the segmental populations of dorsal horn neurons involved in the generation of spontaneous cord dorsum potentials (CDPs) as well as the extent to which these changes could be reverted by therapeutic approaches clinically known to reduce neuropathic pain. In anesthetized, paralyzed and artificially ventilated cats we placed a 12 silver ball electrode array to record the spontaneous CDPs generated along the L4-L7 spinal segments (see Contreras *et al*. *J Physiol* 10, 23: 43-63, 2015 for details in Methods). A machine learning procedure was used to select from continuous recordings made throughout the whole experiment spontaneous CDPs with similar shapes and amplitudes and group them in specific classes (see Martin *et al*., *Front Neuroinform* 26: 9-21, 2015). Analysis of the sequence of classes of CDPs recorded during non-overlapping periods of 10 min allowed us to build, for each segment *i* and time period *t,* a Markovian model $M_{i,t}$ of transition probabilities between the different classes of the recorded CDPs. Finally, we generated a *consensus similarity graph* of Markovian behaviour of the whole network of dorsal horn neurons in different time periods by combining models from all segments in the same time period *t.* Clustering of the similarity graph revealed significant differences between groups of models obtained before and

after the injection of capsaicin, each expressing a particular state of functional neuronal connectivity. The capsaicin-induced changes in functional state could be temporarily reverted to their pre-capsaicin (control) state by the slow systemic injection of clinically relevant doses of lidocaine (5mg/kg). Our observations indicate that the ensemble of dorsal horn neurons involved in the generation of the spontaneous CDPs may acquire specific structured (non-random) patterns of functional connectivity as an adaptive response to the capsaicin-induced nociceptive stimulation. A Markovian approach is presently being used to address the question on whether the same or different structured changes in the state of functional connectivity between the dorsal horn neurones are induced by other types of nociceptive stimulation.

Disclosures: P. Rudomin: None. M. Martin: None. E. Contreras-Hernandez: None. J. Bejar: None. G. Esposito: None. D. Chavez: None. U. Cortes: None. S. Glusman: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.20/CC8

Topic: D.02. Somatosensation: Pain

Title: Activation of spinal STAT6 signaling in M2 like microglia suppressed neuropathic pain

Authors: H. OKUTANI[1], H. YAMANAKA[1], K. KOBAYASHI[1], M. OKUBO[1], M. HIROSE[2], *K. NOGUCHI[3];
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Abstract: An inflammatory process that is mediated by the activated spinal microglia has been considered as an important factor in neuronal sensitization in a neuropathic pain state. Similar to macrophages, microglia adopt two different activation phenotypes, the classical (M1) and alternative activation (M2). In this study we examined the expression of phosphorylation of signal transducer and activator of transcription 6 (STAT6) in order to evaluate M2 responses of spinal microglia, and investigated possible involvement of interleukin 4 signals in the endogenous anti inflammatory responses.
Sprague-Dawley rats, weighing 200-250 g received tibial and common peroneal nerve ligation (spared nerve injury model; SNI) were used in this study. Expression of phosphorylated STAT6 was examined by immunohistochemistry using specific antibody for STAT6. IL4 and IL4 receptor (IL4-R) mRNAs were examined by RT-PCR and in situ hybridization (ISH). The effect of intrathecal IL4 was assessed by the withdrawal threshold of mechanical stimuli to the hind-paw of SNI rats.

We detected injury-induced phosphorylated STAT6 in the spinal cord of SNI model rats. Phosphorylated STAT6 signals were specifically detected in microglia. RT-PCR revealed that peripheral nerve injury increased the expression of IL4-R mRNA in spinal cord. The increase of the IL4-R mRNA began from 12 hours, peaked at 48 hours and continued for at least 14 days after nerve injury. In the double labeling analysis, we observed IL4-R mRNA positive signals exclusively in the Iba1 immunolabeled microglia in the dorsal horn of SNI rats. In contrast, we could not detect expression of IL4 mRNA both in dorsal root ganglia and spinal cord. Intrathecal administration of IL4 up-regulated phosphorylated STAT6 in the spinal microglia and suppressed mechanical hypersensitivity of SNI model rats.
These results suggest that adaptive responses of microglia to nerve injury has an anti-inflammatory signals such as IL4-R and phosphorylated STAT6. Activation of STAT6 signals in spinal microglia has a potential to reduce neuropathic pain. Utilizing these anti-nociceptive mechanisms may provide novel therapeutic strategy for neuropathic pain.

Disclosures: H. Okutani: None. H. Yamanaka: None. K. Kobayashi: None. M. Okubo: None. M. Hirose: None. K. Noguchi: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.21/CC9

Topic: D.02. Somatosensation: Pain

Support: NIH Grant R01 DE021996

Title: The VGF-derived neuropeptide TLQP-62 induces neuroplasticity in the dorsal horn

Authors: *A. G. SKORPUT[1], H. TRUONG[1], W.-J. LIN[2], J. J. WAATAJA[1], S. R. SALTON[2], L. VULCHANOVA[1];
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Abstract: Maladaptive neuroplasticity in the dorsal horn following peripheral nerve damage spurs the transition from acute to chronic pain. While BDNF signaling is heavily implicated in the induction of such plasticity, regulation of its involvement is less well understood. The neurosecretory protein VGF (non-acronymic) is rapidly and robustly upregulated following nerve injury and is proteolytically processed to yield bioactive neuropeptides, including the C-terminal peptide TLQP-62, which has been demonstrated to induce BDNF dependent neuroplasticity in the hippocampus. Therefore, we hypothesized that TLQP-62 may play a

similar role in the dorsal horn following nerve injury. Multiphoton imaging of Ca^{2+} responses in the superficial dorsal horn demonstrated limited direct activation of Flou-4 labeled cells by TLQP-62, yet consistent positive modulation of submaximal glutamate responses, which was blocked by the kinase inhibitor K252a. Further, treatment of spinal cord slices with 500nM TLQP62 increased the ratio of pTrkB/TrkB suggesting induction of BDNF signaling. We are studying the function of TLQP-62 in this context to better understand BDNF induced plasticity in pain, and elucidate regulators of its involvement for targeted pharmacologic intervention.

Disclosures: A.G. Skorput: None. H. Truong: None. W. Lin: None. J.J. Waataja: None. S.R. Salton: None. L. Vulchanova: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.22/CC10

Topic: D.02. Somatosensation: Pain

Support: NIH Grant DK066112S1

NIH Grant MAPP U01 DK82342

Title: Acyloxyacyl hydrolase modulates pelvic pain severity

Authors: *W. YANG[1], R. E. YAGGIE[1], M. JIANG[2], C. N. RUDICK[1], C. J. HECKMAN[2], J. DONE[1], A. J. SCHAEFFER[1], D. J. KLUMPP[1];
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Abstract: Chronic pelvic pain causes significant morbidity to patients and is a bane to clinicians. Using a murine neurogenic cystititis model that recapitulates key aspects of interstitial cystitis/bladder pain syndrome (IC), we recently showed that pseudorabies virus (PRV) induces severe pelvic allodynia BALB/c mice, relative to C57BL/6 mice. Here, we report that a quantitative trait locus (QTL) analysis of PRV-induced allodynia in F_{2CxB} progeny identified a polymorphism on chromosome 13, rs6314295, significantly associated with allodynia (LOD=3.11). The mouse gene for acyloxyacyl hydrolase (*Aoah*), encoded near rs6314295, was induced in the sacral spinal cord of PRV-infected mice. AOAH-deficient mice exhibited increased vesicomoter reflex consistent with spontaneous bladder hypersensitivity in response to bladder distension and increased pelvic allodynia both in neurogenic cystitis and post-bacterial chronic pain models. AOAH deficiency resulted in greater bladder pathology and TNF production in PRV neurogenic cystitis, consistent with increased bladder mast cell activation.

AOAH immunoreactivity was detectable along the bladder-brain axis, including in brain sites previously correlated with chronic pelvic pain in humans. Finally, AOAH-deficient mice had significantly higher levels of urinary and bladder VEGF, an emerging marker of chronic pelvic pain in humans. These findings indicate that AOAH modulates pelvic pain severity and suggest that allelic variation in *Aoah* influences pelvic pain in IC.

Disclosures: W. Yang: None. R.E. Yaggie: None. M. Jiang: None. C.N. Rudick: None. C.J. Heckman: None. J. Done: None. A.J. Schaeffer: None. D.J. Klumpp: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.23/CC11

Topic: D.02. Somatosensation: Pain

Support: Natural Science and engineering Research Council of Canada

Fonds de Recherche du Québec en Santé

Fondation Chiropratique du Québec

Université du Québec à Trois-Rivières

Title: Alteration of neurovascular coupling in the rat spinal cord by systemic blood pressure changes evoked by nociceptive stimulation

Authors: *T. PAQUETTE[1], H. LEBLOND[2,4], M. PICHÉ[3,4];
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Abstract: Neurovascular coupling is a physiological process involving a local hemodynamic response associated with surrounding neuronal activity. Functional magnetic resonance imaging (fMRI) uses this relation to infer neuronal activity by measuring magnetic signals related to changes in deoxyhemoglobin concentration. In general, this neurovascular coupling is relatively stable. However, in the rat primary somatosensory cortex, it is altered during nociceptive processing associated with increases in systemic blood pressure. The objective of this study was to examine neurovascular coupling in the rat spinal cord during nociceptive processing and to determine whether it is affected by systemic blood pressure, as in the primary somatosensory

cortex.
All experimental procedures were approved by the Université du Québec à Trois-Rivières animal care committee, and were in accordance with the guidelines of the Canadian Council on Animal Care. Ten male Wistar rats were anesthetized using isoflurane (1.2-1.5 %). Local field potentials (LFP) and spinal cord blood flow (SCBF) were recorded concurrently in the lumbosacral enlargement, where activity was evoked by sciatic nerve stimulation. The mean arterial pressure (MAP) was recorded continuously with a pressure transducer connected to a cannula inserted in the right carotid artery. Electrical stimulation was applied on the sciatic nerve as constant current at 11 intensities ranging between 0.01 and 9.6 mA. Physiological responses to electrical stimulation were recorded twice, once in intact conditions and once after a spinal transection at the C1 cervical segment of the spinal cord. Spinal transection at C1 was used to abolish MAP changes associated with the nociceptive stimulation in order to test the effect of MAP on neurovascular coupling.
In intact conditions, stimulation between 2.4 and 9.6 mA produced linear MAP changes that were paralleled by similar changes in SCBF, while LFP amplitude followed a similar trend at lower intensities but not at high stimulus intensities, for which it decreased. As expected, spinal transection almost abolished MAP changes. In these conditions, SCBF and LFP amplitude were strongly coupled.
These results imply that in studies involving nociceptive stimulation, estimation of neuronal activity in the spinal cord from the associated hemodynamic changes might lead to inaccurate results due to the influence of MAP on SCBF. This further implies that results from fMRI studies on pain should be interpreted with caution. Future studies should develop methods to control for MAP changes or alternative measures unaffected by MAP.

Disclosures: T. Paquette: None. H. Leblond: None. M. Piché: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.24/DD1

Topic: D.02. Somatosensation: Pain

Title: Resting-state connectivity in the human spinal cord

Authors: *F. EIPPERT[1], Y. KONG[1], J. BROOKS[2], I. TRACEY[1];
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Abstract: Spontaneous fluctuations in the functional magnetic resonance imaging (fMRI) signal have been extensively investigated in the human brain. Recently, this has been extended to the

human spinal cord (Barry et al., 2014; Kong et al., 2014), where the organization of these resting state signals has been characterized both spatially and temporally. Here, we aim to further this characterization by investigating resting-state connectivity both within and across spinal cord segments.
We acquired resting-state fMRI data of the cervical spinal cord from 20 healthy participants on a 3 Tesla MRI-scanner for 7.5 minutes. Initial data processing consisted of slice-wise motion correction, smoothing, physiological noise correction, high-pass filtering, and warping to standard space. Next, we extracted time-courses from spinal cord grey matter voxels and averaged these for each dorsal and ventral horn at each segmental level. Finally, we computed time-course correlations 1) within each spinal level between the dorsal and ventral horns and 2) across the different spinal levels and assessed their significance using non-parametric permutation testing.
We observed highly significant positive correlations between the ventral horns, as well as between the dorsal horns, although ventral horn correlations tended to be stronger. Connectivity between ventral and dorsal horns was significantly weaker, both within each hemi-cord and across. Regarding between-segment connectivity, we observed that correlations became significantly weaker the further two segments were apart. Importantly though, this distance-specific loss of connectivity differed between ventral and dorsal horns: dorsal horns showed significantly stronger inter-segmental connectivity than ventral horns – this effect persisted for a distance of three segments and only then vanished.
Our results replicate a previous observation of ventral horn and dorsal horn connectivity (Barry et al., 2014) – but while this previous finding relied on the advantages of ultra-high field (7 Tesla) data acquisition, we here show that it is possible to observe this connectivity profile also at the clinically relevant field-strength of 3 Tesla. Building on earlier resting-state spinal cord studies (Barry et al., 2014; Kong et al., 2014) we also investigated inter-segmental connectivity, and observed a distance-dependent reduction of functional connectivity that differed between ventral and dorsal horns. It will be interesting to investigate whether the integrity of inter-segmental connectivity can be harnessed in a clinical situation, e.g. when investigating recovery after spinal cord injury.

Disclosures: F. Eippert: None. Y. Kong: None. J. Brooks: None. I. Tracey: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.25/DD2

Topic: D.03. Somatosensation: Touch

Support: NIH grant NS34814

Howard Hughes Medical Institute

Title: Utilizing mouse glabrous skin-specific tactile behavior assays to dissect innocuous touch circuits of the spinal cord dorsal horn

Authors: *A. TOLIVER, L. L. OREFICE, V. ABRAIRA, D. GINTY;
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Abstract: Innocuous touch is detected and processed by neural circuits involving specialized dorsal root ganglion neurons and their respective post-synaptic partners in the spinal cord dorsal horn, comprised of an array of local interneurons as well as long-range projection neuron populations. An obstacle in understanding innocuous touch circuitry is a lack of behavioral assays specific to innocuous touch that may be used for genetic circuit dissection in mice. To this end, we have utilized a battery of behavioral paradigms that enables assessment of the contributions of glabrous skin innervating sensory neurons to tactile behaviors. The tactile assays consist of Von Frey and the brush test, which we use to assess responsivity to non-painful, innocuous touch stimuli. We also assayed the contribution of glabrous skin-innervating sensory neurons using sensorimotor paradigms, including rotarod, balance beam, wire hang, Treadscan gait analysis, and the horizontal ladder test. Nociceptive and thermoreceptive control tests, including Hargreaves and the hot plate test, were used for comparison.
To determine baseline dependency on sensory innervation of glabrous skin using these behavioral measures, we topically applied 20 microliters of 5% lidocaine to the glabrous skin, 20 minutes prior to each behavioral assay. The use of topical lidocaine allows us to determine the cutaneous sensory neuron dependence of the different measures of somatosensory function. This approach will inform us of the utility of tactile and sensorimotor behavioral paradigms for measuring glabrous skin somatosensory functioning. Preliminary findings using the Von Frey and brush tests indicate hyposensitivity following topical lidocaine application to the paws, compared to a control saline gel application. Gait analysis revealed a decrease in stride time following topical lidocaine application to the paws.
Current studies are focused on assessing the contribution of spinal cord interneurons to tactile-based behaviors. We are employing intersectional Cre/Flp genetic tools and a dual recombinase tetanus toxin line, to specifically silence lineages of RorB and CCK expressing interneurons exclusively in the spinal cord. These animals are then tested on the aforementioned battery of behavioral assays in order to determine the effect of silencing various spinal cord interneuron subtypes on somatosensory behaviors. Utilizing multiple behavioral assays will enable a quantitative assessment of the contributions of glabrous skin-innervating sensory neurons to innocuous touch perception and may reveal spinal cord interneuron subtype-specific roles for somatosensory-related behaviors.

Disclosures: A. Toliver: None. L.L. Orefice: None. V. Abraira: None. D. Ginty: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.26/DD3

Topic: D.01. Sensory Disorders

Support: NIH T32 GM108539

NIH R01 NS081707

Title: Synapse specificity in peripheral sensory neurons

Authors: *B. A. COPITS, K. C. MCKENZIE, K. M. WILSON, S. K. VOGT, J. P. GOLDEN, R. W. GEREAU, IV;
Pain Center, Dept of Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Rapid processing and integration of sensory information is critical for the survival of an organism, and permits the discrimination of a tremendous variety of external inputs. Noxious stimuli must be separated from less offensive sensations to ensure potentially dangerous environmental conditions are avoided. However these sensory circuits can become activated in the absence of noxious events following nerve trauma or inflammation, leading to chronic pain states. While we have learned a great deal about the plasticity of nociceptive transmission, we possess a limited understanding of the underlying molecular mechanisms which orchestrate the formation of these neural circuits, and the synaptic adaptations that lead to states of altered sensory perception.

Transsynaptic interactions between presynaptic neurexins (Nrxns) and postsynaptic neuroligins (Nlgns) have been suggested to impart a combinatorial code for neural connectivity. In the peripheral nervous system, sensory neurons are unable to form synapses with each other in culture, suggesting that they lack key signals involved in determining appropriate synaptic partners. To understand the mechanisms governing synaptic connectivity in the somatosensory system, we co-cultured fibroblasts expressing a variety of cell adhesion molecules with both mouse and human sensory neurons. We found that expression of postsynaptic Nlgn isoforms strongly promoted functional hemi-synapse formation, while neurexins were ineffective at inducing presynaptic contacts. Overexpression of Nrxns or Nlgns was unable to induce autaptic connections in these neurons, suggesting that both attractive and repulsive mechanisms exist to specify synapse formation.

Both *Nrxns* and *Nlgns* are subject to extensive alternative splicing, generating thousands of different isoforms, which can specify interactions with various transsynaptic adhesion molecules. Alternative splicing of *Nrxns* at one site in particular, SS4, shifts binding affinities between Nlgns and other postsynaptic partners including leucine-rich repeat transmembrane proteins (LRRTMs) and cerebellins in cortical and cerebellar neurons. We found that the alternative

splicing of all *Nrxn* isoforms is markedly different in spinal and sensory neurons from both mice and humans, compared to those in the cortex. This suggests that the somatosensory system may use these adhesion molecules in functionally distinct ways to specify synaptic connectivity. We are currently focusing on how these interactions might influence synaptic transmission in the spinal cord and regulate sensory behaviors.

Disclosures: B.A. Copits: None. K.C. McKenzie: None. K.M. Wilson: None. S.K. Vogt: None. J.P. Golden: None. R.W. Gereau: None.

Poster

234. Spinal Cord Processing: Anatomy and Physiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 234.27/DD4

Topic: D.03. Somatosensation: Touch

Title: Characterization of Zic2+ dorsal spinal neurons as potential integrators of sensory and motor circuits

Authors: *S. PAIXAO, A. ESCALANTE, R. KLEIN;
Max Planck Inst. of Neurobio., Martinsried, Germany

Abstract: The dorsal spinal cord is the integrative center that processes and transmits a variety of somatosensory modalities such as touch, pain, temperature and itch. It is also becoming clear that the integration of spinal microcircuits, sensory feedback and supraspinal descending circuits necessary for accurate motor performance occurs in the dorsal spinal cord. Nevertheless, the molecular identity and specific function of individual interneuron classes in this circuit is largely unknown.
In our previous work, we identified a population of dorsal spinal neurons marked by the co-expression of EphA4 and the transcription factor Zic2, which we suggested represents a subpopulation of postsynaptic dorsal column (PSDC) neurons (Paixao *et al*., Neuron, 2013). We have recently developed an inducible Zic2-Cre line to genetically target these neurons. Zic2+ neurons reside in laminae III-V in a medial position surrounding the dorsal funiculus, where they partially colocalize with the recently described markers of lamina V interneurons Tcfap2β and SatB1/2. Contrary to what was described for these two populations, Zic2 neurons seem to be exclusively excitatory. Intraspinal cord trans-synaptic rabies virus experiments have confirmed that Zic2 neurons receive sensory input primarily from cutaneous afferents. Preliminary retrograde tracing experiments, indicate that at least a subset of Zic2 neurons send projections to the cuneate nucleus in the medulla, suggesting that Zic2 likely represents a molecular marker of PSDC neurons and could be involved in discriminative touch sensation.

Furthermore, rabies virus experiments have also highlighted that Zic2 neurons receive inputs from ventral spinal cord interneurons and several descending brain centers involved in motor control, like the motor cortex and numerous brainstem nuclei, raising the interesting possibility that Zic2 dorsal spinal neurons could play a role in the integration of sensory and motor spinal circuits.
Ongoing efforts are aimed at investigating the *in vivo* activity of Zic2+ spinal cord neurons during somatosensory stimulation and their requirement for specific motor outputs.

Disclosures: S. Paixao: None. A. Escalante: None. R. Klein: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.01/DD5

Topic: D.03. Somatosensation: Touch

Support: R21NS089084

Title: Somatosensory enhancement of the lips size increases tactile acuity

Authors: *E. AMBRON[1], M. COYLE[2], H. B. COSLETT[2];
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Abstract: Magnifying the view of a body part is known to improve tactile acuity. For the fist time, we tested the effect of somatosensory magnification of a body part on tactile accuracy by applying an anesthetic cream on participants' lips. Tactile discrimination markedly improved after the application of the cream and decreased when the cream was removed. This effect was observed only in participants who perceived an increase of the lips size with the application of the cream. The magnification of body part has beneficial effect on tactile discrimination, not only if it is induced by vision but also in the somatosensory modality.

Disclosures: E. Ambron: None. M. Coyle: None. H.B. Coslett: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.02/DD6

Topic: D.03. Somatosensation: Touch

Support: Vanderbilt/NIMH Silvio O. Conte Center for Neuroscience Research Pilot Award (Cascio)

NIH R01 MH102272 (Cascio)

NIMH F31 MH106291 (Bryant)

Title: Neural signatures of vibrotactile stimulation at 7T MRI and links to autism traits

Authors: *L. K. BRYANT[1], L. CHEN[2], A. MISHRA[2], C. J. CASCIO[3], M. T. WALLACE[4]; [2]Radiology, [3]Psychiatry, [4]Hearing & Speech Sci., [1]Vanderbilt Univ., Nashville, TN

Abstract: It is well known that individuals with autism spectrum disorders (ASD) can display sensory sensitivities across modalities, yet the majority of sensory research in ASD focuses on vision and audition. The sense of touch is comparatively understudied in ASD, despite its central role in early social development and strong links between aberrant tactile processing and clinical symptoms of autism (Foss-Feig et al., 2011). However, the neural basis of such aberrations is not understood. Atypical tactile sensory responsiveness may arise from deviations in sensory cortical organization, as suggested by a magnetoencephelopgraphy (MEG) study of cortical representations of the thumb, lip, and index finger in the primary somatosensory cortex (S1) (Marco et al., 2012). However, MEG lacks the precise millimeter spatial resolution afforded by high-field, 7-Tesla functional magnetic resonance imaging (7T fMRI) to localize aberrant somatotopy to functionally distinct S1 subregions. Such resolution could reveal previously undetected relations between tactile perception, somatotopic organization, and social-communication abilities affected by ASD. Using a combination of psychophysics and high resolution fMRI, we first examined vibrotactile detection ability at the fingertip in neurotypical adults, and then delivered high intensity (200 Hz, 400um) vibrotactile stimulation randomly to the left digits 2-4 during 7T fMRI. Preliminary results suggest the ability to replicate single digit separation (Stringer et al., 2014) using a novel method of vibrotactile stimulation. In addition to quantifying inter-digit distance, ongoing work includes the correlation of psychophysical data with self-report questionnaires targeting behavioral patterns of sensory responsiveness (Sensory Profile (Dunn, 1999)) and subclinical autism traits in daily life (Social Responsiveness Scale-2 (Constantino, 2012)). Emerging relations suggest that increased dynamic range of vibrotactile detection is associated with increased sensation seeking, and with decreased social motivation, in daily life. The ultimate goal of this research is to strengthen our understanding of how the brain

processes tactile information and provide an empirical basis for advancing the treatment of sensory-based symptoms in autism.

Disclosures: L.K. Bryant: None. L. Chen: None. A. Mishra: None. C.J. Cascio: None. M.T. Wallace: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.03/DD7

Topic: D.03. Somatosensation: Touch

Title: Transcranial oscillatory stimulation at individual somatosensory alpha frequency specifically decreases network centrality of S1

Authors: C. GUNDLACH[1], T. NIERHAUS[3], P. RAGERT[2], M. MUELLER[1], A. VILLRINGER[3], *B. S. SEHM[3];
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Abstract: Ongoing alpha oscillations might reflect aspects of functional specific perceptual inhibition. In a previous study we found that transcranial alternating current stimulation applied at an individual somatosensory mu alpha frequency (mu-tACS) induces a phasic decrease of somatosensory perception, suggesting a causal interference with somatosensory processing. Here, we aimed at investigating underlying functional brain changes on a whole brain level by concurrent mu-tACS and resting-state functional MRI. Based on the suggested inhibitory nature of alpha, we hypothesized that mu-tACS decreases functional connectivity of the primary somatosensory cortex.
In a randomized single-blind crossover design, 20 healthy subjects underwent 2 separate sessions of functional magnetic resonance imaging during either 6 minutes of mu-tACS applied over the somatosensory cortex or sham stimulation. Eigenvector centrality mapping (ECM) was used to investigate mu-tACS-induced changes (as compared to sham stimulation) in functional connectivity across the whole brain.
In line with our hypothesis, our results demonstrate that mu-tACS induces a specific decrease in whole-brain centrality of the left primary somatosensory cortex (whole brain analysis, $p<.05$, cluster corrected, voxelwise threshold: $z>2.576$, cluster size: 59 voxels).
These findings (i) add causal evidence to previous correlative findings and underline the inhibitory nature of functional relevant alpha rhythms, and (ii) demonstrate the potential of

transcranial stimulation methods to specifically modulate brain function when adjusting the stimulation to intrinsic oscillatory frequencies.

Disclosures: C. Gundlach: None. T. Nierhaus: None. P. Ragert: None. M. Mueller: None. A. Villringer: None. B.S. Sehm: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.04/DD8

Topic: D.03. Somatosensation: Touch

Support: Swedish Research Council: Grant 3548

Vastra Gotaland Regional Council: ALFGBG-441901

The Knut and Alice Wallenberg Foundation: NeuroSQUID

Title: The event-related somatosensory responses to long lasting gentle touch measured with magnetoencephalography

Authors: *E. J. ERIKSSON[1], D. LUNDQVIST[2], J. SCHNEIDERMAN[3], V. JOUSMÄKI[4,2], J. WESSBERG[1];
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Abstract: The sense of touch is conveyed from the skin to the brain via fast myelinated Aβ afferents and slow unmyelinated C tactile (CT) afferents. Aβs signal discriminative properties of tactile input whereas CTs are believed to code for pleasantness of touch. CTs only exist in hairy skin and single unit recordings show that CT firing is highest when a soft object, e.g., a brush, strokes the skin at a velocity of 3 cm/s. Functional magnetic resonance imaging (fMRI) studies show that CTs project to the insula but not to primary or secondary somatosensory cortices. Nevertheless, how CTs contribute to pleasantness of touch is currently not known. Touch to the hairy skin activates both the fast Aβs and slow CTs; hence tactile sensory perception requires the integration of both types of mechanosensation.
In this study, combined MEG and electroencephalography (EEG) was recorded in 19 healthy volunteers (22-45 years) with an Elekta Neuromag® TRIUX system during 200 ~3 cm/s brush

strokes using a custom-made MEG-compatible brush robot that provided precise and replicable timing, length, and velocity of brush stroke stimuli. A multifilament fiber-optic sensor was attached alongside the bristles of the brush, marking the timing of brush contact with the skin, and a load cell was used to measure the pressure applied on the skin.
Two conditions were performed. In the first condition, brush strokes were delivered to the left upper arm proximal to the elbow. In the second condition, brush strokes were delivered to the left forearm proximal to the wrist. Based on previous EEG work we expected that CT-related brain activity should have a very late onset (>400 ms post stimulation) due to slow peripheral conduction velocity. Altering the stimulation sites i.e., brushing on the upper arm vs the forearm should give rise to a time-delay of CT-related brain activity with approximately 200 ms between the two conditions.
Preliminary results show clear and consistent somatosensory evoked fields (SEFs) to the onset and offset of the brush strokes i.e., contact with the skin, and time-frequency analyses show patterns of early-onset beta and alpha de-synchronization that are most prominent over parietal areas. These patterns reflect the signalling from fast Aβ afferents. Moreover, there are indications of a very late (onset >400 ms post stimulation) CT-related SEF and alpha synchronization over midline parietal sensors, but the postulated time shift between the two conditions is not consistent over subjects.

Disclosures: E.J. Eriksson: None. D. Lundqvist: None. J. Schneiderman: None. V. Jousmäki: None. J. Wessberg: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.05/DD9

Topic: D.03. Somatosensation: Touch

Support: American Legion Brain Sciences Chair

Title: Dynamic cortical network processing of a somesthetic stimulus as revealed by magnetoencephalography (MEG)

Authors: *A. C. LEUTHOLD, C. CHORN, A. P. GEORGOPOULOS;
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Abstract: The dynamic interactions among cortical areas involved in processing somesthetic stimuli over time are not well understood. In this study we used MEG to explore the time-evolving neural interactions following the application of a single stimulus on the right finger.

Healthy participants lay supine with eyes closed, and the right arm to the side and angled slightly away from the body. The palm was up and the stimulus delivered to the right index finger near the tip. The stimulus was an air driven membrane in contact with the skin. The Somatosensory Stimulus Generator (4D Neuroimaging, San Diego, CA) was set to a pressure of 100 kPa and a duration of 20 ms, which produced a reliably perceived stimulus. The stimulus was repeated 100 times with a long (relative to the duration of the stimulus) interstimulus interval (ISI) of 10 seconds. Data were collected with a Magnes 3600 MEG instrument (4D Neuroimaging, San Diego, CA). The system has 248 channels using 1st order axial gradiometers detection coils, with an on-center separation of just under one inch. The MEG signals was recorded continuously with a bandwidth 0.1 to 400 Hz and a sampling rate of 1017.25 Hz (i.e. every 0.974 s). The resulting data file consisted of 248 (sensor) columns and ~10000 rows (every 0.974 s, for 10 s). For each stimulus, MEG time series were prewhitened using a (50,1,3) ARIMA model that we have found most appropriate for such data (Mahan et al., Proc 14th Python In Science Conference, Scipy, Austin, TX, 2015). Next, the crosscorrelation function (CCF; ± 50 lags, corresponding to ± 48.7 ms) was computed for all 30628 sensor pairs and analyzed as a function of sensor location and distance between pairs. The analysis revealed a systematic variation in the values of crosscorrelations at different lags, reflecting lag-dependent strength and polarity of neural interactions following a single stimulus. Results from the 100 independent single stimulus presentations were compared among themselves and summary statistics computed to derive an expected average, time-evolving, dynamic cortical processing network, including strength, polarity and timing of interactions among its elements.

Disclosures: A.C. Leuthold: None. C. Chorn: None. A.P. Georgopoulos: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.06/DD10

Topic: D.03. Somatosensation: Touch

Support: Rutgers University Research Fund

Title: fMRI representation of erotic vs non-erotic genital self-stimulation; attenuation during the post-orgasmic refractory period in men

Authors: *K. ALLEN[1,2], B. KOMISARUK[3];
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Abstract: The neural mechanism underlying the inability of men to have rapid, consecutive orgasms (the "refractory period") is unknown. While prior studies have analyzed neural responses to erotic stimuli, none has compared erotic and prosaic touch, or *imagined* erotic and prosaic touch before/after orgasm. Thus the present study (in healthy men), mapped fMRI responses to genital self-stimulation, self-patterned to feel "erotic" versus "prosaic", prior to orgasm and during the post-orgasmic "refractory" period. Prior to orgasm, a number of brain regions were more active during "erotic" than "prosaic" self-stimulation including the operculum (SII), anterior cingulate, and insula, and we confirmed our previous finding that genital self-stimulation activated the paracentral lobule (i.e., the "genital sensory cortex" of the "Penfield homunculus"). Just "imagining" genital stimulation prior to orgasm also activated the paracentral lobule. By contrast, during the post-orgasmic refractory period, neither the physical nor imagined genital self-stimulation significantly activated the paracentral lobule, whereas the fMRI response to a finger tapping control, was not affected. Moreover, during the refractory period (compared to the orgasm phase), we observed a significant increase in activity of the rostral temporal cortex and the septum. *Conclusion*: As lesions of the rostral temporal lobe induce the Kluver-Bucy "hypersexuality" syndrome, and lesions of the septum have been described as disinhibiting sexual responses in humans and animals*, the activation of these two regions during the refractory period, combined with the attenuation of genital sensory responses in the paracentral lobule during the refractory period, suggest the existence of an active neural inhibitory mechanism underlying the refractory period.
*for review: Komisaruk & del Cerro, Hbk Clin Neurol, 2015:130:109-119.

Disclosures: K. Allen: None. B. Komisaruk: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.07/DD11

Topic: D.03. Somatosensation: Touch

Support: Grant-in-Aid for Scientific Research (A) (#25249026) and (B) (#25303013)

Title: Neural activity in the secondary somatosensory cortex during the tactile working memory delay period

Authors: *Y. YU[1], J. YANG[1], H. SHIGEMASU[2], H. KADOTA[2], K. NAKAHARA[2], H. YAMAMOTO[3], Y. EJIMA[1], J. WU[1];
[1]Okayama Univ., Okayama, Japan; [2]Kochi Univ. of Technol., Kochi, Japan; [3]Kyoto Univ., Kyoto, Japan

Abstract: The human secondary somatosensory cortex (SII) is a cytoarchitectonic region located in the bilateral parietal operculum. Previous non-human primate studies indicated that the SII has sensory-related functions involved in receiving and integrating somatosensory information and mnemonic-related functions linked to the processing of tactile working memory (TWM). However, the mnemonic-related functions of the human SII in the context of TWM processing remain unclear. In the present study, we used delayed tactile orientation-matching tasks (separated by up to 16-s delays) to determine whether the human SII delay period activity reflects the retention of sensory information or the rehearsal of memory traces in TWM. Twenty-six healthy, right-handed subjects (21 males and 5 females; mean age 22.6 years) participated in the fMRI experiment. Participants were asked to memorize a first grating orientation (GO1) pressed onto their right or left middle finger and then to judge whether this was the same as or different from a second tactile grating orientation (GO2) after a random delay interval between 2 s and 16 s. To confirm the activation tendency in the bilateral SII during each delay period, we conducted ROI analysis in the bilateral SII and used the SPM12 to extract BOLD signals from whole anatomical areas of the bilateral OP1 and to calculate percent signal changes. The results showed that the bilateral SII regions were significantly activated by both the GO1 and GO2 phases and that the positive activation related to GO1 extended to the earliest delay period (i.e., the first 2 s). However, the activity of SII differed from other memory retention related areas, such as the persistent delay activity in dorsal prefrontal cortex, which is thought to reflect the sustained internal representations of information in the WM. By contrast, the bilateral SII showed an initial increase in activity followed by decreased activation during the delay period (peak at 12-s temporal interval). As mentioned above, memory retention is often assumed to depend on the persistent neural activity, but recent evidences suggest that the constantly changing delay neural activity seems to reflect the attention-based rehearsal of memory traces. The present result suggests that tactile memory traces are initially sustained in the SII and are subsequently transferred to areas of the prefrontal and parietal areas for sustained memory processing. Therefore, the SII may be linked to the sustained engagement of an attention-based rehearsal mechanisms occurring during the delay period.

Disclosures: Y. Yu: None. J. Yang: None. H. Shigemasu: None. H. Kadota: None. K. Nakahara: None. H. Yamamoto: None. Y. Ejima: None. J. Wu: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.08/DD12

Topic: D.03. Somatosensation: Touch

Title: A new simple objective method to evaluate haptic perception

Authors: *Y.-T. TSENG[1], N. ELANGOVAN[2], J. KONCZAK[2];
[1]Human Sensorimotor Control Lab, Sch. of Kinesiology, [2]Sch. of Kinesiology, Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Haptic perception refers to the ability to perceive an object's shape and texture by active touch, typically by exploring its surfaces by our hands. Existing haptic perceptual assessment techniques often either require specialized laboratory equipment and/or provide not very precise and reliable measurements. Psychophysical somatosensory evaluation has been proven to yield reliable and precise for proprioception but not has been extensively utilized in haptic assessments. This proof-of-concept study evaluated haptic acuity for perceiving object curvature in healthy young adults. The portable assessment tool consisted of 28 small objects of curvature ranging from 0 to 6.6 x 10^{-3} mm^{-1} (lateral deviation (LD): 0 - 34 mm). Using a 2-forced-choice paradigm, 11 healthy young adults (Age, mean ± SD: 23.2 ± 3.1 years) performed two tasks that tested the abilities to 1) detect curvature (*detection task*), and 2) discriminate between two curved surfaces (*discrimination task*). In both the tasks, participants moved the index finger of their dominant hand over the curved surface of a test block with vision occluded. Detection involved identifying whether a block was curved. Discrimination required the exploration of two blocks (standard had LD = 20 mm) and to identify which block was more curved. During testing subsequent stimuli were selected using an adaptive algorithm (Ψ-marginal method) to reduce testing time. After testing, psychophysical thresholds were obtained by fitting a logistic Weibull function to the response data. Test duration for the detection task ranged from 4-6 mins, while the discrimination task required 8-10 mins. Mean haptic threshold for detecting curvature from a straight surface (LD = 0 mm) was 3.72 ± 1.43 mm (Range: 1.76 - 5.97mm). Mean haptic discrimination threshold was 2.85 ± 0.47 mm (Range: 2.06 - 3.77mm). CONCLUSION: The results establish the feasibility and practicality of this novel method to objectively assess haptic perception function. A larger sample size is necessary to establish a normative database against which clinical populations can be compared. Once these steps are completed, the test has the potential to be used in clinical practice

Disclosures: Y. Tseng: None. N. Elangovan: None. J. Konczak: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.09/DD13

Topic: D.03. Somatosensation: Touch

Support: National Center for Complementary and Integrative Health, NIH

Title: H-coil rTMS to S2 and insula affects only S2; alters touch intensity but not pleasantness

Authors: L. K. CASE[1], E. A. RICHARDS[1], C. M. LAUBACHER[1], H. OLAUSSON[2], P. A. SPAGNOLO[3], B. WANG[1], *M. C. BUSHNELL[1];
[1]NCCIH/NIH, Bethesda, MD; [2]Linköping Universiy, Linköping, Sweden; [3]NIAAA/NIH, Bethesda, MD

Abstract: Numerous studies point to divergence in the neural processing of touch discrimination and affect. In line with this, we recently reported that repetitive transcranial magnetic stimulation (rTMS) inhibition of S1 decreased tactile discrimination and increased touch intensity but had no effect on touch pleasantness (Case et al., 2016). In contrast, the posterior insula (pINS) has been implicated in affective touch, in part because it receives projections from c-tactile fibers, sensory afferents whose firing rate correlates with the perceived pleasantness of gentle touch (e.g. Löken et al., 2009).

In the current study, we probed the causal role of S2 and pINS in perception of touch intensity and pleasantness. We used an H-coil designed for deep brain stimulation to target pINS with 1-Hz inhibitory rTMS. Because S2 lies superficially above pINS, S2 and pINS were necessarily stimulated together. We hypothesized that S2-pINS stimulation would decrease perceived touch intensity and touch pleasantness but would not affect sensory discrimination.

Nineteen healthy adults (8 male) completed two 20-minute sessions of 1-Hz rTMS on separate days. One session targeted right hemisphere S2-pINS and the other targeted the vertex (control). Before and after rTMS, subjects rated the intensity and pleasantness of gentle touch (3 and 30 cm/s; palm and dorsum of left hand) and performed a 2-point discrimination task (dorsum). Subjects also completed an fMRI scan while receiving gentle touch to validate H-coil inhibition of S2-pINS.

As predicted, rTMS to S2-pINS decreased ratings of touch intensity (compared to the vertex; $F(1, 132.3 = 2.89)$, one-tailed $p = 0.046$). However, rTMS did not alter ratings of touch pleasantness *(*$F(1, 132.6 = 0.90)$, $p = 0.34$) or affect sensory discrimination ($F(1, 196 = 0.00)$, $p = 1.00$). A whole-brain analysis ($N = 16$) showed no differences in BOLD response to brushing between rTMS sessions, but a region of interest analysis found reduced S2 response to brushing after S2-pINS rTMS (compared with vertex; $t(16) = 1.89$, one-tailed $p = 0.04$). Neither pINS nor the untargeted right hemisphere S1 showed any effect of rTMS ($t(16) = 0.60$, $p = 0.56$; $t(16) = 0.78$, $p = 0.45$).

Our study is one of the first attempts to target the insula with an H-coil. Together, our behavioral and fMRI results suggest that 1-Hz rTMS to S2-pINS reduced activity in S2 and decreased perceived touch intensity, demonstrating the causal role of S2 in touch intensity perception. In contrast, we were unable to detect any effect of rTMS on pINS or on touch pleasantness. This study confirms the role of S2 in touch intensity but not touch discrimination, and it highlights the challenges of reaching the insula with rTMS.

Disclosures: L.K. Case: None. E.A. Richards: None. C.M. Laubacher: None. H. Olausson: None. P.A. Spagnolo: None. B. Wang: None. M.C. Bushnell: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.10/DD14

Topic: D.03. Somatosensation: Touch

Title: Spinal projection pathways of human C-tactile afferents

Authors: *A. MARSHALL[1,2,3], M. SHARMA[4], K. MARLEY[5], F. MCGLONE[2];
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Abstract: Introduction: Microneurographic and psychophysical studies have established the existence of a system of low threshold mechanosensitive C-fibres, so called C-Tactile (CT) afferents, in human hairy skin. CT afferents show velocity dependent spiking in response to gentle stroking touch and are hypothesised to encode the pleasant nature of touch. Currently the CT ascending spinal projection pathways in humans are unknown. Whilst their putative homeostatic function and likely primary cortical target (posterior insula) suggest spinothalamic transmission there is in rodents also evidence of significant integration of primary mechanosensitive information across all nerve classes in the dorsal horn. To address this we assessed for alterations in pleasant touch following therapeutic spinothalamic tract ablation.Methods: Nine patients with intractable unilateral cancer related pain underwent assessment of discriminative and affective touch before and after anterolateral C1/C2 cordotomy. Stroking at velocities optimal (3cm/s) and sub-optimal (0.3 and 30cm/s) for CT afferent activation were performed on the dorsal aspect of the right and left forearm. Patients were asked to rate the pleasantness of touch. Absolute pleasantness ratings and the CT preference index ([rating for 3cm/s x 2 - ratings for 0.3cm/s + 30cm/s] / 2) were calculated. Discriminative touch was assessed using two-point discrimination, tactile detection thresholds and graphesthesia. Results: Ablation of the spinothalamic tract was confirmed by total or sub-total loss of cold and warm sensation contralateral to the cordotomy as well as the complete or partial abolishment of the cancer related pain. Discriminative touch showed no significant difference between lesioned and non-lesioned sides and was unaltered by cordotomy. Pleasantness ratings for CT optimal touch were significantly higher across all conditions ($p<0.005$). A small but significant ($p<0.05$) reduction in CT preferred touch was seen following cordotomy on the side contralateral to lesioning. There was a significant reduction in CT preference index ($p<0.005$) on the side contralateral to cordotomy. No change was seen on the side ipsilateral to lesioning. Conclusion: The alterations in the perception of CT optimal touch contralateral to cordotomy lend support to hypothesis that information salient to affective touch is transmitted in the spinothalamic tract. However, unlike the dramatic changes in temperature and nociception, the effects are relatively

subtle which could reflect spinal integration, incomplete ablation of spinothalamic pathways or top-down processing of dorsal column cortical input.

Disclosures: A. Marshall: None. M. Sharma: None. K. Marley: None. F. McGlone: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.11/DD15

Topic: D.01. Sensory Disorders

Support: 2

Title: Is ineffective processing of sensory information linked to rewired pathways for sensory-motor input? A functional connectivity study on Nerve Growth Factor Beta mutation carriers.

Authors: *H. VAN ETTINGER-VEENSTRA, I. PERINI, I. MORRISON;
Linköping Univ., Linköping, Sweden

Abstract: Deficits in stimulus-appropriate behavioral responses to pain and affective touch caused by a reduction in thin-diameter sensory afferents (C nociceptors and C-tactile afferents) are linked to a rare mutation (R221W) of the human nerve growth factor beta (NGFB). Mutation carriers show more pain and pleasant touch indifference compared to controls, with high individual variability [1,2]. Following findings of [2,3] suggesting a vital executive role for midcingulate cortex (MCC) in context-sensitive behavioral responses to pain, we investigated functional connectivity differences in heterozygous carriers of the R221W mutation. **Methods**: Resting state fMRI of 10 min on 12 R221W carriers and 12 controls at a Philips 3T Ingenia, SENSE head coil, single-shot EPI gradient echo, TR/TE/FA/resolution = 2s/30ms/30°/3.4mm^3, whole-brain coverage. Preprocessing in SPM12 for realigned, normalized and smoothed images (8mm FWHM). ROI-to-ROI functional connectivity analysis (in CONN-toolbox; atlas-based and a 10mm radius sphere at MCC) at $p<0.05$ FDR corrected. For 6 R221W carriers, a quantification of C-fiber density was obtained using corneal confocal microscopy. **Results**: R221W carriers showed increased connectivity between nucleus accumbens (NAc) and left temporal fusiform gyrus compared to controls. In the R221W group, lower C afferent density was associated with increased connectivity between MCC and sensorimotor cortices on the pre- and postcentral gyri. Carriers, but not controls also showed increased connectivity between MCC and putamen, left temporal fusiform and right lingual gyrus. **Discussion:** NAc is suggested to integrate cognitive and affective information to engage in action selection [4]. Fusiform gyrus contains a multisensory input region [5] modulating emotional responses [6]. Interestingly, no altered

functional activation in NAc was found during pain for the R221W group (post-hoc analysis, see [2] for dataset). The R221W carriers may have a functional rewiring involving basal ganglia, sensorimotor regions and MCC, and fusiform gyrus. As a compensatory mechanism, MCC and sensorimotor connections might be increased as an effect of lack of reduced sensory afferent input, and reflected in compensatory functional connectivity between other affective modulatory regions, as well as and altered behavioral responses to pain [2]. **References:** [1] Morrison et al. Brain. 2011;134(4):1116-26. [2] Perini et al. J Neurophysiol 2016; DOI: 10.1152/jn.00667.2015. [3] Perini et al. J Neurosci. 2013;33(40):15930-9. [4] Floresco. Annu Rev Psychol. 2015;66:25-52. [5] Kassuba et al. Front Psychol. 2014;5. [6] Pehrs et al. SCAN. 2014;9(11):1770-8.

Disclosures: H. Van Ettinger-Veenstra: None. I. Perini: None. I. Morrison: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.12/DD16

Topic: D.01. Sensory Disorders

Title: The influence of testosterone on pain perception differences between males and females exposed to the cold pressor test

Authors: M. ARCHEY[1], S. GARDON[1], E. CROCKETT[3], K. GOLDEY[1], *J. A. BOYETTE-DAVIS[2], *J. A. BOYETTE-DAVIS[2];
[2]Psychology, [1]St. Edward's Univ., Austin, TX; [3]Southwestern Univ., Georgetown, TX

Abstract: Pain is a complex and demanding sensation that affects millions every year and represents one of the leading causes for health care intervention and costs. Research indicates that women are more susceptible to pain than men, both in acute and chronic forms. While the reasons for these sex differences are not well defined, it is thought that hormones may have a modulating effect. Indeed, several lines of literature indicate that estrogen and progesterone are especially important in female pain responding. Interestingly, testosterone has not received adequate consideration in this literature, despite the fact that females have lower levels than males. In the present study, saliva samples were collected from males and females completing a cold pressor test (CPT) and analyzed for testosterone concentration levels. The CPT is a common tool used for experimentally inducing pain, in which healthy participants place their hand in a container of very cold water (2^0 C) and report the level of pain perceived using a visual analog scale. Fifty-six participants (16 males, 40 females) completed the CPT and provided saliva that was later analyzed with hormone immunoassay kits. The results support previous findings that females have lower pain thresholds than males which can be explained in terms of gender

differences in salivary testosterone levels. This study therefore adds to the body of literature investigating the mechanisms of pain, and has interesting implications for possible treatments for pain.

Disclosures: M. Archey: None. S. Gardon: None. E. Crockett: None. K. Goldey: None. J.A. Boyette-Davis: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.13/DD17

Topic: D.03. Somatosensation: Touch

Support: ERC-2013-StG-336050 (FP7)

Title: Neural correlates of distorted body representations underlying tactile distance perception

Authors: *L. TAMÈ[1], M. I. SERENO[2], R. SADIBOLOVA[1], M. R. LONGO[1];
[1]Dept. of Psychological Sci., Birkbeck, Univ. of London, London, United Kingdom; [2]Univ. Col. London/Birkbeck, Univ. of London, London, United Kingdom

Abstract: Tactile distance perception is believed to require that immediate afferent signals be referenced to a stored representation of body size and shape (the *body model*). For this ability, recent studies have reported that the stored body representations involved are highly distorted, at least in the case of the hand, with the hand dorsum represented as wider and squatter than it actually is. Here, we investigated whether we can elicit the same type of distortions by using an MR-compatible system for somatosensory stimulation (airpuff) and in turn define the neural basis of this phenomenon. In each trial, participants received sequential tactile stimulations via airpuff on two out of nine points on a square grid (5x5cm) on the dorsum of their hand. In a behavioural experiment (Exp. 1) they estimated the distance between the two points by adjusting the length of a visually-presented line on the screen. The technique of multidensional scaling (MDS) was used to reconstruct a perceptual map of tactile space. Analysis of spatial distortion using Procrustes alignment showed that maps were stretched in the mediolateral hand axis. These behavioural results are consistent with previous studies, in which there were clear biases to overestimate distances oriented along the mediolateral axis of the hand compared to the proximodistal axis. In order to determine the neural correlates of these body distortions, we performed an fMRI study (Exp. 2) - functional scans in a random block design and a tactile localiser - in which the same nine points were this time individually stimulated. For each participant, we measured the response pattern generated by each stimulated point by contrasting

each stimulation condition (e.g., repeated stimulation of point 1, 2, etc) against baseline. In order to relate the representations between the different points and to computational models, we compare response-pattern dissimilarity matrices in several regions of interests (ROIs). ROIs were defined using both anatomical (i.e., probabilistic map) and functional (i.e., functional localiser) criteria at individual level. Similar to the behavioural experiment, we used MDS to reconstruct maps of the neural representation of tactile space using the values from the dissimilarities matrices. We expect to find a similar pattern of results (i.e., distortions) in the brain areas, of the tactile representation processing, that generate the distorted body representations we found in the behavioural experiment.

Disclosures: L. Tamè: None. M.I. Sereno: None. R. Sadibolova: None. M.R. Longo: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.14/EE1

Topic: D.03. Somatosensation: Touch

Support: FWO grant G065715N

BOF-ZAP Startup financing

Title: Functional MRI responses in monkey somatosensory cortex during grasp and touch observation.

Authors: S. SHARMA, *K. NELISSEN;
Dept. of Neurosciences, KU Leuven, Leuven, Belgium

Abstract: Background: Numerous studies in human and non-human primates have shown that merely observing other's actions can modulate activity in the observer's motor cortices. Similar automatic responses have also been suggested in human somatosensory cortices when actions involving touch are observed, with some studies suggesting an involvement of primary S1 (Pihko et al., 2010) and/or secondary S2 somatosensory regions (Keysers et al., 2004), while others emphasize the involvement of more posterior parietal regions (Chan and Baker, 2015). Methods: Here we investigated brain responses during grasp or mere touch observation using contrast-enhanced functional MRI (Siemens 3 Tesla) in monkeys. We first localized hand representations of S1 and S2 in three macaques (male, 4-6 kg) by stimulating the hand region with a brush in anaesthetized fMRI scans. In subsequent awake experiments, we scanned these monkeys while they observed videos of various actions displaying different instances of touch (grasping or

simple touching of objects or hands). Control videos depicted the same actors performing reaching movements towards the same objects/hands without interacting with them. In an additional experiment, videos of different actions (grasp and touch) towards a hand displaying obvious skin displacements (Ferri et al., 2015) were also presented. Results: A region-of-interest analysis of S1 and S2 hand regions responding during passive tactile stimulation did not yield significant fMRI signal increases during either grasp or touch observation (compared to controls), nor did observing actions showing obvious skin displacements. Posterior parietal regions, including AIP and PFG, as well as premotor F5, in addition to early visual and STS regions, showed significant responses during observation of both grasp and mere touch (compared to controls). Interestingly, a more anterior S2 sector, that mainly responds during monkeys' active hand manipulation tasks like grasp execution (Nelissen and Vanduffel., 2011; Ishida et al., 2013), showed consistent responses during touch observation, particular those actions involving a grasp. Conclusion: Our results suggest that somatosensory sectors of monkey S1 and S2 that respond significantly during passive tactile stimulation do not show modulations during grasp and touch observation. However, a more anterior portion of S2 responds during touch observation. These observations are in line with recent evidence of single-cell responses in macaque S2 during human action observation (Hihara et al., 2015).

Disclosures: S. Sharma: None. K. Nelissen: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.15/DP03 (Dynamic Poster)

Topic: D.03. Somatosensation: Touch

Support: ERC grant n.323606 "Parietalaction"

Title: Unravelling somatotopy in time

Authors: ***P. AVANZINI**[1,3], F. CARUANA[2,3], V. PELLICCIA[4], L. TASSI[4], G. LO RUSSO[4], G. A. ORBAN[1], G. RIZZOLATTI[2,3];
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Abstract: We recently reported that a number of cortical areas respond to median nerve stimulation, far beyond the primary (SI) and secondary (SII) somatosensory areas, describing also their temporal pattern of activity. Whether these areas are somatotopically arranged or not is still a matter of debate. In this study, we examined the intracerebral recordings collected in 104

hemispheres (L=49, R=55) of 96 patients during the electrical stimulation of three peripheral nerves, namely the median, tibial and trigeminal (mandibularis branch, V3) nerves. Over 11680 leads exploring grey matter (L=5678, R=6002), we identified 381 responding for tibial (L=178, R=203), 1089 for median (L=489, R=600) and 258 for trigeminal (L=82, R=176) stimulation. Outside SI, we report a clear somatotopical arrangement of primary motor and dorsal premotor cortex, as well as a partial somatotopy for supplementary motor areas and anterior parietal operculum (PR). The comparison of the three maps highlighted several areas housing multiple nerve representations, and two regions responding to all three stimulations: the first included OP1, OP2 and insular long gyri, bilaterally, while the second was located in the hand area of right primary motor cortex. The comparison between left and right hemispheres revealed no lateralization for tibial nerve representation, a greater responsiveness of right premotor cortex for median nerve representation and, notably, a large right-sided bias in the whole motor system for trigeminal nerve. To examine the time course of the responsive areas, we clustered all responsive leads on the basis of their time course, separately for each stimulation dataset. Results showed prototypical patterns for the three nerves, with 3 phasic clusters (strong, middle and weak), a prolonged activity peaking later with respect to the strong phasic response and a tonic cluster. The backmapping of these clusters on the cortical sheet showed that the strong phasic maps yield a precise somatotopical organization of primary somatosensory cortex, that the leads with delayed activity are mainly exploring the dorsal motor and premotor cortices and, finally, that the tonic activity is generated by a peri-sylvian network including mostly OP1, OP2, human PR and posterior insular cortex. The different time course of afferents from hand and mouth in the hand-part of motor cortex may subserve hand-mouth interactions, giving a functional meaning to the so-called imprecisions of somatotopy. Supported by ERC Grant Parietalaction and a grant from Fondazione Cariparma to GR.

Disclosures: P. Avanzini: None. F. Caruana: None. V. Pelliccia: None. L. Tassi: None. G. Lo Russo: None. G.A. Orban: None. G. Rizzolatti: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.16/EE2

Topic: D.01. Sensory Disorders

Support: Wellcome Trust Grant 095939

Wellcome Trust Grant 098362

Wellcome Trust Grant 096185

Wellcome Trust Grant 100227

Wellcome Trust Grant 091593

Title: Knowing about gut feelings

Authors: *J. S. WINSTON[1,2], A. V. EMMANUEL[1,2], E. ATHANASAKOS[2], K. OHRNBERGER[1], R. J. DOLAN[1], S. M. FLEMING[1], G. REES[1];
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Abstract: Homeostasis depends not only upon knowledge of the external environment provided by *exteroceptive* senses (the traditional five senses) but also upon awareness of the state of the body. Such *interoceptive* awareness is likely to be of great importance in the experience of medical symptoms, the majority of which arise from internal or visceral sensation. Despite its biological and clinical importance, interoceptive awareness is understudied and poorly understood, particularly with respect to higher levels of cognition. We used rectal electrosensation in healthy human participants (n=51) to establish the first psychophysically-controlled study of interoceptive awareness. This allowed us to explore not only basic interoceptive perceptual processes (such as stimulus detection or magnitude estimation) but also the higher order cognitive process of awareness of basic task performance, known as "metacognition". We show that exteroceptive and interoceptive metacognitive abilities are dissociable, and that poorer interoceptive metacognition is associated with a greater burden of subjective-health complaints. This implies that the study of metacognitive processes is an important avenue for developing new mechanistic and diagnostic insights into the expression of medical symptoms, including many challenging clinical disorders where symptoms are reported in the absence of objective markers of illness.

Disclosures: J.S. Winston: None. A.V. Emmanuel: None. E. Athanasakos: None. K. Ohrnberger: None. R.J. Dolan: None. S.M. Fleming: None. G. Rees: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.17/EE3

Topic: D.03. Somatosensation: Touch

Title: Interaction of attention, instructions, and proprioception in a joint matching and N-Back task

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Abstract: *Background*
Application of vibration to the muscle tendon is understood to produce an illusion of movement at the joint, but not in all people at all times. Thus it is possible that this phenomenon is not purely a physiological response, but instead may be influenced by factors such as attention and comprehension of instructions.
Purpose
The purpose of this study was to examine the influence of investigator provided instructions, locus of attention, and distraction on a joint matching task during tendon vibration.
Methods
10 healthy, young adults between the ages of 20-32 were asked to continuously match the position of their elbows for 10 seconds while vibration was applied to the biceps tendon of their dominant arm while their vision was blocked. The following conditions were tested 1) no instructions about the direction or magnitude of the anticipated illusion 2) correct instructions and 3) false instructions; 4) direction to an external focus of attention 5) direction to an internal focus of attention and 6) a simultaneous N-back task. The N-back task requires the participant to repeat the previous word, every time a new word is provided. The maximum absolute difference between the two elbow joints was recorded and the absolute error (AE) as a measure of the magnitude of the illusion and variable error (VE) as a measure of the variability of participant responses between trials was calculated. The percent of correct responses and reaction time during the N-back task alone and during the joint matching task was also obtained.
Results
A repeated measures MANOVA and subsequent univariate analysis confirmed a significant effect of instruction type on both AE and VE. The condition of providing No Instructions lead to the least VE and the greatest AE of all instruction conditions. Instructions to an external focus of attention lead to the least AE. The distraction task did not have an effect on either AE or VE. However, participants reported significantly more correct responses when simultaneously performing both the N-Back task and the joint matching task than the N-Back task alone.
Conclusion
The results of this study suggest that illusions induced by tendon vibration are influenced by instructions and are likely not a purely physiological response. However, it is probable that directed attention toward the perception of vibration is not required. The data suggest that investigators should carefully select the language provided in their instructions and maintain consistency across participants when investigating human responses to tendon vibration.

Disclosures: A. Forrest: None. B. Lee: None. A. Thrasher: None. R. Kabbaligere: None. C. Layne: None.

Poster

235. Somatosensation: Human and Non-Human Primates

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 235.18/EE4

Topic: D.01. Sensory Disorders

Support: NSF GRFP Grant DGE-1256260

Title: Gender differences in kinesthesia

Authors: *Y. ACOSTA-SOJO[1], D. E. ADAMO[2], B. J. MARTIN[1];
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Abstract: Vibration-induced movement illusions are widely used to investigate the role(s) of proprioceptive information. In a recent study we showed that a dominant non dominant asymmetry in movement perception asymmetry was gender dependent and females appeared to be more sensitive than males Hence, the aim of the study was to determine whether these asymmetries/symmetries and sensitivity could be frequency dependent and use this data as baseline for further comparison with stroke patients. Our hypothesis is that the effect of frequency would significantly differ between stroke patients and healthy participants. Six strongly right handed young adults (3 males, 3 females, aged matched), free from any neurological disorders, participated in this pilot study. Vibration-induced Illusions of elbow flexion were matched concurrently with the opposite arm for 40, 60, 80, 120 or 150 Hz frequencies with a constant displacement amplitude of 100 μm. Vibration was applied for 10 seconds or less when the matching forearm flexion reached the torso. The preliminary results indicate as expected an increase in velocity of the matching movement as a function of the vibration frequency. In addition, a gender difference was also observed at all vibration frequencies. Asymmetries between dominant and non-dominant hand could not be clearly assessed. These results confirm a difference in proprioceptive sensitivity between females and males and may also strengthen our previous hypothesis proposing a feed forward control of matching movements.

Disclosures: Y. Acosta-Sojo: None. D.E. Adamo: None. B.J. Martin: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.01/EE5

Topic: D.05. Audition

Support: NIH Grant DC013501

NIH Grant DC013073

Title: Synaptic or intrinsic, that is the question; parsing out synaptic currents in mapping the auditory corticocollicular inputs with glutamate uncaging

Authors: *B. SLATER[1], S. K. SONS[2], D. A. LLANO[2];
[1]Neurosci., Univ. of Illinois Syst., Urbana, IL; [2]Mol. and Integrative Physiol., Univ. of Illinois, Urbana, IL

Abstract: In the auditory cortex, a subset of neurons in layers 5 and 6 project to the inferior colliculus. These projections have been shown to have a wide variety of effects when stimulated *in vivo*. Very little is currently known about nature of the inputs from the rest of the auditory cortex onto these cells. To investigate these inputs, we use laser photo-uncaging of glutamate to stimulate the cells that synapse onto the layer 5 and layer 6 corticocollicular cells in brain slices taken from adult mice. Pre-identified cells were recorded in a whole cell patch configuration then stimulated with a larger grid covering the area from the white matter to the pia. In this preparation we use a low calcium artificial cerebral spinal fluid method to isolate synaptic responses, and contrast this method with the more commonly used time window method. In identified layer 5 and layer 6 corticocollicular recordings, cells show spatial differences in their respective input maps with layer 5 having inputs coming from various layers compared to layer 6 which almost exclusively receives input from layer 6. Previously, layer 5 and layer 6 have been shown to have different electrophysiological properties and we find evidence that they differ in the nature of their inputs. Differences in these properties will likely then play different roles in modifying ascending information at the IC. These differences may explain the varied results seen in the inferior colliculus during *in vivo* stimulation of the auditory cortex.

Disclosures: B. Slater: None. S.K. Sons: None. D.A. Llano: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.02/EE6

Topic: D.05. Audition

Support: NSFC 20131351192

NSFC 20151311567

Title: Nmda receptor-dependent temporal processing plasticity in the developing auditory cortex requires $gaba_b$ receptor-mediated inhibition

Authors: D. CAI, R. HAN, M. LIU, F. XIE, L. YOU, Y. WANG, Y. YUE, *K. YUAN; Tsinghua Univ., Beijing City, China

Abstract: Faithful tracking of rapidly successive acoustic signals in the primary auditory cortex (A1) is vital to processing of temporal information and perception of natural sounds such as human speech and animal vocalizations. However, how cortical stimulus-tracking capacity developmentally emerges remains poorly understood. Using *in vivo* whole-cell recordings, we found that inhibition in the developing rat A1 was initially prolonged and strong, leading to cortical responses with considerably poor temporal resolution. Unexpectedly, exposure to repeated acoustic stimulation produced significant and long-lasting shortening of inhibition duration, resulting in strongly improved periodicity of cortical responses. Selective interruption of the signaling pathway of postsynaptic $GABA_B$ receptors shortened inhibition duration, prevented exposure-induced plasticity and improved cortical stimulus-tracking ability. Furthermore, the blockade of NMDA receptors abolished exposure effect. These results identified $GABA_B$ receptor as a key player in NMDA receptor-dependent , non-map plasticity of temporal processing in the developing cortex.

Disclosures: D. Cai: None. R. Han: None. M. Liu: None. F. Xie: None. L. You: None. Y. Wang: None. Y. Yue: None. K. Yuan: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.03/EE7

Topic: D.05. Audition

Support: NIMH Silvio Conte Center (P50MH094271)

Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program) initiated by the Council for Science and Technology Policy

Title: Modular innervation of primary auditory cortex (A1) by morphologically diverse thalamocortical axons

Authors: *E. E. DIEL[1], S. KUROKI[2], S. ITOHARA[2], T. K. HENSCH[1,2,3];
[1]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [2]RIKEN Brain Sci. Inst., Wako-shi, Saitama, Japan; [3]Dept Neurol, Boston Children's Hosp., Boston, MA

Abstract: Primary auditory cortex (A1) reflects the tonotopic layout of sound frequency within the peripheral organ of corti relayed by thalamocortical (TC) projections originating in the ventral medial geniculate body (MGBv) and terminating within cortical layer IV. While roughly topographic, TC axonal anatomy has not been fully characterized, especially in the context of their contributions to the tonotopic map. Here, we describe a strategy to label TC axons by targeted injection of AAV-Brainbow into mice expressing Cre recombinase in thalamic nuclei. We find that MGBv projections to A1 are non-uniformly distributed across the extent of the tonotopic axis, giving rise to domains of axonal innervation. This modular structure is recapitulated in functional connectivity using voltage sensitive dye (VSD) imaging of an acute TC slice preparation. Upon stimulation of MGBv, cortical responses best correlated with ΔF/F in thalamus are non-homogeneously distributed across the horizontal tonotopic axis in layer IV, revealing a modular pattern reminiscent of TC axonal labeling. Further leveraging the multi-colored Brainbow labeling to partially reconstruct several TC axons spanning these domains, we reveal a wide morphological diversity of branching patterns with respect to macroscopic modules. Many axons run along vertical trajectories between layers in largely columnar fashion, restricting branches within a domain to send collaterals into both layers IV and I. Other axons instead extend horizontal branches within layer IV to span axonal domains. These two TC axon subtypes, ‘columnar’ and ‘horizontal’, may differ in their contribution to domains and tuning properties in A1 or MGB subdivision of origin. Single arbor tracing by combined AAV-Brainbow and dilute AAV-Cre injection in C57Bl6/J mice, as well as various sound-rearing paradigms to alter tonotopy may offer further insight. Together, these results describe an unanticipated modular structure and the underlying contribution of individual axons to the auditory TC axon map.

Disclosures: E.E. Diel: None. S. Kuroki: None. S. Itohara: None. T.K. Hensch: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.04/EE8

Topic: D.05. Audition

Support: NIH MARC-USTAR grant 2T34GM007663-32

NIH/NIDCD grant RO1-04318

Title: Laminar distribution of calcium binding proteins in macaque auditory cortex.

Authors: *L. A. DE LA MOTHE[1], C. R. CAMALIER[2], H. CANSLER[3], S. M. HUBBARD[1], T. A. HACKETT[4];
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Abstract: Inhibitory interneurons play an important role in cortical computation, and characterizing their histochemical diversity and laminar distribution in cortex is key to understanding computational differences between areas and species. One major family are the GABA-ergic interneurons that express calcium binding proteins (CBP) parvalbumin (PV), calbindin (CB), and calretinin (CR). Though these groups of neurons have received attention in some species and cortical areas (Cruikshank et al., 2001; Kawaguchi and Kubota, 1997; Markram et al., 2004), their distributions remain to be fully characterized in primate auditory cortex, an important model for human communication. To address this, cortical depth distributions were examined for each CBP in the primary core region of macaque auditory cortex. Preliminary data suggests that PV neurons have comparable cell expression in both supragranular and infragranular layers, whereas CB and CR expression is located predominantly in the supragranular layers. In addition, these distributions were compared to depth distributions of fast and regular-spiking neurons classified from extracellular waveforms recorded in the same region. Interestingly, fast-spiking neurons exhibit similar distribution patterns as the PV expression, consistent with the identification of fast-spiking as a marker for PV cells in sensory cortex. Multi-fluorescent immunohistochemistry revealed that CBP were found to express largely distinct patterns of expression with limited incidence of co-localization.

Disclosures: L.A. de la Mothe: None. C.R. Camalier: None. H. Cansler: None. S.M. Hubbard: None. T.A. Hackett: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.05/EE9

Topic: D.05. Audition

Support: NIH R01DC04682

Title: Imbalanced excitation and inhibition in pyramidal cells in awake mouse auditory cortex.

Authors: *H. K. KATO, J. S. ISAACSON;
UCSD Sch. of Med., La Jolla, CA

Abstract: In the primary auditory cortex (A1), sound representations in principal pyramidal cells are shaped by inhibitory inputs provided by a variety of inhibitory neuron subtypes. Revealing the interplay between excitation and inhibition in individual neurons is of critical importance in order to understand how sound is encoded in the brain. Studies in anesthetized mice suggest that approximately balanced inhibition counteracts excitation to sharpen the tuning properties of pyramidal cells. While both parvalbumin-expressing neurons (PV cells) and somatostatin-expressing neurons (SOM cells) are thought to contribute to sound-evoked inhibition, the tuning properties of these interneuron subtypes are a matter of debate.
Here, we use cell type-specific large-scale calcium imaging to reevaluate the excitatory and inhibitory receptive fields in major neuronal subtypes in A1 of awake mice. We found that a large fraction of pyramidal cells show either purely inhibitory receptive fields or mixed excitatory/inhibitory receptive fields, suggesting an imbalance between excitation and inhibition in individual cells. In contrast to the coarse alignment of excitatory receptive fields to the A1 global tonotopic axis, inhibitory receptive fields showed high spatial heterogeneity, further suggesting a mismatch between excitation and inhibition. Furthermore, we observed stronger and broader excitation in SOM cells compared to PV cells or pyramidal cells. The broad tuning properties of SOM cells make them well poised to provide untuned inhibitory input onto pyramidal cells. Currently, we are investigating how inputs from distinct inhibitory neuron subtypes differentially shape tuning properties of pyramidal cells.

Disclosures: H.K. Kato: None. J.S. Isaacson: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.06/EE10

Topic: D.05. Audition

Support: NIH Grant UO1 NS090569

Title: Avalanche dynamics during spontaneous and sound evoked activity in the auditory cortex of awake mice

Authors: *D. E. WINKOWSKI[1], Z. BOWEN[2], S. SESHADRI[3], T. RIBEIRO[3], D. PLENZ[3], P. O. KANOLD[2];
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Abstract: In sensory cortical areas, thalamic afferents synapse primarily on mid-cortical layer neurons (L4) which in turn project to neurons in upper (L2/3) and lower (L5/6) cortical laminae. Within L2/3, neurons incorporate ongoing cortical activity, usually in the form of reverberating activity from within or distant cortical regions that reflect the state and behavioral context of the animal. These features, among others, may contribute to the heterogeneous organization of stimulus preference in L2/3. A well-established hypothesis is that cortical activity, especially in L2/3, reflects a complex system operating at or near criticality in order to maintain network stability while optimizing information processing. Prior studies have shown that spatiotemporal patterns of spontaneous neuronal activity organize into neuronal avalanches that exhibit signatures of critical dynamics; that is, the probability distribution of avalanche sizes can be fit with a power law of specific parameters. Here we study neuronal avalanche dynamics preceding as well as during sensory stimulation in primary sensory cortex. We investigate neuronal avalanches using in vivo 2-photon Ca^{2+} imaging of ongoing and sound evoked activity of primary auditory cortex (A1) in awake mice using GCaMP6s. We explore the relationship between properties of sensory stimuli, single cell responses, and population responses with respect to avalanches. We investigate the spectral tuning properties of neurons participating in avalanches in order to probe the relationship between criticality and sensory coding. On the single cell level, many neurons were tuned to a particular sound frequency but responded unreliably. On the population level, we found that avalanche statistics varied depending on the stimulus frequency and intensity of the presented sound. We speculate that stimulus frequency and intensity dependence reflected in avalanche size suggests that stimuli are encoded in distinct local subpopulations; not just single neurons. Furthermore, stimulus-evoked avalanches often contained neurons that are not overtly tuned to the presented stimulus. The role of these neurons in sensory coding is unclear. Our investigation provides insight into how neural networks

containing differing populations of neurons with varying firing rates stably encode information about sensory stimuli in the context of self-organized criticality.

Disclosures: D.E. Winkowski: None. Z. Bowen: None. S. Seshadri: None. T. Ribeiro: None. D. Plenz: None. P.O. Kanold: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.07/EE11

Topic: D.05. Audition

Support: NIH Grant DC006013

NIH Grant T32-GM007507

NIH Grant GM109086

Department of Anesthesiology, University of Wisconsin School of Medicine and Public Health

University of Wisconsin-Madison Office of the Vice Chancellor for Research and Graduate Education, with funding from the Wisconsin Alumni Research Foundation

Title: Control of cortical network activity by somatostatin- and parvalbumin-positive interneurons

Authors: *B. M. KRAUSE[1], D. J. UHLRICH[2], M. I. BANKS[1];
[1]Dept. of Anesthesiol., [2]Dept. of Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Experiments *in vivo* and in brain slices suggest that that the vast majority of spiking in auditory cortex in response to feedforward inputs occurs in the context of network bursts corresponding to synchronous, brief UP states in cortical neurons. Previous work also has shown that subtypes of inhibitory interneurons participate in this network activity to different degrees, and their involvement varies between distinct cortical regions. However, the degree to which these subtypes control the occurrence and magnitude of these network events, and the timing of pyramidal cell spikes within UP states, remains unclear. Here, we studied two types of cortical interneurons in slices of mouse auditory cortex, parvalbumin (PV+) and somatostatin (SOM+) interneurons. We found that both PV+ and SOM+ interneurons fired considerably more than pyramidal cells, but differed in timing: PV+ cells fired earlier in UP states, whereas SOM+ cells

were active later. PV+ cells were more precise and reliable relative to thalamocortical stimuli, whereas individual pyramidal cells were more precise relative to network activity; SOM+ cells were precise in neither sense. Although optogenetic suppression of either interneuron population increased overall population activity, suppression of PV+ cells reduced the onset latency and threshold of network bursts, whereas suppression of SOM+ cells increased burst duration. Surprisingly, the precision of spiking in pyramidal cells did not deteriorate upon suppressing either interneuron population. These data suggest that spike timing in auditory cortex is not tightly controlled by interneurons during network events, when cortical activity is at its highest level in the column.

Disclosures: B.M. Krause: None. D.J. Uhlrich: None. M.I. Banks: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.08/EE12

Topic: D.05. Audition

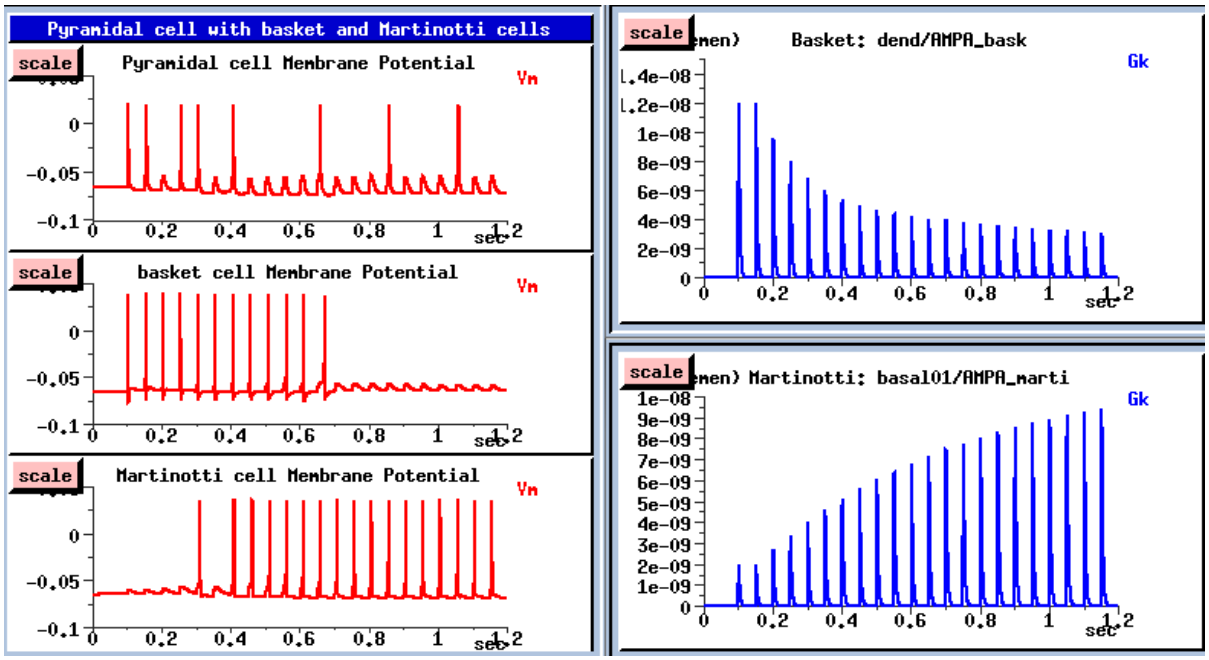
Support: NSF IGERT Grant 1144807

Title: A role for Martinotti cells in auditory cortex dynamics: A modeling study

Authors: *D. BEEMAN, L. NATALE;
Univ. of Colorado Boulder, Boulder, CO

Abstract: The importance of the role of inhibitory neurons in cortical dynamics and in auditory processing has long been recognized and has been the subject of many modeling studies. The ACnet2 model (genesis-sim.org/GENESIS) is a simple model of primary auditory cortex layer with 2304 multi-compartmental spiking pyramidal cells coupled to 576 inhibitory basket cells and tonotopically arranged thalamic inputs. The use of structurally realistic cell models provides proper dendritic locations of synapses and allows modeling of field potentials in auditory evoked potential simulations.
Martinotti cells are the next most common cortical inhibitory interneuron after basket cells and have significantly different properties that could affect cortical dynamics. They fire at low thresholds and produce adapting rather than tonic fast spiking spike trains. Their excitatory synapses are strongly facilitating, rather than depressing. Their axons contact pyramidal cell dendrites on different locations.
To study the effect of including Martinotti cells in the ACnet2 model, an 8 compartment model was created with GENESIS 2.4. The elongated bitufted morphology was based on published cell

images. Passive cell parameters and ion channel parameter fits were based on published electrophysiology data.
As a first step, a simple circuit was modeled consisting of a pyramidal, basket, and Martinotti cell, each receiving a 20 Hz spike train, and with the latter two inhibiting the pyramidal cell. The figure shows how the depressing synapses on the basket cells and the facilitating ones on the Martinotti cells affect their firing and that of the inhibited pyramidal cell during the three periods of separate and overlapping inhibition. This suggests a behavior that will broaden the spectra calculated from field potentials in the ACnet2 network.



Disclosures: D. Beeman: None. L. Natale: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.09/EE13

Topic: D.05. Audition

Support: NSF Grant DMS-1042134

NIH Grant R21DC012894

Title: L5 corticocollicular and L6 corticothalamic neurons support a parallel and complementary analysis of auditory stimulus features

Authors: *R. S. WILLIAMSON[1,2], D. B. POLLEY[1,3];
[1]Eaton Peabody Labs., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Ctr. for Computat. Neurosci. and Neural Technol., Boston Univ., Boston, MA; [3]Dept. of Otolaryngology, Harvard Med. Sch., Boston, MA

Abstract: Neurons in layers (L) 5 and 6 of the auditory cortex (ACtx) give rise to a massive subcortical projection that innervates all levels of the central auditory pathway as well as non-auditory areas including the amygdala, striatum, and basal ganglia. L5 and L6 neurons feature distinct morphology, connection patterns, intrinsic membrane properties and synaptic properties, yet little is known about how these differences relate to sensory selectivity *in vivo*. Here, we focused on two distinct ACtx L5 and L6 projection neurons; L5 corticocollicular neurons (L5CCol), and L6 corticothalamic neurons (L6CT).
We developed a dual-channel antidromic optogenetic "phototagging" strategy to isolate single L5CCol and L6CT units from extracellular recordings in awake, head-fixed mice. We injected two adeno-associated viral constructs (AAV) into ACtx of Ntsr1-Cre transgenic mice, in which Cre-recombinase is expressed only in L6CT neurons. One Cre-dependent AAV encoded Chrimson (a red-shifted channelrhodopsin) and a second non-specific AAV encoded hChR2. One optic fiber was then implanted near the surface of the inferior colliculus and a second near the medial geniculate body. By evoking antidromic spikes from L5CCol neurons with blue light and L6CT neurons with red light, we could simultaneously isolate and characterize both types of projection neurons with a single multi-channel recording probe in ACtx.
L5CCol neurons exhibited shorter response latencies and broader frequency tuning than L6CT neurons. Linear spectrotemporal receptive field (STRF) fits were able to explain a higher percentage of response variance in L5CCol neurons, indicating a higher degree of linearity in their responses when compared to L6CT units. Finally, we used a closed-loop evolutionary stimulus optimization strategy to identify the best stimulus for L5CCol and L6CT neurons across a 4-dimensional stimulus manifold. The evolutionary search strategy manipulated the modulation frequency, level, spectral bandwidth, and center frequency of noise tokens in real time based on spike feedback to identify an optimal stimulus. We found that L5CCol neurons featured a lower multi-dimensional sparseness index, indicating a reduced stimulus selectivity and a broader response distribution than L6CT neurons. These findings suggest a functional dichotomy in the form of stimulus-related modulation imposed by L5 and L6 neurons to subcortical targets. Future work will entail recording from these projection neurons during task engagement to establish how these functional differences are adaptively used in service of goal-directed behavior.

Disclosures: R.S. Williamson: None. D.B. Polley: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.10/EE14

Topic: D.05. Audition

Support: NIH Grant GM109086

NIH Grant GM116916

UW Department of Anesthesiology

Title: Anesthetic induced changes in cortical spike timing across awareness states

Authors: *N. S. MORAN[1,2], J. KAKES[1], S. M. GRADY[1], M. I. BANKS[1];
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Abstract: Introduction: The mechanisms of loss of consciousness (LOC) under anesthesia are poorly understood. Theoretical and experimental evidence indicates that network connectivity, and especially cortical feedback connectivity, is impaired upon LOC. However, anesthetics also affect stimulus representation in cortex. Anesthetics impair temporal representations in the ascending auditory pathway, but have also been reported to improve spike precision and decrease response variability. Here, we tested the effects of anesthesia LOC on spontaneous and stimulus-evoked spiking activity in auditory cortex. We chose three anesthetics with distinct molecular targets: Isoflurane (iso), which acts at multiple pre- and postsynaptic loci, propofol (pro), which acts primarily on $GABA_A$ receptors, and dexmedetomidine (dex), an α_2 adrenergic agonist. **Methods:** Spontaneous and click train-evoked (3-100Hz) multiunit activity was recorded from auditory cortex in rats chronically implanted with 16 channel microwire arrays under four conditions: waking (ctrl), sub-LOC and just-LOC doses of iso, pro and dex, and recovery. Spike timing precision and reliability was evaluated using the Spike Time Tiling Coefficient (STTC; Cutts & Eglen 2014 J. Neurosci 34:14288). This method was used to evaluate consistency across trials of single channel click train responses (within channel STTC), and the similarity (i.e. connectivity), during spontaneous activity, of spike trains on different channels (across channel STTC). **Results:** STTC peaked at click frequencies of 10-20Hz. All anesthetics caused a reversible decrease in within channel STTC. For iso and pro, the majority of this effect occurred at sub-LOC doses (STTC ctrl - just-LOC = 0.045 and 0.047, iso and pro respectively; $p < 0.03$). For dex, the decrease in STTC was only significant at the just-LOC dose (STTC ctrl - sub-LOC = 0.042, $p = 0.17$; ctrl - just-LOC =0.085, $p =0.007$). Although anesthesia decreased the STTC at all click frequencies, 10-20Hz clicks responses were preferentially impaired. In ctrl, between channel STTC decreased with increased distance between recording sites, but there were no

consistent effects of anesthetics on this measure. All three agents caused reversible increases in first spike latency (just-LOC - ctrl = 0.70, 3.5 and 5.3 ms for iso, pro and dex, respectively). **Conclusions:** All anesthetic agents had profound effects on the encoding of temporal information in auditory cortical responses, but these effects appear to be unrelated to LOC. Agent-specific effects likely arise due to diverse mechanisms of action. There was little effect on millisecond-scale spike precision in spontaneous activity.

Disclosures: N.S. Moran: None. J. Kakes: None. S.M. Grady: None. M.I. Banks: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.11/EE15

Topic: D.05. Audition

Support: NIH Grant R01DC013102

Title: Dopaminergic modulation of vocalization-selective neurons in the inferior colliculus acts via D2-like receptors

Authors: *J. M. HOYT[1], R. A. FELIX, II[1], D. J. PERKEL[2], C. V. PORTFORS[1];
[1]Integrative Physiol. and Neurosci., Washington State Univ. Vancouver, Vancouver, WA; [2]Univ. of Washington, Seattle, WA

Abstract: Background: The ability to understand speech relies on the accurate processing of these sounds by the auditory system. A variety of factors may impede accurate representation, including disorders associated with neuromodulatory systems. For example, individuals with Parkinson's disease suffer from deficits in speech perception, suggesting that dopamine may be involved in the normal encoding of behaviorally relevant sounds. The inferior colliculus (IC) receives inputs from multiple auditory and non-auditory sources and is rich in both dopaminergic terminals and D2-like receptors. Recent studies in our lab demonstrated that dopamine heterogeneously modulates responses of individual neurons to tones and noise in the IC of mice. However, it is currently unknown which type(s) of dopamine receptors is/are involved in such alteration of neuronal responses to auditory stimuli, and whether dopamine affects neuronal responses to communication sounds. In this study, we tested the following hypotheses: dopamine acts via D2-like receptors to alter auditory evoked neuronal responses in the IC; dopamine alters neuronal responses to conspecific vocalizations in the IC.
Methods: We recorded extracellular responses of single neurons in the IC of awake mice. We compared neuronal responses to tones and mouse vocalizations before and after iontophoretic

application of dopamine and D1- or D2-like agonists or antagonists. We quantified how activating or blocking dopamine receptors changed the rate and timing of action potential spikes.
Results: We found that the effects of both dopamine and a D2-like agonist on spiking rate in IC neurons were heterogeneous as both similarly increased or decreased auditory-evoked responses to pure tones, while a D2-like antagonist reversed the effects of dopamine. Moreover, both dopamine and a D2-like agonist similarly affected responses of IC neurons to vocalizations in the same relative direction as responses to tones, while a D2-like antagonist reversed the effects of dopamine.
Conclusions: Our study increases the understanding of neurophysiological mechanisms underlying hearing and auditory-based communication. We found that dopaminergic neuromodulation in the IC acts on responses to behaviorally relevant sounds, and that such modulation occurs via D2-like receptors. Understanding how dopamine modulates auditory processing will ultimately provide insight into mechanisms underlying specific communication- and auditory-based neurological disorders.

Disclosures: J.M. Hoyt: None. R.A. Felix: None. D.J. Perkel: None. C.V. Portfors: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.12/EE16

Topic: D.05. Audition

Support: JSPS KAKENHI Grant No. 26830008

a grant for the Promotion of Medical Science and Medical Care No. 15KI149 from the Ichiro Kanehara Foundation

a grant for Basic Science Research Projects No. 140254 from the Sumitomo Foundation

Title: Functional properties in the secondary auditory field are derived from structural organization of primary auditory thalamus and thalamocortical projections in mice

Authors: *H. TSUKANO[1], K. SHIBUKI[2];
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Abstract: Optical imaging studies revealed multi-frequency organization in the mouse auditory cortex. A higher-order auditory field, the secondary auditory field (AII), is also tonotopically

arranged. Frequency organization of the auditory cortex reflects cochleotopy that generates in the ears and is kept through the course of the auditory ascending pathway. In a prevailing dogma, fundamental tonal information is relayed to the primary auditory cortex (AI) via the primary thalamus, the ventral division of the medial geniculate body (MGv, MGB). In contrast, the AII is believed to receive thalamic inputs from the higher-order thalamus, the dorsal division of the MGB (MGd), although the MGd is not tonotopically arranged. To reconcile the presence of tonotopy in the AII and the absence of tonotopy in the MGd, we precisely investigated distribution of thalamic neurons projecting to the functionally identified AII using a retrograde tracer, Alexa Fluor-conjugated CTB. Contrary to our expectation, the AII received dense projections from the caudal part of the MGv, and only a small number of neurons in the MGd gave rise to projections to the AII. In addition, triple injection of fluorescent tracers along the frequency gradient of the AII revealed topological organization in the caudal part of the MGv that run ventrodorsally. We noticed that MGv neurons labeled by injection to a particular site in the AII were distributed wider than MGv neurons labeled by similar injection to the AI. We conducted double injection of fluorescent CTB into a low or high frequency area of the AI and AII, and found that two neuronal groups projecting to a low or high frequency area of the AII were not clearly separated compared with neurons projecting to a low or high frequency area of the AI. These data suggest that an AII neuron receives a wider range of tonal information from the MGv compared with an AI neuron. To validate this, we used two-photon calcium imaging and observed tonal responses of neurons in cortical input layers stained with Cal520. As expected, band width of neurons in layer 4 was broader in the AII than that in the AI, and tonotopic arrangement of layer 4 in the AII was less ordered than that in the AI. Furthermore, we found a possibility that the mouse MGd is also tonotopically arranged, albeit only weakly. Although MGd neurons projecting to a low, middle, or high frequency area of the AII were highly intermingled, the average coordinate of each neuronal group was slightly but significantly shifted. These findings suggest that functional properties of the AII are largely determined by the thalamocortical projections from the MGv, but not those from the MGd.

Disclosures: H. Tsukano: None. K. Shibuki: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.13/EE17

Topic: D.05. Audition

Support: CIHR Grant MOP274494

the Natural Sciences and Engineering Research Council of Canada (Discovery Grant)

Campbell McLaurin Chair for Hearing Deficiencies

Title: Activation of auditory cortex induces excitatory postsynaptic potential in midbrain neurons

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Abstract: Neuroanatomical studies reveal a great amount of descending (corticofugal) projections from the primary auditory cortex (AI) to all subcortical regions such as the central nucleus of the inferior colliculus (ICc). In the past 20 years, physiological studies show that corticofugal system implements a highly specific modulation of the sound information processing in the ascending auditory system. In the frequency domain, for example, focal electrical stimulation of the AI (ES_{AI}) facilitates subcortical neurons when the best frequency (BF) of subcortical neurons is identical to the BF of cortical neurons (physiologically matched). In contrast, ES_{AI} suppresses subcortical neurons when their BFs are different (unmatched). In addition, ES_{AI} shifts the frequency tunings (e.g., BFs) of unmatched subcortical neurons towards the BF of the stimulated AI neurons. It is noted that all previous physiological findings are achieved by extracellular recording. To date, little is known about the cellular mechanism of corticofugal modulation. An immediate question raised here is if the postsynaptic membrane potential is depolarized or hyperpolarized following focal cortical stimulation. Since the inactivation of the entire auditory cortex reduces tone-evoked spike number of ICc neurons by 30-50%, our hypothesis is that ES_{AI} should primarily induce excitatory postsynaptic potential (EPSP) in the ICc neurons. We used the C57 mouse as animal model. The membrane potential of ICc neurons was recorded by in vivo whole cell patch current-clamp. We found that ES_{AI} induced EPSP of ICc neurons. The range of the threshold current was from 8 to 64 μA, with an average of 34.38 ± 18.94 μA for inducing ICc EPSPs. Further analysis indicated that the ES_{AI}-induced ICc EPSP was related to the BF difference of stimulated AI and recorded ICc neurons; at the 64 μA level, ES_{AI} induced ICc EPSP was larger in matched neurons than in unmatched neurons. Our data suggests that corticocollicular synapses are excitatory. Additionally, corticofugal synaptic strength relies heavily on the frequency tuning relationship between the stimulated AI and recorded ICc neurons.

Disclosures: **J. Qi:** None. **J. Yan:** None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.14/EE18

Topic: D.05. Audition

Support: NIH Grant GM109086

NIH Grant GM116916

UW Department of Anesthesiology

Title: Cortical network activity in posterior parietal cortex following feedforward and feedback afferent stimulation

Authors: *C. MURPHY, R. D. SANDERS, B. K. KRAUSE, M. I. BANKS;
Dept. of Anesthesiol., Univ. of Wisconsin, Madison, WI

Abstract: Introduction:
Evidence suggests that information flow in neocortex relies on coordinated activity within local ensembles, and that this activity arises on the platform of depolarization synchronized across cells in the network (UP states). However, little is known about the contribution of feedforward (FF) versus feedback (FB) pathways in triggering network activity. Posterior parietal cortex (PPC) integrates information from primary sensory and higher order cortical areas. Because activity in PPC is specifically linked to awareness, understanding the integration of FF and FB inputs has important implications for elucidating the neural basis of consciousness and its disorders. We investigated properties of FF- and FB-induced UP states in PPC.
Methods:
Coronal slices (500 μm) containing primary somatosensory (S1), PPC, and retrosplenial (RSC) cortices were prepared from mice (4-10 weeks old). Multiunit activity (MUA) signals were recorded from PPC using 16-channel electrode arrays oriented orthogonally to the cortical laminae. Electrical stimuli were applied to supragranular (SG) layers of S1 and infragranular (IG) layers of RSC using bipolar tungsten electrodes. UP states were defined as positive-going excursions of the MUA signal above a pre-stimulus baseline. Previous results indicate that UP state activity in SG & IG layers is distinct, and thus properties of UP states were compared between SG & IG layers.
Results:
Stimulation of both FF (S1) and FB (RSC) afferents to PPC induced UP states. Increasing stimulus intensity led to monotonic decreases in latency, and increases in magnitude and duration, for UP states induced by S1 stimulation; UP states induced by RSC stimulation were more variable. S1-induced UP states occurred with higher probability and were of shorter latency

and greater duration, amplitude, and integral compared to RSC- induced. Differences in UP state properties between SG and IG layers were also evident, especially for S1-induced UP states: UP states occurred with higher probability in IG layers, and were shorter latency and greater duration, amplitude, and integral than those in SG layers.
Conclusions:
These data support a model in which propagation of information across the cortical hierarchy via coordinated network activity is more efficacious in the FF compared to FB direction. That FF stimulation results in less variable, more robust UP states suggests that FF connections may be responsible for driving the network into an active, highly synchronous state, while FB connections are more apt to modulate the receptivity of the network to these inputs.

Disclosures: C. Murphy: None. R.D. Sanders: None. B.K. Krause: None. M.I. Banks: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.15/FF1

Topic: D.05. Audition

Support: NIH Grant GM109086

NIH Grant GM116916

UW Department of Anesthesiology

Title: A computational modelling study of cortical network activity

Authors: *A. RAMAMOORTHY[1,2], N. MORAN[3], B. KRAUSE[2], M. I. BANKS[2];
[2]Dept. of Anaesthesiology, [3]Neurosci. Training Program, [1]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Introduction: Auditory cortical networks exhibit periods of coordinated network activity (50-200 ms), both spontaneously and in response to acoustic stimuli, similar to brief UP-states, that impact sensory responses. Previously, we examined the cellular and circuit-level mechanisms of UP-state generation *in vitro* and observed their occurrence *in vivo* in chronically implanted animals. Here, we developed a computational model aimed at simulating UP-states in an artificial cortical circuit. **Methods:** A model representing L2/3, L4 and L5 of rodent auditory cortex was set up using the Brian2 simulator for spiking neural networks. Each layer consisted of excitatory and inhibitory units, modelled as conductance-based leaky-integrate and fire neurons. Excitatory connections were all-to-all, with connection strength decreasing as a function of

distance. Inhibitory connections were confined to the layer of origin. Non-zero mean white noise generated and sustained spontaneous network activity. Network activity was also driven via simulated thalamic synaptic input. We compared network activity in the model to data from 1x16 laminar probe recordings in rat auditory cortex. Spike-trains were analyzed to determine interval statistics and the presence and properties of network bursts. Burst-detection analysis was performed using the logISI method described in Pasquale et al. (J Comput Neurosci 29:213, 2010). **Results:** The model reproduces the firing patterns observed in both spontaneous and stimulus-driven recorded data. More specifically, the network, in the absence of an extrinsic stimulus, generates coordinated bursts confined to the granular and infragranular layers, as well as bursts that encompass the entire column. Over a wide range of simulated stimulation intensities, model responses to stimuli correspond to all-or-none network bursts. Increasing stimulus strength increases burst response probability. The inter-spike-interval histograms from the data and the model deviate from an exponential distribution in a similar fashion, suggesting the presence of bursts and pauses. Burst detection analysis on the units in the network suggests that bursts typically range from 50-220 ms. **Conclusions:** The overarching goal of this computational modelling effort is to test presumptive mechanisms of anaesthesia with a specific focus on how global activity in cortical networks is mediated by depth of anaesthesia. The model described above reproduces typical activity profiles observed in cortical networks of interest (murine auditory cortex) and is a suitable theoretical tool for the intended purpose.

Disclosures: A. Ramamoorthy: None. N. Moran: None. B. Krause: None. M.I. Banks: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.16/FF2

Topic: D.05. Audition

Support: Israel Science Foundation

European Research Council (project RATLAND)

Title: Stimulus-specific adaptation in distinct inhibitory populations in auditory cortex

Authors: *T. S. YARDEN, A. MIZRAHI, I. NELKEN;
Hebrew Univ., Jerusalem, Israel

Abstract: Neurons in primary auditory cortex exhibit stimulus-specific adaptation (SSA), the decrease in responses to a common (standard) stimulus that does not generalize fully to another,

rare stimulus (deviant). Here we use *in vivo* two-photon targeted loose-patch recordings in mice to investigate how inhibitory populations shape SSA. We recorded in primary auditory cortex from identified neurons of the three main inhibitory populations, parvalbumin- (PV), somatostatin- (SST) and vasointestinal polypeptide-(VIP) positive neurons. PV neurons, which form the major inhibitory population in the cortex, exhibited early-onset SSA with responses that terminated about 30 ms after stimulus onset. Responses of VIP neurons lasted up to 150 ms following stimulus onset, i.e. substantially beyond stimulus offset, maintaining SSA for nearly the whole time. SST neurons were less responsive to sound stimuli. Those that did respond to pure tones showed SSA, but with somewhat weak deviant responses. Our findings of SSA in all inhibitory populations suggest that the temporal dynamics of inhibitory responses are important in shaping the dependence of cortical responses on stimulus history. In particular, different populations of interneurons can control deviance sensitivity during different temporal windows following stimulus onset. In addition, as inhibitory neurons are a main locus of top-down and neuromodulatory regulation, modulation of their deviant responses can be a means for controlling the level of deviance detection according to the behavioral state.

Disclosures: T.S. Yarden: None. A. Mizrahi: None. I. Nelken: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.17/FF3

Topic: D.05. Audition

Title: Anaesthetic choice modulates basic auditory processing: A combined EEG/LFP study in guinea pigs

Authors: *O. WOOLNOUGH, J. I. BERGER, B. COOMBER, M. N. WALLACE, A. R. PALMER, C. J. SUMNER;
MRC Inst. of Hearing Res., Nottingham, United Kingdom

Abstract: Anaesthesia is widely used in *in vivo* studies of sensory neural processing. Previous studies of the effects of general anaesthesia on neurons in the auditory cortex have shown significant changes to frequency tuning and responses to basic features within the stimulus such as onsets and offsets. Studies of this nature have typically used a single anaesthetic agent, comparing awake and anaesthetised states, and it remains unclear to what extent the choice of anaesthetic agent will affect basic response properties of cortical neurons to sensory stimulation. Electrophysiological recordings were made with chronically implanted, extradural electrodes, positioned over auditory and visual cortices, and penetrating electrodes in auditory cortex.

Recordings were made both while awake and under a range of anaesthetic regimes including opiates, NMDA antagonists and GABA potentiators. Recordings of spontaneous oscillations replicate the results of previous human studies, showing a rapid increase in power of low-frequency (<10Hz) oscillations at loss of consciousness, suppression of high frequency activity and a shift toward criticality, with a higher proportion of unstable oscillatory modes being observed - effects which appear mostly independent of anaesthetic regime. Responses to a range of basic sensory stimuli, such as auditory clicks and tones, display substantive differences in population level processing of even basic auditory stimuli between anaesthetic regimes. These modulations range from -20% to +50% changes in onset response amplitude, up to 25ms peak latency shifts and up to 600% increases in offset response amplitudes. Neural responses to visual flashes demonstrated universal suppression of visually evoked potentials under all anaesthetics and we also observed, under all anaesthetics tested, near total abolishment of auditory-visual cross-modal interactions. Adapter-probe stimuli were also tested showing substantial modulation of adaptation recovery time constants, with diazepam slowing the release from adaptation and ketamine allowing faster recovery than when awake. Local field potentials were used to isolate changes occurring in primary auditory cortex, helping to explain the phenomena seen in the EEG recordings and show changes in frequency tuning resulting from each anaesthetic. In conclusion, we have demonstrated that anaesthesia has significant effects on systems level sensory processing and that the choice of anaesthetic used for recording can have grossly different effects on the response to even simple sensory stimuli.

Disclosures: O. Woolnough: None. J.I. Berger: None. B. Coomber: None. M.N. Wallace: None. A.R. Palmer: None. C.J. Sumner: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.18/FF4

Topic: D.05. Audition

Support: NIMH Silvio Conte Center (P50MH094271)

Nancy Lurie Marks Family Foundation

Title: A layer 1 map in primary auditory cortex (A1)

Authors: *A. E. TAKESIAN[1,2], E. E. DIEL[2], T. K. HENSCH[1,2];
[1]Neurol., Boston Children's Hosp., Boston, MA; [2]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Cortical sensory maps are remodeled throughout life to adapt to a changing environment. Both sensory and neuromodulatory signals are important in sculpting these maps, but how these inputs converge within thalamocortical circuits to trigger plasticity remains unknown. Here, we reveal that a layer (L) 1 map within mouse primary auditory cortex (A1) is a site of convergence of neuromodulatory and topographically-organized thalamic inputs. Surprisingly, we found that L1 cells receive strong, monosynaptic connections from the auditory thalamus (ventral division of the medial geniculate body; MGBv). Electrical stimulation of the MGBv in thalamocortical slices evoked minimum- and maximum- evoked excitatory postsynaptic potentials (EPSPs) in L1 cells as large as those recorded in L4 pyramidal cells. Anatomical studies using targeted AAV-Brainbow injections into mice expressing Cre recombinase in thalamic nuclei revealed abundant MGB axonal projections up to L1. Furthermore, voltage-sensitive dye imaging in thalamocortical slices to examine functional connectivity between MGBv and A1 confirmed a topographic map in L1 along the tonotopic rostro-caudal axis that mirrored the map in L4. L1 cells were also robustly activated by serotonergic and cholinergic input, suggesting that L1 integrates precise sensory information with neuromodulatory state. Functional and anatomical studies further demonstrate developmental and experience-dependent changes in this L1 map. Both MGBv and neuromodulatory activation of L1 are enhanced during an early critical period when passive sound exposure robustly restructures A1 tonotopic maps. Interestingly, L1 and L4 exhibit distinct topographic alterations following early tone exposure, suggesting that the MGBv connections onto L1 cells may be mediated by specific plasticity mechanisms. Together, our studies reveal an unexpected function of L1 cells within the auditory thalamocortical circuit. Namely, L1 cells are activated by both neuromodulatory tone and temporally- and spatially-precise sensory signals. Such inputs to L1 cells may evolve over postnatal development and in response to sensory experience, controlling the manner in which these cells orchestrate activity and plasticity in the cortical layers below.

Disclosures: A.E. Takesian: None. E.E. Diel: None. T.K. Hensch: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.19/DP04 (Dynamic Poster)

Topic: D.05. Audition

Support: NIDCD R21012894

Title: Corticothalamic neurons control modes of cortical processing and perception by entraining columnar oscillations

Authors: *W. GUO[1], A. R. CLAUSE[1], A. N. BARTH-MARON[2], B. G. SHINN-CUNNINGHAM[3], D. B. POLLEY[1];
[1]Eaton Peabody Lab., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Dept. Neurobio., Harvard Med. Sch., Boston, MA; [3]Ctr. for Computat. Neurosci. and Neural Technol., Boston Univ., Boston, MA

Abstract: The cortical column is the canonical unit of information processing in the cerebral cortex. Until recently, the neural circuitry that imposes real-time modulation on columnar sensory processing has remained obscure. Here, we characterize thalamocortical response dynamics by activating a subtype of layer 6 (L6) neuron that makes a massive projection to the thalamus and also has abundant connections within the local column. We used a cre-dependent viral construct to selectively express ChR2 in L6 of primary auditory cortex (A1) of Ntsr1-cre transgenic mice. We explored the modulatory effects of L6 activation on thalamocortical sound processing by simultaneously recording from the ventral subdivision of the medial geniculate body (MGBv) and all layers of A1 in awake, head-fixed mice while optogenetically activating L6 corticothalamic (L6CT) neurons. In current source density (CSD) traces, photoactivating L6CT neurons induced robust gamma oscillations in deep layers (at 100-120 Hz) and superficial layers (at 40-50 Hz). Cessation of L6CT activation induced a delta-theta oscillation (2-6 Hz) that persisted for hundreds of milliseconds. The delta-theta offset oscillation exhibited a stereotypical spatial-temporal pattern such that CSDs in each layer were reset to the same phase, independent of the duration or amplitude of laser power. Sound responses across the column were modulated with either divisive or multiplicative gain, depending on the phase of the oscillation, which enhanced either discrimination or detection behavior of mice in a perceptual task. Granger causality analysis revealed that delta-theta oscillations did not originate from L6, but from L4 and L5, suggesting that L6CT neurons do not influence columnar neural activity directly, but rather engage distributed networks of excitatory and inhibitory neurons throughout the cortical column that could dynamically transform afferent sensory traces. By analyzing spontaneous spiking activity in relation with the CSDs, we identified a group of neurons that were linked with columnar CSD resetting, including fast-spiking (FS) neurons and regular-spiking (RS) neurons in cortical L4 and L5, as well as neurons in MGBv. We found that L6CT neurons strongly activated the cortical FS resetter neurons, whose activity persisted for an extra 10-20 milliseconds post L6CT activation, inducing the observed columnar CSD phase reset. In sum, by driving cortical FS neurons that entrains columnar CSDs, L6CT neurons can dynamically and bi-directionally modulate sound processing and perception, providing a powerful tool for analyzing time-varying stimuli in complex auditory scenes.

Disclosures: W. Guo: None. A.R. Clause: None. A.N. Barth-Maron: None. B.G. Shinn-Cunningham: None. D.B. Polley: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.20/FF5

Topic: D.05. Audition

Support: NSFC 973 grant 2013CB530900

HK GRF grant 561313M

HK GRF grant 11101215M

HK TBRS grant T13-607/12R

HK CRF grant C1014-15G

NSFC grant 31371114

HK HMRF grant 01121906

Title: Rewiring the deafferented cortical neurons to the remaining auditory inputs: A model for treatment of tinnitus patients

Authors: *P. JENDRICHOVSKY, J. FENG, J. HE;
Dept. of Biomed. Sci., City Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Tinnitus is an auditory phantom sensation when no external sound is present. Our working theory is that tinnitus is caused by the positive feedback oscillation in the thalamo-cortical circuit [1] that becomes hypersensitive and causes tinnitus after hearing loss [2]. Cholecystokinin (CCK) has plasticity enabling properties towards cortical neurons [3]. In the present study, we aim to rewire weak connections from the remaining inputs towards the deaff erented ones. We hypothesize that this will suppress the hypersensitivity and therefore silence tinnitus permanently. The first main objective was to verify whether the auditory cortical neurons could be changed to retune to other frequency in the presence of CCK. The rat was chronically implanted with recording electrodes and injection cannula for CCK delivery in the auditory cortex. After successful pairing of a previously low-responsive or non-responsive sound stimulus with cortical stimulation after CCK infusion, the neuron shows higher firing rates to the paired sound. The second objective is to establish appropriate hearing loss and tinnitus model. Our results so far indicated that neural network could be rewired in the presence of CCK.
Key references:
[1] Andersen RA, Knight PL, Merzenich MM: The thalamocortical and corticothalamic connections of AI, AII, and the anterior auditory field (AAF) in the cat: evidence for two largely

segregated systems of connections. J Comp Neurol., Dec. 1980.
[2] Eggermont JJ , Roberts LE.: The neuroscience of tinnitus., Trends Neurosci., (27)11: 676-682, Dec. 2004.
[3] Li X., Yu K., Zhang Z., Sun W., Yang Z., Feng J., Chen X., Liu CH., Wang H., Guo YP., He J.: Cholecystokinin from the entorhinal cortex enables neural plasticity in the auditory cortex., Cell Research, 24(3), Mar. 2014.

Disclosures: P. Jendrichovsky: None. J. Feng: None. J. He: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.21/FF6

Topic: D.05. Audition

Title: Reafference integration in an escape circuit.

Authors: *C. R. VON REYN[1], G. M. CARD[2];
[1]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [2]Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Reafferent signals, sensory cues generated by an animal's motor actions, are indistinguishable from externally generated sensory signals at the first stage of sensory processing. Nonetheless, nervous systems have devised ways to identify and properly integrate reafferent signals to avoid ambiguity and saturation of sensory pathways, to determine whether a performed motor action produced intended consequences, and to guide future motor actions. Substantial evidence supports the necessity for reafference integration in sensory processing and motor coordination, however we still know little about the neural circuits that underlie these computations. Here, we provide evidence for reafference integration within the Giant Fiber (GF) escape circuit of the fruit fly *Drosophila melanogaster*. Voluntary leg motion, like that experienced during walking, significantly increased GF EPSPs and spikelets recorded in whole-cell, current-clamp mode in behaving animals. Since passive antennal deflections have been observed during walking behaviors, we hypothesized that the increase in GF activity occurred through stretch activation of mechanosensory neurons located in the Johnston's Organ of the antenna that make direct synapses onto the GFs. We found that antennal ablation significantly reduced EPSPs and spikelets during leg movements, supporting that the GFs receive reafference from the antenna during self-motion. Antenna ablation additionally uncovered a significant hyperpolarization within the GFs, also synchronized to leg motion. Our data suggest that the GF circuit is organized to recognize and diminish reafferent signals, avoiding an energetically

unfavorable self-stimulation of an escape pathway. This work establishes the GF circuit as a model for dissecting the neural architecture behind reafference integration using the neuroengineering toolkit of *Drosophila melanogaster*.

Disclosures: C.R. von Reyn: None. G.M. Card: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.22/FF7

Topic: D.05. Audition

Support: NIMH Division of Intramural Research Programs

Title: Mapping auditory information flow in primate cortex

Authors: *B. H. SCOTT[1], Y. KIKUCHI[2], K. S. SALEEM[1], M. FUKUSHIMA[1,3], M. MISHKIN[1], R. C. SAUNDERS[1];
[1]Lab. Neuropsychol, NIMH, Bethesda, MD; [2]Inst. of Neurosci., Newcastle Univ. Med. Sch., Newcastle Upon Tyne, United Kingdom; [3]Lab. for Marmoset Neural Architecture, RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: The flow of information in the primate auditory cortex follows a hierarchical processing scheme, with projections in the medial-lateral dimension from core, to belt, to parabelt. We recently showed that rostral auditory cortex in the rhesus monkey receives input via stepwise serial projections in the caudal to rostral dimension: through the primary, rostral, and rostrotemporal core areas (AI, R, and RT) on the supratemporal plane (STP), continuing to the rostrotemporal polar area RTp (Scott et al., *Cerebral Cortex* 2015). In addition to this serial cascade of corticocortical connections, every region of auditory cortex receives parallel thalamocortical projections from the medial geniculate nucleus (MGN), with the caudal core (AI and R) being the primary recipient of input from the ventral division (MGv, i.e. the lemniscal auditory pathway). Because few studies have placed tracer injections into the rostral STP beyond AI and R, we injected anterograde and retrograde anatomical tracers into AI, R, RT, and RTp to quantify the thalamic inputs to these areas and address several outstanding questions about the organization of auditory cortex. Whereas AI and R both receive nearly 90% of their thalamic inputs from the MGv, RT receives only ~45% from MGv, and an equal share from the dorsal subdivision, MGd (Fig. 1). Area RTp receives ~25% of its inputs from MGv, but ~30% of its thalamic inputs arise from multisensory areas outside the MGN (e.g., medial pulvinar). In accord with the laminar patterns evident in corticocortical connections in the same cases, these

thalamocortical connections support a model in which AI and R lie at the same hierarchical level, but RT and RTp lie at a higher level, perhaps between that of the core and belt (Fig. 1). These results will be presented in the context of an expanded hierarchical model that aims to capture the complexity of the primate's ventral *auditory* stream, which may well exceed the complexity of the primate's ventral *visual* stream.

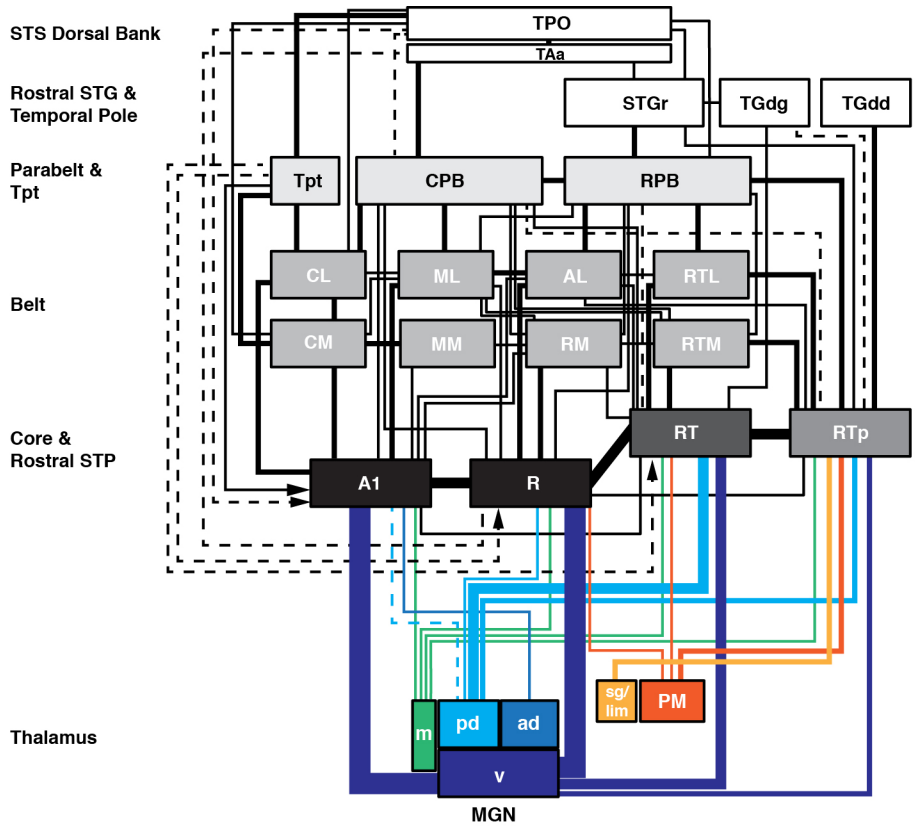

Figure 1: *Connections of the auditory thalamus and temporal cortex. Line weight reflects connection strength. Only thalamocortical connections to AI, R, RT, and RTp are shown.*

Disclosures: B.H. Scott: None. Y. Kikuchi: None. K.S. Saleem: None. M. Fukushima: None. M. Mishkin: None. R.C. Saunders: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.23/FF8

Topic: D.05. Audition

Support: CIHR Grant MOP274494

the Natural Sciences and Engineering Research Council of Canada (Discovery Grant)

funds from the Campbell McLaurin Chair for Hearing Deficiencies

Title: Thalamic inactivation does not prevent auditory cortical plasticity induced by intracortical stimulation

Authors: *L. KONG, S. WANG, X. LIU, J. YAN;
Dept. of Physiol. and Pharmacology, Cumming Sch. of Med., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: Auditory learning induces frequency-specific plasticity in the auditory cortex. However, the role of the intracortical circuitry for the development of the cortical plasticity remains unclear until the potential contribution of the corticothalamic and thalamocortical loop is excluded. Here, we examine the frequency-specific plasticity in the primary auditory cortex (AI) by electrical stimulation of AI (ES_{AI}) with and without inactivation of the auditory thalamus. We found that ES_{AI} induced cortical frequency-specific plasticity in the control condition when the auditory thalamus was not inactivated. Similar pattern of cortical plasticity was induced by ES_{AI} even when the auditory thalamus was inactivated during the ES_{AI}. The best frequencies (BFs) of the recorded cortical neurons shifted towards the BFs of the electrically stimulated ones. In addition, the BF shifts were linearly correlated to the BF differences between the recorded and stimulated cortical neurons. More importantly, the ratio of the linear function with thalamic inactivation was nearly the same as the ratio of the linear function in the control condition. Our data suggest that the induction of frequency-specific plasticity in auditory cortex does not rely on the corticothalamic and thalamocortical loop, thus the intracortical circuitry appears to independently underlie the cortical frequency-specific plasticity.

Disclosures: L. Kong: None. S. Wang: None. X. Liu: None. J. Yan: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.24/FF9

Topic: D.05. Audition

Support: Autifony Therapeutics

NIDCD grant R01 DC009836

Lauer Tinnitus Research Center

Title: Opposing shifts in the transcription of AMPA and GABAA receptor subunit genes support homeostatic gain adjustments in the auditory cortex and midbrain following cochlear denervation

Authors: *P. BALARAM[1], D. B. POLLEY[1,2];
[1]Eaton-Peabody Labs., Massachusetts Eye and Ear Infirmary, Boston, MA; [2]Dept. of Otology and Laryngology, Harvard Med. Sch., Boston, MA

Abstract: A loss of cochlear hair cells or afferent synapses induces a host of intracellular changes at every stage of the central auditory pathway. These changes are often compensatory, allowing central auditory neurons to recover threshold sensitivity and support robust sound perception despite a loss of inputs from the inner ear. One common compensatory response involves a homeostatic 'rescaling' of excitatory and inhibitory neurotransmission. Under normal conditions, neurons maintain a stable ratio of excitatory and inhibitory synaptic transmission (E/I balance) that allows them to preserve a set point of activity in the face of large drops or surges in afferent drive. Following the loss of auditory nerve afferent fibers or inner hair cells, sound-evoked activity is reduced in the auditory nerve and brainstem nuclei. However, recordings at higher stages of the auditory pathway, including the midbrain, thalamus, and auditory cortex, show significant increases in sound-evoked activity despite diminished input from the periphery. This increase in central gain points toward a shift in the underlying E/I balance in these structures, which could provide greater sensitivity toward spared inputs that remain following peripheral insult.
Here, we describe transcriptional changes that contribute to altered E/I balance following unilateral round window application of ouabain, a Na+/K+ ATPase inhibitor that lesions Type 1 afferent fibers in the auditory nerve. Selective denervation of afferent fibers without hair cell dysfunction was confirmed with ABR and DPOAE measurements. Several weeks following cochlear denervation, neurons in the auditory cortex, medial geniculate body and inferior colliculus all showed increased mRNA expression of the GluR2 AMPA receptor subunit (*Gria2)* and decreased expression of the GABAα1 receptor subunit (*Gabra1).* Using high-sensitivity fluorescence *in situ* hybridization, we compared *Gria2* and *Gabra1* counts in individual neurons, and conclude that more pronounced changes in E/I were found in auditory cortex than in subcortical areas. These changes may support the persistence of rudimentary sound perception in the face of extreme afferent loss but may also underlie auditory pathologies typified by hyperexcitability, such as tinnitus and hyperacusis.

Disclosures: P. Balaram: None. D.B. Polley: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.25/FF10

Topic: D.05. Audition

Support: NIDCD R01DC009607

Title: Influence of sensory activity on the development of subplate neuron circuits

Authors: *X. MENG[1], P. O. KANOLD[2];
[1]Dept. of Biol., [2]Biol., Univ. of Maryland, College Park, MD

Abstract: Subplate neurons (SPNs) are among the earliest generated neuronal population in the cerebral cortex. They provide a transient excitatory relay of thalamic information to layer 4. Ablation studies have shown that SPNs play an instrumental role in the establishment and refinement of early thalamocortical and intracortical circuits.
Sensory experience, esp. sensory deprivation, has a profound influence on cortical development. Since SPNs are the first cells to receive sensory activity, sensory deprivations could alter SPN function. This altered SPN function could influence the cortical circuit, and thereby cortical development, even past the duration of the sensory disruption. Thus we here test if sensory experience influences SPN function. We use genetic deafness model (TMC1,2 double-knockout mice) and investigate if synaptic circuits associated with SPNs in the auditory cortex (ACX) are disrupted.
To investigate synaptic circuits we use laser-scanning photostimulation (LSPS) in thalamocortical slices of ACX at postnatal days 6-14. We compare the spatial pattern of excitatory inputs to SPNs in cells from genetically deaf mice with those in normal controls. The analysis of a limited sample of cells suggests that the pattern of excitatory inputs to SPNs in cells from genetically deaf mice. These results indicate that early sensory experience might sculpt SPN circuits and thereby potentially alter subsequent development.
Since in humans the effects of deafness on SPNs would happen in utero before deafness can be diagnosed even at the earliest possible diagnosis cortical circuits could have already been affected by deafness.

Disclosures: X. Meng: None. P.O. Kanold: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.26/FF11

Topic: D.05. Audition

Support: NIH Grant F32DC013482

Title: $GABA_A$ and $GABA_B$ mediated inhibition display distinct critical periods in auditory cortex.

Authors: *T. M. MOWERY[1], J. DIMIDSCHSTEIN[2], G. FISHELL[2], V. KOTAK[1], D. SANES[1]; [2]Langone Med. Ctr., [1]New York Univ., New York, NY

Abstract: Introduction
Sensory deprivation can induce profound changes to central processing during developmental critical periods (CPs). For example, loss of visual or auditory function during development commonly leads to reduced $GABA_A$ receptor-mediated inhibitory synaptic responses in cortex. However, the effect of hearing loss on $GABA_B$ receptor-mediated inhibition is not known. Here, we used a novel recombinant adeno-associated virus (rAAV) that restricts channelrhodopsin expression to telencephalic GABAergic interneurons, permitting us to optogenetically activate only inhibitory interneurons while recording from pyramidal cells. Using this approach, we investigated whether auditory cortex $GABA_A$ and $GABA_B$ receptor function was differentially altered by a period of developmental hearing loss.
Methods
Bilateral earplugs were inserted in gerbils (*Meriones unguiculatus*) on postnatal day 11, and were subsequently removed at successively later ages (P13, P17, or P18). On P48, auditory cortex was injected with rAAV-mDlx-ChR2-mCherry (50 nl). Adult thalamocortical brain slices were generated and whole-cell current clamp recordings were obtained from unlabeled L2/3 pyramidal cells. Inhibitory postsynaptic potentials (IPSPs) were evoked by stimulating with 1 ms pulses of blue light (470 nm).
Results
Light stimulation reliably evoked IPSPs that were composed of a short latency $GABA_A$ and a long latency $GABA_B$ receptor-mediated component. In a subset of experiments, each component was validated with selective antagonists to either $GABA_A$ (bicuculline) or $GABA_B$ (SCH-50911) receptors. When hearing loss was reversed at P13, both $GABA_A$ receptor amplitude components were normal in adulthood ($p's > 0.1$). When hearing loss was reversed at P17, $GABA_A$ergic IPSPs were smaller in adults (mean mV ± SEM: Ctrl – 9.4 ± 0.9 vs. EP11 to 17 – 6.0 ± 0.4, $p < 0.01$). In contrast, $GABA_B$ receptor amplitude was normal (mean mV ± SEM: Ctrl – 5.8 ± 0.9 vs. EP11 to 17 – 6.1 ± 0.5, $p > 0.1$). Finally, when hearing loss was reversed at P18, then both

$GABA_A$ (mean mV ± SEM: Ctrl – 9.4 ± 0.9 vs. EP 11 to 23 – 3.9 ± 0.4, $p < .001$), and $GABA_B$ receptor-mediated IPSPs were significantly diminished (mean mV ± SEM: Ctrl – 5.8 ± 0.9 vs. EP 11 to 23 – 1.9 ± 0.2, $p < .001$).

Conclusion

Our results support the concept that a brief period of sensory deprivation during developmental CPs has a long-lasting effect on synaptic inhibition. However, the two major classes of GABAergic transmission display unique CPs. Specifically, the effect of hearing loss on $GABA_B$ergic transmission requires a longer period of auditory deprivation.

Disclosures: T.M. Mowery: None. **J. Dimidschstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NYU. **G. Fishell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NYU. **V. Kotak:** None. **D. Sanes:** None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.27/FF12

Topic: D.05. Audition

Title: Mice with reduced alpha7 nicotinic receptors demonstrate auditory processing EEG abnormalities relevant to schizophrenia

Authors: *A. M. PATINO[1], D. J. GRAZIANO[1], D. FEUERBACH[2], M. M. SIDOR[1]; [1]Novartis, Cambridge, MA; [2]Novartis, Basel, Switzerland

Abstract: Recent studies have associated the α7 nicotinic acetylcholine receptor (nAChR) subunit gene, CHRNA7, with the pathophysiology of schizophrenia. In addition to abnormalities in cognition and mood, individuals with schizophrenia show robust deficits in inhibiting or suppressing incoming sensory input, termed sensory gating. Sensory gating is a normal physiological response observed across species and is measurable using electroencephalography (EEG). Such event-related potentials have been used to examine both normal and disease states, making them robust translatable biomarkers between humans and rodents. Understanding how these deficits in processing environmental stimuli arise may yield insight into the underlying circuit abnormalities in schizophrenia.

Heterozygous mice with a null mutation in the α7 nAChR gene have a ~ 50% reduction in nAChRs. Here, we systematically phenotype α7 heterozygous mice (α7 mice) through a battery of EEG-based auditory sensory paradigms, including auditory gating, mismatch negativity and

the auditory steady-state response. EEG recordings were performed with male α7 mice (B6.129S7-Chrna7<tm1Bay>/J , n=10) and age-matched wild type (WT) controls (C57BL/6J , n=10) using the Data Sciences International wireless telemetry system with skull screws implanted over the hippocampus for signal and cerebellum for reference. For auditory gating, paired 10kHz tones (10ms pulse width, 500ms interstimulus interval, 10s trial length) were presented for a total of 180 trials. The amplitude of the response to the first tone stimulus (S1) was compared to that of the second tone stimulus (S2). Gating was calculated as the S2/S1 ratio. We observed a significant deficit in auditory gating in α7 mice relative to WT controls. This deficit was primarily driven by reduced S1 P20 (most positive deflection between 10-30ms) and N40 amplitudes (most negative deflection between 35-65ms); no differences in S2 amplitudes were detected. This suggests that α7 nicotinic receptors primarily modulate the S1 response. To determine if gating deficits were due to reduced α7 receptors, nicotine was used to increase receptor activity. In both groups, nicotine (2.3mg/kg, sc) increased the P20 amplitude while decreasing N40 amplitudes, resulting in no overall S1 response change. However, nicotine decreased the S2 response, thus reversing the gating deficit in α7 mice to WT levels. Subtle deficits in the auditory steady-state response were also observed in α7 mice, suggesting broad network impairment. No abnormalities were observed in resting-state EEG. This work gives insight into the potential cortical circuit abnormalities involved in schizophrenia.

Disclosures: A.M. Patino: None. D.J. Graziano: None. D. Feuerbach: None. M.M. Sidor: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.28/FF13

Topic: D.05. Audition

Title: An inhibitory corticostriatal circuit

Authors: *C. ROCK, H. ZURITA, C. WILSON, A. APICELLA;
Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: It is very well established that cortical neurons regulate the activity of spiny projection neurons (SPNs) in the striatum through long-range glutamatergic/excitatory projections, while inhibition is thought to be mediated by local feed-forward and feed-back circuits. Striatal neuronal activity has been shown to be involved in movement, learning, and goal-directed behavior, therefore it is crucial to understand the cortical connectivity pattern and dynamics that shape the flow of information in the striatum. Although anatomical and physiological studies have led to the assumption that the dorsal striatum receives exclusively excitatory afferents from

the cortex, here we demonstrate that the dorsal striatum also receives GABAergic projections from the cortex. We addressed this by taking advantage of optogenetics and directly examining the functional effects of cortical GABAergic inputs to SPNs from the mouse auditory and motor cortex. We found that the cortex, via corticostriatal somatostatin neurons (CS-SOM), has a direct inhibitory influence on the output of the striatal SPNs. Our results describe a previously unknown corticostriatal long-range inhibitory circuit (CS-SOM inhibitory projections → striatal SPNs) underlying the control of spike timing/generation in SPNs and attributes a specific function to a genetically defined type of cortical interneuron in corticostriatal communication. These findings may suggest that the timing and ratio of excitation and inhibition, two opposing forces in the mammalian cerebral cortex, can dynamically affect the output of the dorsal striatum, providing a general mechanism for motor control driven by sensory stimuli.

Disclosures: C. Rock: None. H. Zurita: None. C. Wilson: None. A. Apicella: None.

Poster

236. Auditory Processing: Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 236.29/FF14

Topic: H.01. Animal Cognition and Behavior

Title: Isoflurane anesthesia attenuates cortical responses to sounds in rats

Authors: M. M. HOLDFORD[1], Z. R. HOLLOWAY[1], J. J. SABLE[1,2], F. ANDRASIK[1], *H. J. SABLE[1];
[1]Dept. of Psychology, Univ. of Memphis, Memphis, TN; [2]Dept. of Behavioral Sci., Christian Brothers Univ., Memphis, TN

Abstract: The N100 component of the auditory event-related brain potential (ERP) represents the initial negative peak occurring approximately 80-120 ms after the onset of sound. The N100 is generated during sleep, but it is delayed. Anesthesia reduces the amplitude of the N100, but research examining anesthesia-related effects on N100 amplitude and latency is limited. In this study, we used subdermal needle electrodes in Wistar rats to examine isoflurane effects on auditory N100 amplitude and latency over a 20-minute session. The stimuli were pairs of 50-ms complex tones, presented with onset-to-onset intervals of 500 ms. The inter-pair interval (IPI) was randomized between 1 and 5 seconds. The isoflurane was then discontinued, and subjects were allowed to fully awaken, at which point the recording procedure to the tone pairs was repeated. Peak-to-peak amplitude of the N100 was reduced by isoflurane. These results confirm that the characteristics of the N100 are modified by anesthesia. Other ERPs were similarly

affected. Thus, measuring the N100 for diagnostic purposes in anesthetized patients is not advisable, but may provide information about optimal levels of sedation/anesthesia.

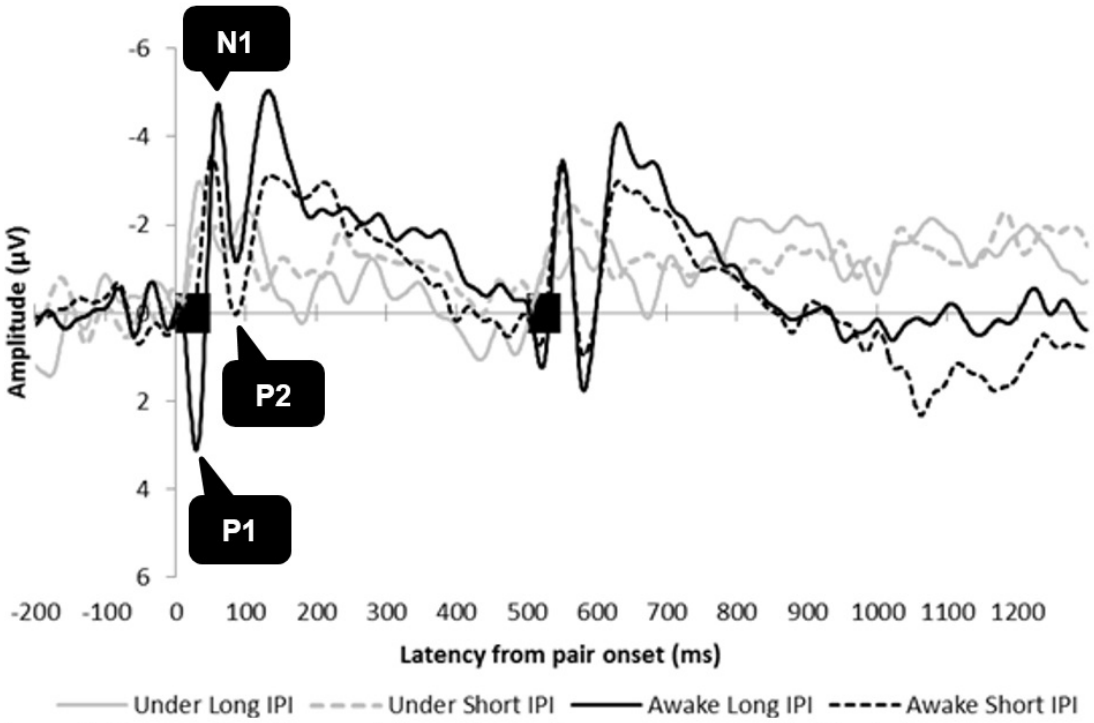

Figure 1. Event-related potentials to the auditory two-tone task. Black boxes represent the presentation of each tone in the pair. The short interpulse interval (IPI) was 1 second, while the long IPI was 5 seconds.

Disclosures: M.M. Holdford: None. Z.R. Holloway: None. J.J. Sable: None. F. Andrasik: None. H.J. Sable: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.01/FF15

Topic: D.05. Audition

Support: NINDS R37NS21135

HHMI International Predoctoral Fellowship (YMF)

NIH EB00856

NIH EB006356

NIH EB018783

US Army Research Office W911NF-08- 1-0216

US Army Research Office W911NF-12-1-0109

Title: Omissions of expected phonemes generate stimulus specific predictions in superior temporal gyrus, an ecog study

Authors: *Y. M. FONKEN[1], A. MUKERJI[2], P. BRUNNER[3,4], G. SCHALK[3,5], R. T. KNIGHT[2,6];
[1]Knight Lab. - Univ. of California, Berkeley, Berkeley, CA; [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; [3]Dept. of Neurol., Albany Med. Col., Albany, NY; [4]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Center, New York state department of health, Albany, NY; [5]Natl. Ctr. for Adaptive Neurotechnologies, Wadsworth Center, New York state department of health, Albany,, NY; [6]Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: Recent evidence suggests that higher-level predictions inform perception at the level of early sensory processing. The underlying mechanism of how predictions influence sensory processing is unknown. Studying omissions of expected sounds provides a unique way of isolating top-down prediction effects on auditory cortex. Evidence from electroencephalography (EEG) has revealed early (< 100ms) responses to unexpectedly omitted stimuli. In the current study we aimed to 1) establish whether auditory cortex responds to omitted stimuli by delineating the source of early activation and 2) define if this response is specific to the content of the prediction of the sound that was expected. We investigated these questions by recording electrocorticography (ECoG), while subjects listened to a sequence of phonemes in a regular pattern La-La-Ba; La-La-Ga, in which two of the phonemes ('Ba' and 'Ga') were infrequently omitted. To determine if an omission response was stimulus-specific, a binary classifier was applied to distinguish which phoneme was omitted. In one subject with a high-density grid (3mm; 250 channels) located over superior- and middle temporal gyrus (STG and MTG respectively), we found an early positivity (< 100ms) in the event-related potential (ERP) over the MTG in response to omitted stimuli, as well as a high-gamma band (70-150Hz) response in the STG (100-500ms). A logistic regression classifier trained on high-gamma in phoneme responsive electrodes successfully predicted (61.4%, compared to null distribution; 95% C.I. at 58.3%) which stimulus was omitted based on high-gamma band traces from 0-400ms relative to the omission 'onset' (i.e. when the omitted sound was expected). This study isolates a pure top-down influence on auditory cortex when an omission of a predicted event occurs. Our results localize previously reported omission positivity observed in EEG to auditory cortex. In addition, we confirm that expectation activates a representation of the expected stimulus in STG. In summary, our results suggest that the brain is generating higher-order predictions that influence auditory processing.

Disclosures: Y.M. Fonken: None. A. Mukerji: None. P. Brunner: None. G. Schalk: None. R.T. Knight: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.02/FF16

Topic: D.05. Audition

Support: TINNET STSM Grant

Title: Can transcranial direct current stimulation (tDCS) modulate auditory cortical oscillations? Simultaneous acquisition of tDCS and magnetoencephalography (MEG)

Authors: *M. HOLDING[1], P. ADJAMIAN[1], N. WEISZ[2], G. DEMARCHI[2], D. HALL[3]; [1]MRC Inst. of Hearing Res., Nottingham, United Kingdom; [2]Ctr. for Cognitive Neurosci., Univ. of Salzburg, Salzburg, Austria; [3]Nottingham Biomed. Res. Unit, Nottingham, United Kingdom

Abstract: Abnormal neural oscillations in the auditory cortex have been implicated in the generation of tinnitus. This has been shown several times in both magento- and electro-encephalography (MEG/EEG) studies. Given the problems that people with tinnitus face such as increased anxiety and depression, and the lack of treatments and therapies, anything that could be capable of helping tinnitus should be considered of importance. tDCS has been shown to be able to proactively modulate certain neuronal oscillations. However, those oscillatory frequencies studied were not the same as the ones implicated in tinnitus. When combined with residual inhibition (the temporary suppression of tinnitus following presentation of a masking noise) tDCS has been found to have the potential to increase the suppression of tinnitus. However, it is unclear what neural effects the tDCS and white noise combination is having. Both tDCS and masking noise devices are becoming increasingly available to consumers so it is imperative to fully understand what effects tDCS is having on neuronal oscillations. This study aimed to investigate the effects of tDCS on auditory cortical oscillations using MEG. We carried out a combined tDCS and MEG experiment on 13 non-tinnitus participants. Participants were stimulated using either a sham tDCS or anodal stimulation on alternating trials in conjunction with a white noise masker known to induce residual inhibition. MEG data was recorded during this process using an Elekta Neuromag 306 channel system. The data presented are the results of this investigation, and show modulations in alpha and delta frequency oscillations. This would indicate that tDCS is capable of modulation oscillatory frequencies implicated in the generation of tinnitus. Further work is needed to assess how this would convert to a tinnitus population, but offers a promising avenue of exploration.

Disclosures: M. Holding: None. P. Adjamian: None. N. Weisz: None. G. Demarchi: None. D. Hall: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.03/FF17

Topic: D.05. Audition

Support: JSPS Grant 16K12456

Title: Entrainment of EEG during the music listening

Authors: Y. KUMAGAI, *T. TANAKA;
Dept. of Electrical and Electronic Engin., Tokyo Univ. of Agr. and Technol., Koganei-shi, Japan

Abstract: Introduction: It has been reported that music perception has an important role in developments of human cognitive functions such as intelligence, language, and memory (Schellenberg et al., 2005). Recently, the relationship between music and cortical activities have been well investigated. Nozaradan et al. reported that cortical entrainment to periodic tone (Nozaradan et al., 2012). Meltzer et al. showed cortical entrainment to periodic rhythm of music (Meltzer et al., 2015). However, it is unclear whether or not the celebral cortex entrains to natural music, aperiodic stimuli. Meltzer et al. also demonstrated that the cerebral cortex more strongly responded to the periodic rhythm of unfamiliar music than to that of familiar music. On the other hand, Jacobsen et al. reported that a deviant sound among a sequence of familiar sounds enhanced the mismatch negativity (MMN) compared to that among a sequence of unfamiliar sounds (Jacobsen et al., 2005). Therefore, it remains unclear how the familiarity of music affects the cerebral cortex. To better understand the mechanism of music perception, we investigated by using electroencephalogram (EEG) the cortical entrainment to natural music, and the relationship between cortical response and familiarity of music.
Methods: Eight subjects in their twenties participated in the experiment. All subjects were healthy and had normal or correctedtonormal vision. They were given an informed consent, and the study was approved by the research ethics committee of Tokyo University of Agriculture and Technology. We prepared single-tone melody as simple and natural music, and recorded EEG while a subject was listening to them. The experiment consisted of 30 trials, each of 32 seconds in length, while listening to one of the music stimulus. After each trial, subjects were instructed to answer whether the listened music was familiar or not. Each music was labeled with either “familiar” and “unfamiliar” according to the answers of subjects. The cross-correlation function was calculated between filtered EEG (2-8 Hz) and envelope of music for each label, based on the

finding that perception low-frequency is important for music perception (Farbood et al., 2013). Results: The cross-correlation function averaged across trials, channels, and subjects showed a peak at time lags around 150 ms. Moreover, the value of cross-correlation function at the peak while listening to unfamiliar music was larger than listening to familiar music. These findings suggest that at a low-frequency band, the EEG is entrained to natural aperiodic music, and the response to the music is stronger to unfamiliar music than to familiar music.

Disclosures: Y. Kumagai: None. T. Tanaka: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.04/FF18

Topic: D.05. Audition

Support: National Natural Science Foundation of China (31470987)

The‘973’ National Basic Research Program of China (2011CB707805),

Title: Different cortical oscillation patterns effectively distinguish auditory selective and non-selective attention in multi-talker speech perception

Authors: *Y. GAO, L. LI;
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Abstract: Human listeners are able to selectively attend to the target speech in the noisy natural environment. The current study focuses on different cortical oscillation pattern of internal speech representation, long-term neural activities and cortical causal connections between selective and non-selective attention. The Event-Related Potentials (ERPs) signals were recorded from participants listening to multi-talker speech under selective or non-selective attention states. The results show that selective attention enhances the causalities from temporal area in alpha band oscillation and also enhances both causalities to right motor area and internal target speech representation in beta band oscillation, but non-selective attention improves both causality from motor area to frontal area long-term neural activities in gamma band oscillation. The findings suggest that different frequency bands of neural recording distinguish selective and non-selective attention by different cognitive processing.

Disclosures: Y. Gao: None. **L. Li:** None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.05/GG1

Topic: D.05. Audition

Support: ERC Consolidator Grant (AUDADAPT)

Volkswagen Foundation

Title: Neural noise in the human electroencephalogram predicts perceptual decisions

Authors: *L. WASCHKE, M. WÖSTMANN, J. OBLESER;
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Abstract: Perceptual decisions close to threshold are error-prone and unstable. With less Information to base a decision on, the impact of spontaneous variations in brain activity increases. This is taken to extremes when discriminative information is absent altogether: When comparing two identical stimuli, pre-stimulus differences in brain activity and varying post-stimulus neural responses to the stimuli (together referred to as “neural noise”) must be driving the ensuing decision. Evidence from both modeling and experimental work on the discrimination of identical stimuli suggests that neural noise could indeed lead to varying percepts. Here, we investigated the potency of different indicators of neural noise in the electroencephalogram (EEG) to predict the later perceptual decision. We recorded the 24-electrode scalp EEG while human subjects (n = 16; age 19-74) discriminated two identical, consecutively presented pure tones (SOA = 900ms, 650 Hz) and reported whether the first or second tone was higher in pitch. Since evoked responses did not differ between decisions, we pursued two routes of analysis to address trial-by-trial variations of pre-stimulus noise and systematic differences in the neural post-stimulus response. First, we analyzed trial-wise Weighted Permutation Entropy (WPE) of the broadband EEG signal (1-100 Hz), an information-theoretic measure quantifying the regularity of time series data. WPE around presentation of the first tone allowed classification whether participants would later report the first versus the second tone to be higher in pitch (above-chance classification in 12 out of 16 subjects). Second, Theta band (6-9 Hz) Inter-Trial Phase Coherence (ITPC) as a measure of stimulus-evoked activity around the presentation of the first tone was higher over central electrodes when this first tone was later chosen as compared to trials of the opposite decision. Lastly, we quantified differences in phase concentration around tone onset with a phase bifurcation index. Higher phase concentration (i.e., lower neural noise) in the Delta- (1- 4 Hz, before onset of the first tone) and Theta-Band (at onset of the first tone) at fronto-central sensors marked decisions for the first tone. In sum, both time-domain, information-theoretic measures and time-frequency, phase-coherence measures suggest that auditory decisions in the absence of physical stimulus evidence can serve as a proxy for the

degrees to which neural noise is involved in varying percepts and to which neural noise can promote perceptual learning.

Disclosures: L. Waschke: None. M. Wöstmann: None. J. Obleser: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.06/GG2

Topic: D.05. Audition

Support: national science foundation of china (61473169)

national program on key basic research projects of china (2011CB933204)

Title: Neural representation and hierarchical processing of Chinese phonemes in temporal cortex revealed by intracranial EEG

Authors: *C. SONG[1], R. XU[2], B. HONG[2];
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Abstract: An important procedure in human speech recognition is to recognize the segmental units, e.g. phonemes and syllables, from the continuous speech sound stream. In English, these segmental units, especially phonemes and their acoustic features were represented in temporal cortex (Mesgarani, 2014). In this study, we used intracranial EEG signal from both surface and depth electrodes on human cortex to explore the representation of segmental units in Chinese, e.g. phonemes and combination of phonemes, which are supposed to be fundamental units in previous phonological studies. Subjects were instructed to listen to short stories. Chinese rhymes, which are defined to be composed of 3 phonemes named glide, nucleus and coda in phonology (Lee, 2003; Duanmu, 2000), were manually segmented from the stories. High gamma responses showed clear selectivity to Chinese rhymes on some electrodes throughout auditory pathway such as Heschl's gyri (HG), superior temporal gyri (STG) and superior temporal sulci (STS). To further explore the hierarchies along auditory pathway, we constructed both acoustic model and identity model for the Chinese rhymes. In the acoustic model, features for the rhymes were derived from principal components of their spectrograms. These models showed significantly different explained variance along the auditory pathway, which clearly dissociate the low level areas (HG) and high level areas (STG, STS). High level areas also showed stronger speaker invariance in their selective response. In short, the representation of speech units clearly exists in continuous speech perception, and distinct features of speech were processed along the

hierarchies of auditory pathway, with high level representation biased to the identity of rhymes rather than acoustic features.

Disclosures: C. Song: None. R. Xu: None. B. Hong: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.07/GG3

Topic: D.05. Audition

Support: Max Planck Society

Volkswagen Foundation

Title: Effects of L-dopa on the benefit from attention to memory

Authors: *S.-J. LIM[1,2], C. THIEL[3], B. SEHM[2], L. DESERNO[2], J. LEPSIEN[2], J. OBLESER[1,2]; [1]Dept. of Psychology, Univ. of Luebeck, Luebeck, Germany; [2]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [3]Dept. of Psychology, Univ. of Oldenburg, Oldenburg, Germany

Abstract: Selective attention to working memory (i.e., retrospective attention) is known to facilitate recall performance. We previously showed that retrospective attention to syllable objects in auditory working memory improves representational quality of the attended object. However, the extent to which multiple attention-related top-down brain networks and sensory (i.e., auditory) cortical regions are modulated by auditory retrospective attention is relatively unknown. Furthermore, dopamine is known to enhance working memory and cognitive flexibility. Yet, it is still unclear whether increased level of dopamine modulates neural activity that underlies retrospective attentional benefits. By using functional magnetic resonance imaging (fMRI) and pharmacological manipulation, we investigated the neural basis of auditory retrospective attention benefits in respect to dopaminergic modulation. Human participants (N=22, aged 25-35) underwent two separate scanning sessions, each with either 150 mg of L-dopa (Madopar) or placebo, while performing a syllable pitch-discrimination task. On each trial, participants encoded and maintained two sequentially presented syllables. During maintenance, a valid or neutral visual retro-cue was presented to guide participants' attention to a to-be-probed syllable in memory. Behaviourally, directing attention to the to-be-probed syllable via exogenous valid retro-cues led to faster and more precise detection of pitch change occurred at probe. However, under L-dopa the response-time benefit from valid retro-cues was reduced as L-dopa

increased the speed of response even in the uninformative neutral cue trials. Neurally, during the cue and maintenance period the primary auditory cortical regions were suppressed regardless of cue conditions. Overall, retrospective attention to a specific syllable led to enhanced activations in language-related networks, such as left inferior frontal gyrus and left posterior superior temporal regions as well as top-down attentional control networks, such as fronto-parietal and cingulo-opercular networks. However, L-dopa attenuated the extent of neural modulation in attentional networks, especially in the right dorsolateral prefrontal cortex and right insula by enhancing neural activity in the neutral cue trials. Overall, our results suggest that retrospective attention to auditory working memory engages top-down networks to actively select and maintain attended objects in memory, but increased dopamine level generally reduces attentional benefits from the exogenous cue to direct attention to memory.

Disclosures: S. Lim: None. C. Thiel: None. B. Sehm: None. L. Deserno: None. J. Lepsien: None. J. Obleser: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.08/GG4

Topic: D.05. Audition

Support: NIH R01-DC04290

NIH UL1RR024979

NSF CRCNS-1515678

The Hoover Fund

Hearing4All Cluster of Excellence

Title: Investigating decay in human auditory sensory memory with direct intracranial recordings

Authors: *A. WILSCH[1], A. RHONE[2], K. V. NOURSKI[2], C. S. HERRMANN[3], J. OBLESER[4]; [1]Univ. of Oldenburg, Oldenburg, Germany; [2]Dept. of Neurosurg., The Univ. of Iowa, Iowa City, IA; [3]Exptl. Psychology lab, Universitiy of Oldenburg, Oldenburg, Germany; [4]Dept. of Psychology, Univ. of Lübeck, Lübeck, Germany

Abstract: Working memory is characterized by two limitations: First, the amount of information that can be retained in memory (i.e., memory load) and second, the duration of retention (i.e.,

memory decay). Neural oscillations in the alpha frequency range (8-13 Hz) have been shown to increase in power with increasing memory load during memory retention (e.g. Jensen et al., Cereb Cortex. 2002, 12:877-882). However, it is unclear whether alpha power plays a more direct role in sensory memory decay and in potential counteracting factors, such as temporal expectations during sensory encoding. This study sought to characterize the degree to which neural oscillatory responses in human superior temporal cortex and its vicinity co-vary with sensory memory decay, and whether oscillatory decay would be modulated by temporal expectations. Subjects were neurosurgical patients undergoing chronic invasive electrocorticographic (ECoG) monitoring for remediation of medically refractory epilepsy. In an auditory delayed matching-to-sample task temporal expectations were manipulated (fixed vs. jittered cue-stimulus time interval) for a pair of pure tone sequences (S1-S2) embedded in white noise. Memory decay was manipulated with variable S1-S2 delays (1, 2, 4 s). ECoG data were simultaneously recorded from Heschl's gyrus (HG) and perisylvian cortex including supramarginal gyrus (SMG) using depth and subdural grid electrodes, respectively. Time-frequency analysis of neural responses focused on activity during the delay phase. Linear mixed models on the factors temporal expectations and memory decay revealed that alpha power increased with increasing delay phase duration at HG, independent of temporal expectations. Additionally, there was an interaction of temporal expectations and memory decay on left SMG indicating that alpha power increased with delay phase duration when S1 was temporally expected and decreased when S1 was not expected. Notably, the expected event-related high gamma power (80-150 Hz) increase at stimulus presentation was only present at the very onset of a trial but not during encoding of S1 and S2, most likely due to the ongoing noise masker. These findings imply that alpha power in auditory cortex (HG) serves as a neural marker of the inhibitory or protective processes required for sensory memory. This supports a domain-general interpretation of alpha as a modulatory top-down signal. Moreover, alpha power in SMG, a region relevant for pitch memory, indicates facilitated stimulus retention during memory decay for temporally expected sensory events.

Disclosures: A. Wilsch: None. A. Rhone: None. K.V. Nourski: None. C.S. Herrmann: None. J. Obleser: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.09/GG5

Topic: D.05. Audition

Title: Ongoing dynamics of frequency-specific large-scale brain networks predict the speed of auditory decisions

Authors: *M. ALAVASH[1,2], C. DAUBE[2], M. WÖSTMANN[1,2], A. BRANDMEYER[2], J. OBLESER[1,2];
[1]Auditory Cognition Group, Inst. of Psychology I, Luebeck, Germany; [2]Max Planck Res. Group "Auditory Cognition", Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Combining human magnetoencephalography (MEG) and source localization with large-scale network analyses allows us to explore the dependence of perception and cognition on frequency-specific brain network states. It is unknown to what degree the neural implementation of perceptual decisions relies on the brain functional connectivity networks that process the information available, for example from the auditory modality. Here, we investigate how ongoing brain network states relate to listeners' perceptual decisions in two distinct yet challenging auditory tasks. We measured MEG signals in human listeners (N = 20, age 20-38) who judged brief (400-ms) acoustic textures along one of two distinct dimensions. The acoustic textures consisted of densely layered tone sweeps and varied in the proportion of coherent tones in terms of sweep rate (upwards or downwards; local feature), as well as their overall pitch (global feature). Oscillatory power of the MEG source signals were derived within -0.5-1.5 sec relative to stimulus onset for the frequencies 1,2,4 Hz, together with the alpha and beta bands (8-32 Hz). To assess neural interactions in terms of graph-theoretical measures, Pearson's correlations between the power of all pairs of sources were computed per trial. Binary brain graphs were built at 10% of density, from which graph metrics were extracted per trial. To predict trial-by-trial decision speed from the ongoing brain network states, we used a general linear model approach, controlling the effects of the acoustic features (i.e. coherence and pitch). The standardized regression weights obtained from this model were averaged over subjects and compared with a null distribution. On the whole-brain level and for both tasks, faster decisions were correlated with lower global integration and higher segregation of dynamic brain networks. This effect was found prominently in the beta band (peaking at 21 Hz; false coverage-statement rate corrected). Interestingly, on the regional level we also found task-specific effects: Higher local efficiency in the vicinity of right supramarginal gyrus correlated with faster decisions in the pitch task. In contrast, higher local efficiency within the right anterior cingulate and left middle frontal gyrus correlated with faster decisions in the coherence task. Our results suggest that higher segregation of brain functional connectivity networks in mainly extra-temporal cortices speeds up auditory decisions. This might be due to more efficient local communication within the task-relevant networks. Depending on the task, this network configuration can emerge in distinct areas within the parietal and frontal cortices.

Disclosures: M. Alavash: None. C. Daube: None. M. Wöstmann: None. A. Brandmeyer: None. J. Obleser: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.10/GG6

Topic: D.05. Audition

Support: Oticon Foundation

Title: Scalp EEG predicts listener's attentional focus and attentional demands under continuously varying signal-to-noise ratio

Authors: *L. FIEDLER[1,2], M. WÖSTMANN[2], S. HERBST[2], C. GRAVERSEN[3], T. LUNNER[3], J. OBLESER[2];
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Abstract: In natural auditory scenes, the sound pressure level of the many sound sources varies over time. If a listener attends to one sound source (i.e., the signal) and ignores the other sound sources (i.e., the noise), the time-varying signal-to-noise ratio (SNR) can be extracted. In concurrent-speaker paradigms, the low-frequency oscillatory responses (~1-5 Hz) in the magneto- and electroencephalogram (M/EEG) phase-lock to the slow amplitude fluctuations (i.e., the temporal envelope) of speech. Importantly, this neural phase-locking is enhanced for attended versus ignored speech and furthermore for higher versus lower signal-to-noise ratios. In addition, the power of neural alpha oscillations (~10 Hz) correlates with the top-down demands of effortful listening and might indicate the inhibition of task-irrelevant perceptual input and neural processes. However, it is unclear in how far listening depends on the interplay of low-frequency phase and alpha power in time.
In order to extract the time courses of low-frequency phase-locking and alpha power in an approximated real-life listening scenario, four human subjects (aged 23-47) were asked to listen to one of two simultaneously presented audiobooks while the EEG was recorded. Over time, the two audiobooks followed two uncorrelated level time courses, yielding a highly variable underlying SNR time course varying between -6 and +6 dB. We employed regularized regression to predict (i.e., to forward-model) the EEG responses from the acoustic input signals. In detail, first, spectro-temporal profiles of attended and unattended speech were used to predict low-frequency phase-locking. Second, the underlying SNR time course was used to predict the power of induced alpha oscillations.
Under these continuously varying listening conditions, the attended speaker could be identified with an accuracy significantly above chance in all individuals by predicting the low-frequency phase-locking both of the attended and unattended speaker from the spectro-temporal representations. Best prediction of the listener's attentional focus was found at fronto-central

scalp channels. Furthermore, a fronto-central alpha power enhancement followed increases in the SNR with a temporal delay of several seconds.
These data pose a new approach to identifying a listener's attentional focus from EEG data applying a combined model for the prediction of low-frequency phase and alpha power. In this respect, our results suggest that low-frequency phase and alpha power reflect the enhanced neural encoding of attended acoustic input and the ensuing deployment of attentional control to overcome listening demands, respectively.

Disclosures: **L. Fiedler:** None. **M. Wöstmann:** None. **S. Herbst:** None. **C. Graversen:** A. Employment/Salary (full or part-time): Oticon A/S. **T. Lunner:** A. Employment/Salary (full or part-time): Oticon A/S. **J. Obleser:** None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.11/GG7

Topic: D.05. Audition

Support: JSPS Grant 16K12456

Title: Imagined rhythm can be decoded from EEG

Authors: *H. OKAWA, K. SUEFUSA, T. TANAKA;
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Abstract: Introduction : The neural mechanisms of rhythm perception have been studied for many years. A related work reported that rhythm imagery elicits a periodic electroencephalogram (EEG) response tuned to the imagined rhythm frequency (Nozaradan et al., 2011). They recorded EEG while subjects listened to periodic sounds and imagined rhythm. Their study revealed the neuronal entrainment to imagined rhythm by investigating neural responses to auditory rhythmic stimulus. However, brain activity during rhythm imagery without any auditory stimulus has not been revealed. Therefore, in this study, we recorded EEG while subjects imagined beat and meter under the condition that they were not presented any auditory stimulus, and investigated neuronal entrainment to imagined beat and meter by analyzing amplitude spectra of EEG. Methods : Nine subjects in their twenties participated in the experiment. All subjects were healthy and had normal or corrected-to-normal vision. They were given an informed consent, and the study was approved by the research ethics committee of Tokyo University of Agriculture and Technology. Subjects were asked to perform a no imagery task and three types of rhythm imagery tasks. In the rhythm imagery tasks, subjects were asked

to imagine beat by imaging tones at regular intervals and imagine the meter by changing strength of imaging tones. To give subjects a reference tempo for rhythm imagery, we used a movie like a rhythm game. In the no imagery task, subjects were asked to watch the movie without thinking about anything. This task was performed to compare the results with the rhythm imagery tasks. The EEG was recorded using 30 Ag/AgCl active electrodes located on the scalp following the international 10-10 system. We analyzed EEG epochs lasting 10 s during tasks as follows. Firstly, EEG epochs were filtered using the third-order Butterworth bandpass filter with a passband of 0.5-15 Hz. Secondly, EEG epochs were segmented into four epochs lasting 2.5 s, and then, they were averaged. Thirdly, for each subject and task, averaged EEG epochs lasting 2.5 s were averaged across trials. Finally, the trial-averaged EEG epochs were transformed into the frequency domain using the discrete Fourier transform. Results : EEG amplitude at imagined rhythm frequency clearly increased in each of the rhythm imagery tasks. We found that EEG amplitudes at imagined rhythm frequency in each of the rhythm imagery tasks were significantly greater than that in the no imagery task. Conclusions : We investigated neuronal entrainment to rhythm which was imagined without any auditory stimulus. We found that rhythm imagery elicited a periodic EEG response at imagined rhythm frequency.

Disclosures: H. Okawa: None. K. Suefusa: None. T. Tanaka: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.12/GG8

Topic: D.05. Audition

Support: DFG SFB TRR 31

Title: Predictability modulates excitation in the auditory cortex of macaques

Authors: *N. C. AGGELOPOULOS[1], E. SELEZNEVA[1], S. KNYAZEVA[1], A. G. GORKIN[2], M. BROSCH[1];
[1]Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Russian Acad. of Sci., Moscow, Russian Federation

Abstract: In a previous study (Selezneva et al, 2013), neuronal responses from the macaque primary auditory cortex had sensitivity to the type of isochronous sequence being presented. We defined isochrony as the occurrence of all tones with a strict periodicity, in our study 400ms apart. Stimuli consisted of complex tones of either 50 or 200 ms (also referred to as “short” and “long” respectively). Short and long tones were presented either in regular sequences of short-

short-long triplets or in irregular sequences in which they were randomly permuted (scrambled). The modulation of neuronal responses was in particular evident in a higher firing rate during the long tone in the regular isochronous sequence.
§
In this experiment we parametrically varied isochrony and regularity to determine whether either or both factors were important in the facilitation of neuronal responses by the rhythmical sequences. We presented 6 types of sequences with regular isochronous, regular partially isochronous and regular non-isochronous tone sequences, as well as irregular sequences where the long tones were randomly permuted among the short tones. The macaques were passively listening to these sequences and were not required to make a behavioural response.
§
A total of 101 multiunits with excitatory responses were analysed from two monkeys. A repeated measures ANOVA with 2 fixed factors, isochrony and regularity (i.e. whether the stimuli were arranged in short-short-long triplets or were randomly scrambled) was carried out. Both factors were significant in modulating the response of the population of neurons to the long tone. The responses to the 200ms stimuli were highest in the two regular isochronous sequences without scrambling.
§
We conclude that neural activity in the auditory cortex was modulated by regularities in a sound sequence. The modulation led to higher network excitation during the regular isochronous and partially isochronous sequences, the most rhythmical sequences with the highest degree of temporal predictability.
§
References
Selezneva E, Deike S, Knyazeva S, Scheich H, Brechmann A, Brosch M. (2013) Rhythm sensitivity in macaque monkeys. Front Syst Neurosci. 7:49.

Disclosures: N.C. Aggelopoulos: None. E. Selezneva: None. S. Knyazeva: None. A.G. Gorkin: None. M. Brosch: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.13/GG9

Topic: D.05. Audition

Support: KAKENHI 26330175

KAKENHI 15H05880

KAKENHI 15H05875

Title: Subconscious detection of the pitch change direction

Authors: *K. SAITO[1,2], M. MATSUHASHI[1,4], T. AWAYA[2], T. KATO[2], T. MIMA[1,5], A. IKEDA[3], H. FUKUYAMA[1,4], T. HEIKE[2];
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Abstract: [Introduction] The ability to discriminate emotion is so primitive that infants before speaking words are partly able to recognize emotions of the speaker. A distinction of emotion is sometimes represented by an alteration of pitch change direction of prosody. So we hypothesized that alterations of pitch change direction were detected in an early stage in perception. To clarify this hypothesis, we recorded mismatch fields of pitch change directions in unattended condition. [Method] Subjects were 20 healthy volunteers, age 19 - 26, without history of neurological or audiological diseases. Auditory multi-feature odd-ball paradigm with one standard and two types of deviants were performed. Each sound stimulus consisted of a common 600 ms tone-burst and a successive 200 ms that had different pitch from the preceding part, and presented with a 1200 ms stimulus onset asynchrony. The direction of pitch change of the latter 200 ms part of deviant 1 sound was the same as standard sound but with larger difference, and that of deviant 2 sound was opposite to standard sound but the same difference. Mismatch fields were recorded by 306 ch whole head magnetoencephalography (MEG). Source dipoles of deviant 1 and deviant 2 over left and right temporal areas were estimated from respective averaged waveforms in the time window of 75 ms to 225 ms from the beginning of the change of pitch, then mismatch responses were calculated as the largest differences between source activities of deviant stimuli and those of standard-like stimuli, which consisted of only the corresponding deviant sound presented with a 1200 ms stimulus onset asynchrony. After the MEG recording, behavioral tests to detect dissimilarity of stimuli pairs were performed with similar sound stimulus sets used in MEG. [Results] Two subjects were excluded from MEG analysis because of large and poorly controlled artifacts. ANOVA of the mismatch responses revealed a main effect of the pitch change direction ($p = 0.0002$), and the mismatch responses of deviant 2 (opposite direction) were larger than that of deviant 1(same direction). Behavioral study of 19 subjects excluding one subject because of lack of understanding showed that the pitch change directions significantly affected accuracy ($p < 2.2 \times 10^{-16}$) and response time ($p = 2.3 \times 10^{-9}$), and the accuracy was higher and the response time was shorter when the pitch change directions of the paired stimuli were opposite to each other. [Discussions] The results show that discrimination of pitch change direction is processed in the early, subconscious stage. This explains the result of behavioral data that it is easier to discriminate the pitch changes with a alteration of direction.

Disclosures: **K. Saito:** None. **M. Matsuhashi:** None. **T. Awaya:** None. **T. Kato:** None. **T. Mima:** None. **A. Ikeda:** Other; Endowed department by UCB, GSK, NihonKoden, Otsuka. **H. Fukuyama:** None. **T. Heike:** None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.14/GG10

Topic: D.05. Audition

Support: Action On Hearing Loss PhD studentship

Cochlear UK PhD studentship

Title: Cyborgs have problems too: measuring the cognitive cost of assistive technologies for the deaf

Authors: ***H. WILLIS**, S. ROSEN, D. VICKERS;
Speech, Hearing and Phonetic Sci., Univ. Col. London, London, United Kingdom

Abstract: In this century, those with hearing impairment have become cyborgs: their physiological functioning is dependent upon, or aided by, an electronic device. It is often assumed that, because many of these technologies are able to significantly improve speech perception, the negative impact of the disability has been ameliorated too. However, it must be borne in mind that none of these assistive technologies are able to restore perfect hearing, meaning that extra cognitive processing is required to compensate. This additional cognitive load has been specifically identified as listening effort. Alarmingly, listening effort has recently been discovered to have a direct and negative impact on physical and mental health. Yet, despite these acknowledged health consequences, there is no clinical test available to quantify and monitor this pernicious phenomenon. This study piloted a prototype of the first clinical test of listening effort on 6 normal hearing controls listening to simulations of the cochlear implant, as well as 4 experienced cochlear implant users. This cohort of deaf individuals were selected because cochlear implant users are often deemed to be the closest to being cured of their disability. This prototype was based on a novel dual task paradigm comprising of a primary task of auditory recall (in either quiet or in noise) that was to be simultaneously executed alongside a secondary task of visual recall. The intention was that secondary task performance will become the index of listening effort in the cochlear implant user. To validate the sensitivity and reliability of this listening effort index, a physiological process known to be intimately related to listening effort was measured: the pupil dilation response. In addition, a thorough assay of cognitive, executive,

intellectual and hearing function was performed to separate out any confounding variables. Results showed that normal hearing controls exhibited a decrease in secondary visual recall when listening to cochlear implant simulations compared to normal speech, suggesting increased listening effort. However, the cochlear implant users were unable to perform in noise (i.e. signal-to-noise ratio of -5dB SPL), despite being exemplary users of the cochlear implant. The pupil dilation response indicated that the cochlear implant users were overwhelmed by the auditory stimulation, with pupil constriction being exhibited instead of the predicted dilation. Therefore, there are indications that: 1) listening effort is being detected by this prototype clinical test; and 2) cochlear implant users are particularly vulnerable to excessive listening effort levels.

Disclosures: H. Willis: 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; Action On Hearing Loss, Cochlear UK. **S. Rosen:** 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; Action On Hearing Loss, Cochlear UK. **D. Vickers:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Action On Hearing Loss, Cochlear UK.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.15/GG11

Topic: D.05. Audition

Support: NIH R01-DC04290

NIH UL1RR024979

NSF CRCNS-1515678

The Hoover Fund

Title: Electrophysiology of the human superior temporal sulcus during speech and language processing

Authors: *M. STEINSCHNEIDER[1], K. V. NOURSKI[2], A. E. RHONE[2], H. KAWASAKI[2], M. A. HOWARD, III[2];
[1]Neurol., Albert Einstein Col. of Med., Bronx, NY; [2]Neurosurg., Univ. of Iowa, Iowa City, IA

Abstract: The superior temporal sulcus (STS) is a crucial hub in the cortical system subserving speech perception. To date, this area has been primarily examined with non-invasive functional neuroimaging (e.g. fMRI). Electrocorticography (ECoG) offers a unique opportunity to study this region with both high spatial and temporal resolution. Here, we examined ECoG activity evoked in the STS by a variety of stimulus classes under multiple task conditions. Our goal was to identify fundamental electrophysiological properties of this region. Subjects were neurosurgical patients undergoing chronic invasive monitoring for medically intractable epilepsy. All procedures were approved by the University of Iowa Institutional Review Board and NIH, and all subjects gave informed consent for this study. Direct recordings from middle and anterior portions of the STS were made using multicontact depth electrodes that targeted mesial temporal lobe structures (amygdala and hippocampus). ECoG data were simultaneously acquired from the auditory cortex in Heschl's gyrus (HG) and lateral superior temporal gyrus (STG) using penetrating depth and subdural grid electrodes, respectively. Stimuli were non-speech and speech sounds, presented in passive-listening and target detection tasks. Additional data were acquired during performance of the Mini Mental State Exam (MMSE; Folstein et al., J Psychiatr Res, 1975, 12:189-98) and other cognitive tasks. ECoG frequencies between 4 and 150 Hz were examined. The STS exhibited response profiles distinct from those in auditory cortex. The STS was less responsive to non-speech stimuli compared to speech. Prominent activity occurred in low gamma (30-70 Hz) and beta (14-30 Hz) bands. Responses had longer onset latencies than those in HG and on STG, except its most anterior portion. Activity within the STS preceded subjects' behavioral responses during active tasks. In a minority of sites, activity preferentially increased to target stimuli, including complex tones. Responses increased with intelligibility when speech was manipulated using a noise vocoder. Comparable degrees of activity were elicited in the language-dominant and non-dominant hemispheres. Finally, a conversation-based paradigm (MMSE) revealed sites in STS that preferentially responded to the subject's own speech over that of the interviewer's, reflecting their possible involvement in the dorsal audiomotor processing pathway. We conclude that human STS can be effectively probed with ECoG, offering unique insights into its response properties and the transformations that occur from earlier processing stages in auditory cortex.

Disclosures: M. Steinschneider: None. K.V. Nourski: None. A.E. Rhone: None. H. Kawasaki: None. M.A. Howard: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.16/GG12

Topic: D.05. Audition

Title: Biological motion sensitivity in deaf adults:an fMRI study

Authors: *M. SIMON[1], L. LAZZOUNI[1], E. CAMPBELL[1], F. CHAMPOUX[2], A. NEWMAN[3], F. LEPORE[1];
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Abstract: BACKGROUND: After an auditory privation, plasticity changes occur in the brain that make reference to a cross-modal reorganization. For individuals who use sign language as their primary means of communication, it is necessary to be more sensitive to the different types of motions (communicative *vs* non-communicative). Recently, research with deaf adult signers has uncovered an enhancement for visual motion detection. Also, the neural substrates for sign language requires activations of neuronal regions used in the processing of spoken language. However, it is not strictly identical as structural and hemispheric distinctions are noticeable on deaf subjects. OBJECTIVE: The aim of this study is to explore the influence of these processes (linguistic and visual detection) on the acute sensitivity of deaf subjects to detect and identify communicative *vs* non-communicative motions. METHOD: Functional MRI data were acquired on normal hearing and deaf signer participants. The task consisted of identifying the type of motion (communicative sign for "eat"), non-communicative ("playing piano") and scrambled (random motion) represented by point-light animations. Lasting approximately six minutes, the task was completed twice by each participant in a randomized order. RESULTS: Group differences were found between deaf and normal hearing participants in all conditions. More activity in temporal and frontal regions is found in deaf participants. Normal hearing participants were significantly less sensitive and slower to the presentation of communicative gestures than to the presentation of non-communicative movements, whereas deaf signers were equally sensitive and prompt to both types of motion. DISCUSSION: Consistent with previous research, these findings suggest that deaf and normal hearing participants use different systems for processing motion that may be result of an adaptative brain plasticity.

Disclosures: M. Simon: None. L. Lazzouni: None. E. Campbell: None. F. Champoux: None. A. Newman: None. F. Lepore: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.17/GG13

Topic: D.05. Audition

Support: Iowa State University College of Human Sciences Research Enhancement Grant

Title: Listening to music increases motor evoked potential amplitude in healthy older adults

Authors: *P. IZBICKI[1], S. ANDERSON[1], E. STEGEMOLLER[2];
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Abstract: Music has the ability to evoke various emotions as well as physical movement. However, there is only anecdotal experience that suggests that music influences the intent to produce a movement. Previous results from our lab using EEG have shown an increase of power in the upper Beta and lower Gamma band when moving in time with music. Moreover, there was a differential effect of style of music on the sensorimotor cortical activity when moving at fast rates. The use of transcranial magnetic stimulation (TMS) may provide further insight into these findings. Thus, the aim of this study was to examine motor evoked potential (MEP) amplitude induced by TMS in healthy young adults listening to different styles of music. Participants were exposed to three listening conditions during TMS: no music, relaxing music, and activating music. Twenty single pulses of TMS were applied over the hand area in the primary motor cortex approximately every 8 seconds at 120% resting motor threshold intensity for each condition. MEPs were recorded from the first dorsal interossieous muscle (FDI) using bipolar surface electromyography (EMG) (Delsys). Mean MEP amplitude was obtained for each condition. Repeated measures ANOVA was completed to compare MEP amplitude between the music and non-music conditions. Results thus far have revealed no statistically significant differences. However, mean MEP amplitude was greater for both music conditions compared to rest, suggesting that music listening may increase motor cortical excitability. Increasing the number of participants may reveal significant differences between music conditions.

Disclosures: P. Izbicki: None. S. Anderson: None. E. Stegemoller: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.18/GG14

Topic: D.05. Audition

Support: MRC Doctoral Training Award Studentship

MRC MC-A060-5PQ70

Title: A neural signature of segregation of tone sequences, independent of the attended stream

Authors: *A. J. BILLIG[1,2], M. H. DAVIS[2], R. P. CARLYON[2];
[1]Brain and Mind Inst., London, ON, Canada; [2]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom

Abstract: Repeated ABA- triplets of pure tones are often initially heard as arising from a single source but after several seconds split into two streams, each containing tones of one frequency. The percept then alternates between “integrated” and “segregated” forms. Characterising the neural response associated with these contrasting percepts is valuable for two reasons. First, it may reveal the loci and timing of mechanisms underlying perceptual organisation. Second, it provides an objective signature that can be used to probe how segregation is affected by cognitive factors, such as intention. Scalp recordings of evoked electric and magnetic fields have revealed percept-related differences in phase-locked activity between the B and final A tone of each triplet (Gutschalk et al., J Neurosci, 2005; Hill et al., Front Hum Neurosci, 2012; Szalárdy et al., Bio Psychol, 2013). However, whether this reflects modulation of native components (P1/N1), perhaps due to attention, or instead represents a new streaming component remains unclear. Divergent results may be due in part to different recording modalities and signal processing approaches. Here, 24 participants with normal hearing listened to 150-second ABA- sequences (A tones four or six semitones higher than B tones, 600-ms between-triplet onset asynchrony) while continuously reporting their percept. Cortical activity was recorded with concurrent EEG and MEG. In some sequences participants listened neutrally, while in others they attempted to bias their perception (by attending to the whole pattern, or to the A or B tones exclusively). The 20 sensors with the highest signal-to-noise ratio (for power at the triplet repetition rate, 1.67 Hz) in each recording modality were selected for each participant, and a weighted average evoked response calculated for each condition over 600-ms epochs. The continuous presentation of stimuli does not allow for an activity-free baseline period, so various alternative high-pass filters and baselines were applied to emphasise activity on different timescales. Percept differences in the neutral condition were revealed more strongly in magnetic than electric fields, and their timing depended on the signal processing. In general, segregation was reflected in an extended positivity beginning around 90 ms after the B tone, and distinct

from the canonical P1/N1 responses. Importantly, this signature persisted when comparing "attempt segregation" to "attempt integration" trials, and did not depend on whether the A or B tones were the focus of attention during attempts to segregate. This suggests the signature is truly one of streaming, and that listeners can influence their percept.

Disclosures: A.J. Billig: None. M.H. Davis: None. R.P. Carlyon: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.19/HH1

Topic: D.05. Audition

Support: Wellcome Trust WT091681MA

NIDCD R01 DC004290-11

Title: Direct human electrical recordings demonstrating fundamental bases for auditory figure-ground analysis

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Abstract: The ability to detect a relevant sound by filtering out irrelevant sounds in the environment is crucial in day-to-day listening. This ability requires that the features of the relevant sound be grouped together as a single source (figure) and segregated from the features (background) of other competing sounds in the environment. How the auditory system performs this task is not completely understood. In the current experiment we recorded local field potentials (LFPs) from human subjects undergoing invasive ECoG monitoring for pre-surgical localization of their epileptic foci while they were listening to a stimulus in which the salience of the figure was varied systematically against a background. The subjects were implanted with depth electrodes in auditory cortex along the axis of Heschl's Gyrus (HG) and subdural grids covering the superior temporal gyrus (STG).
The subjects listened to a stimulus in which the salience of the figure was varied systematically against a background. We used a stochastic-figure-ground (SFG) stimulus developed in our lab that has been previously characterized using psychophysics and modelling. The SFG consisted of

a sequence of simultaneously presented tones that were randomly distributed in log frequency space and varied from one 25 ms time frame to the next. During one time segment, a certain proportion of the tones remained the same over several consecutive time frames. This causes a figure to emerge from the background in which the salience is determined by number of tones kept fixed (the coherence) and the number of time frames over which this occurred (duration). In the current experiment, the first part (700ms) of the stimulus consisted of only the background with no figure, and in the second part the coherence of the figure was varied across trials (between 0, 2, 4 and 8). The overall duration of the figure was 28 individual 25ms time frames (700ms).
We measured event-related potentials (ERPs) and carried out single-trial time-frequency analysis using a wavelet transform. In HG responses to the figure when compared to the acoustically matched background were minimal or non-existent. In contrast, we observed a power change in the high gamma band (60-120 Hz) that peaked 200-300 ms and varied with the coherence of the stimulus on STG for all tested subjects.
The data demonstrate a neural correlate of auditory figure-ground segregation in the form of high-frequency local oscillations in human non-primary auditory cortex.

Disclosures: P.E. Gander: None. S. Kumar: None. K.V. Nourski: None. H. Oya: None. H. Kawasaki: None. M.A. Howard: None. T.D. Griffiths: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.20/HH2

Topic: D.05. Audition

Support: NIH R01-DC04290

NIH UL1RR024979

NSF CRCNS-IIS-1515678

The Hoover Fund

Washington Research Foundation

Title: Cortical mechanisms of auditory streaming in humans: An intracranial electrophysiology study

Authors: *X. WANG[1], R. CURTU[1], B. BRUNTON[2], K. NOURSKI[1];
[1]The Univ. of Iowa, Iowa City, IA; [2]Univ. of Washington, Seattle, WA

Abstract: Grouping and segregation of sounds within an auditory scene into coherent "streams" is a key component of auditory perception. To date, the neural mechanisms of auditory streaming in humans have been primarily investigated with non-invasive functional neuroimaging (e.g. EEG, MEG and fMRI). This study utilized electrocorticography (ECoG), which offers a unique opportunity to study auditory cortical processing in humans with high spatial and temporal resolution. ECoG activity was recorded during an auditory streaming paradigm, including stimuli that support auditory perceptual bistability, to characterize the transformation of acoustic stimulus attributes into auditory percepts. Subjects were neurosurgical patients undergoing chronic invasive monitoring for medically intractable epilepsy. Stimuli were 5-minute sequences of pure tones, presented in a classical ABA_ auditory streaming paradigm, where A and B are pure tones with a frequency difference ΔF, and "_" is a silent pause. Triplets presented with 2 and 12 semitone ΔF were used as control conditions to elicit stable 1- and 2-stream percepts, respectively. Triplets presented with 6 and 8 semitone ΔF were used as perceptually bistable stimuli. Subjects reported perceptual switches between 1 and 2 streams using a button press. ECoG recordings were made simultaneously from depth electrodes implanted in Heschl's gyrus (HG) and subdural grid electrodes implanted over perisylvian cortex, including superior and middle temporal gyrus (STG, MTG). Auditory cortical activity was characterized in the time domain as averaged evoked potentials (AEPs). In HG, responses to tone B were both positively correlated with stimulus acoustic attributes (ΔF) and, for perceptually bistable stimuli, were significantly larger to triplets reported as 2-stream vs. 1-stream. In contrast, no consistent correlation between responses to B-tones and ΔF was observed on STG and MTG, yet these areas exhibited a significant effect of percept (larger AEPs to 2-stream vs. 1-stream percept). The maximum difference between 2-stream and 1-stream percepts occurred at 60-100 ms after tone B onset in HG and at 100-140 ms in STG and MTG. The present findings confirm the results and expand the understanding of previous human functional neuroimaging studies. Effects of acoustic and perceptual stimulus attributes on cortical responses and their latency demonstrate differential contributions of hierarchically organized auditory and auditory-related cortical areas to auditory scene analysis. This work lays the foundation for further studies addressing the influence of top-down mechanisms such as attention on auditory scene analysis.

Disclosures: X. Wang: None. R. Curtu: None. B. Brunton: None. K. Nourski: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.21/HH3

Topic: D.05. Audition

Support: NIH F32 DC014192

NIH R01 DC012379

NIH DP2 OD008627

Title: Cortical encoding of speech intonation on human superior temporal gyrus

Authors: *C. TANG, L. S. HAMILTON, E. F. CHANG;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Speech intonation plays a crucial role in vocal communication, conveying linguistic information that is not explicit in word choice or syntax. For example, in English, rising pitch can turn a statement into a question and local changes in pitch can change sentence semantics through emphasis. Despite the importance of intonation in language comprehension, it is not known how different intonation contours are represented in human auditory cortex or how intonation affects the neural encoding of phonetic content. To investigate these questions, we created a set of sentences with linguistically distinct pitch contours and manipulated baseline pitch and formant values for each to create three speakers (two female, one male). Using electrocorticography, we recorded neural activity (high-gamma, 70-150Hz, analytic amplitude of the local field potential) from the cortical surface of human participants as they listened to these sentences. We found that local neural populations on the superior temporal gyrus (STG) showed activity that differentiated intonation contours regardless of phonetic content or speaker identity. These populations were spatially distinct from regions that encoded phonetic content or speaker identity. In addition, intonation did not affect the representation of phonetic features, such that phonetically selective electrodes had the same response to phonetically identical sentences with different intonation contours. Because neural activity patterns on intonation electrodes were similar in response to speakers with different baseline pitch values, the results cannot be attributed solely to differences in absolute pitch level between intonation conditions. However, they could still be explained by encoding of relative pitch, a measure of pitch which is normalized by speaker. Using a separate speech corpus (TIMIT) containing sentences spoken by hundreds of male and female speakers, we fit temporal receptive field models that predicted neural activity from absolute and relative pitch values simultaneously. We found that neural discriminability of intonation contours on single electrodes was significantly correlated with relative pitch encoding, while discriminability of speakers was associated with absolute pitch encoding. Our results indicate that the cortical representation of speech intonation is locally encoded in STG, is phoneme and speaker invariant, and is associated with the encoding of relative pitch, a more abstract feature than absolute pitch.

Disclosures: C. Tang: None. L.S. Hamilton: None. E.F. Chang: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.22/HH4

Topic: D.05. Audition

Title: The contribution of cognition in speech-in-noise perception in younger and older normal hearing listeners

Authors: *A. DRYDEN[1,2], H. A. ALLEN[1], H. HENSHAW[1,3], A. HEINRICH[1,2];
[1]Univ. of Nottingham, Nottingham, United Kingdom; [2]MRC Insitute of Hearing Res., Nottingham, United Kingdom; [3]NIHR Nottingham Hearing BRU, Nottingham, United Kingdom

Abstract: This study aimed to investigate the contributions of cognition in Speech-in-Noise (SiN) perception in a systematic way for a range of speech situations in a cross-section of younger and older adult listeners with age-normal hearing.
We hypothesised the strength of associations between intelligibility and cognition to vary depending on the choice of fore- and background sounds. For instance, sentences and words invoke semantic processing for intelligibility to different extents, and therefore might be expected to differ in their engagement of cognition. A similar reasoning may apply to informational and energetic maskers. Finally, by examining younger and older adults, we can investigate how cognitive contributions to speech perception change with age and hearing loss.
In the SiN tests, the target speech consisted of semantically low- and high-predicable (LP/HP) sentences, and of single words. Background noise was either speech-modulated noise or 3-talker babble. The speech conditions were chosen to vary the extent of semantic processing (words versus LP/HP sentences); the background noise was chosen to invoke more energetic (speech-modulated noise) or informational masking (3-tallker babble).
Cognitive assessments were selected to reflect the components of Baddeley's model of working memory: The central executive (Test of Everyday Attention subtests 1, 6 and 7, the Stroop test, reading span test, letter-number sequencing and digit span backward); the visual-spatial sketchpad (Corsi blocks forward and reverse); the episodic buffer (digit span forward and word list recall), and the phonological loop (two versions of a rhyme verification task).
Using principal component analyses, cognitive measures were reduced to four latent variables corresponding to the four sub-domains of the Baddeley model. Individual differences in latent variable scores were then related to each SiN condition using linear mixed models.
Young adults showed no predictive effect of central executive processing for any SiN measures, but did show an overall predictive effect of phonological loop processing. They also showed a predictive effect for visuo-spatial sketchpad as a main effect and in interaction with masker type, and for the episodic buffer component in interaction with masker type. Data collection for the older participant group is ongoing, results will be available in the poster presentation.

For normal hearing younger adults the results indicate that non-executive cognitive processes are more important in speech-in-noise perception, and that underlying cognitive processes may differ between listening in energetic and informational masking.

Disclosures: A. Dryden: None. H.A. Allen: None. H. Henshaw: None. A. Heinrich: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.23/HH5

Topic: D.05. Audition

Support: JSPS KAKWNHI 15K12069

Title: How to create the intelligible speech perception by cochlear nerve stimulation?

Authors: *Y. TAMAI, S. HIRYU, K. I. KOBAYASI;
Grad. Sch. of Life and Med. Sci., Doshisha Univ., Kyoto, Japan

Abstract: For compensating the hearing sense in a deaf person, a cochlear implant is generally used. While, the method has surgical invasiveness and infection risk. Stimulating cochlear nerves by a probe (an electrode, glass fibers, etc.) placed outside of the cochlea has been investigated as one possible solution for the problem. Most of these methods, however, has limited capacity to differentially stimulate auditory nerves comparing to a cochlear implant, and is virtually same as a single channel stimulation. The purpose of this study was to investigate whether the single channel stimulation enable the subjects to perceive speech sounds. Because mono channel stimulation evokes action potentials from several cochlear nerves simultaneously, the stimulation may create the perception that resemble the sensation evoked by a click sound. Therefore, click vocoded speech sounds were synthesized. The sounds were repetitive click, whose pitch (repetition rate) and amplitude followed formant frequency and amplitude envelope transition of an original speech sound respectively. Peak frequencies of first and second formant were extracted every 15 msec and amplitude envelope was extracted using half-wave rectification and low pass filter. Four-mora Japanese words with high familiarity (FW03: Japanese voice data set of familiarity-controlled word lists made by NTT speech resources consortium) were used as original sound; fifty words were randomly selected. Each stimulus was calibrated to 60 dB SPL. Five Japanese native speakers participated in the experiment as subjects. Tow session were conducted. In first session, subjects listened click vocoded speech sounds and answered what they perceived from four alternative choices. In second session, they wrote down their perception in the Roman alphabet. Consequently, the percentage of correct answers was significantly higher

than chance level in both the alternative choice test and the writing test. The result demonstrated that click vocoded sounds were at least partially intelligible as speech sound. In conclusion, with our speech coding algorithm, mono channel stimulation may enable the subjects to comprehend speech.

Disclosures: Y. Tamai: None. S. Hiryu: None. K.I. Kobayasi: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.24/HH6

Topic: D.05. Audition

Support: NIH Grant R01 EY018923

Title: Auditory-motor integration in the dorsal stream of the early blind

Authors: *J. PHILLIPS-SILVER[1], J. P. RAUSCHECKER[2];
[2]Neurosci., [1]Georgetown Univ. Med. Ctr., Washington, DC

Abstract: The dorsal auditory stream is crucial for sensorimotor control and integration (Rauschecker, 2011), auditory spatial localization and motion in space (Rauschecker & Tian, 2010), and musical anticipation, auditory and motor sequential processing (Leaver et al., 2009; Rauschecker, 2014). The ultimate expression of auditory-motor integration in humans is musical rhythm, using hierarchical temporal processing from auditory and motor channels and their interaction (Chen et al., 2007; Phillips-Silver & Trainor, 2005). Using fMRI we investigate the role of the dorsal stream in sensorimotor integration during the multisensory experience of 'feeling the beat' of music. We examine activation of the dorsal pathway in sighted and early blind populations, as the latter exhibit sensorimotor plasticity in auditory, motor and spatial processing (Fiehler & Rösler, 2010; Renier et al., 2010). Early blind and sighted individuals were trained to 'feel the beat' of an ambiguous drum rhythm pattern, by bending their knees on every second or third beat (from Phillips-Silver & Trainor, 2007). Following training, subjects entered the scanner and completed a beat recognition task, listening to paired renditions of the rhythm pattern with acoustic strong beats on every second or third beat. Patterns were played on varying percussion timbres; subjects identifed the rendition with the familiar strong beat based on their movement training. In a control task, subjects determined whether the pairs of stimuli were played on instruments of identical or different timbres. We predicted that the beat recognition task would recruit the auditory dorsal stream and other areas for musical beat processing (Merchant et al., 2014) in the sighted and blind. In contrast, we predicted that the timbre

discrimination task would recruit areas of the ventral stream in both groups. Preliminary data analyses in sighted (N=10) and blind (N=5) groups reveal predicted activations: dorsal stream areas (posterior auditory cortex, inferior parietal lobule, premotor and dorsal prefrontal areas including supplementary motor areas), as well as subcortical beat processing regions (cerebellum, thalamus and putamen), for the beat task; ventral areas light up during the timbre task. Findings from this study will illuminate sensory cortical organization and functional specialization in the dorsal stream of the early blind, and identify the neural circuitry for auditory-motor integration that is necessary and sufficient for musical anticipation, temporal processing and action.

Disclosures: J. Phillips-Silver: None. J.P. Rauschecker: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.25/HH7

Topic: D.05. Audition

Support: DFG LA 3080/1-1

Title: Cortical plasticity for own and partner material after short- term musical duet training

Authors: *C. LAPPE, S. BODECK, M. LAPPE, C. PANTEV;
Univ. Muenster, Muenster, Germany

Abstract: Predictive mechanisms in the human brain can be investigated using markers for prediction violations like the mismatch negativity. Short-term piano training increases the mismatch negativity for melodic and rhythmic deviations in the training material. This increase occurs only when the material is actually played, not when it is only perceived through listening, suggesting that learning predictions about upcoming musical events are derived from motor involvement. However, music is often performed in concert with others. In this case, predictions about upcoming actions from a partner are a crucial part of the performance. In the present MEG experiment we trained non-musician subjects in a piano-duet task and measured mismatch components elicited by melodic and rhythmic errors within the pianist's own and the partner's material before and after training. The training exercise was a short piano piece based on the C-major chord progression. Training sessions were scheduled for each pair separately on 8 days over the course of three weeks and lasted 30 min each. For the pre- and post-training mismatch negativity measurement in the MEG, the intro of the piano exercise was used. To generate a melodically elicited mismatch negativity within the primo and secundo musical material, 80

deviant sequences were altered by lowering the sixth tone, which was part of the primo training material, by a minor third. In the remaining 80 deviant sequences of that run the eighth tone was lowered by a minor third. This tone was part of the secundo training material. To generate a mismatch negativity to a rhythmic change, the sixth (primo part) and eighth tones (secundo part) were presented 70 ms earlier. ERP results revealed that the mismatch negativity increased significantly both for own and partner material suggesting a neural representation of the partner's part in a duet situation. Source analysis using beamforming revealed common activations in auditory, inferior frontal and parietal areas, similar to previous results for single players but also a pronounced contribution from the cerebellum. In addition, activation of the precuneus and the medial frontal cortex was observed, presumably related to the need to distinguish between own and partner material.

Disclosures: C. Lappe: None. S. Bodeck: None. M. Lappe: None. C. Pantev: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.26/HH8

Topic: D.05. Audition

Support: SNSF P1ZHP1_158642

Title: Auditory aversion in subjects with absolute pitch

Authors: *L. ROGENMOSER, H. LI, G. SCHLAUG;
Beth Israel Deaconess Med. Ctr., Harvard Med. Sch., Boston, MA

Abstract: Musicians with absolute pitch (AP) have the advantageous ability of correctly identifying or producing pitches without making use of reference tones. However, they are known to become unusually disturbed when hearing tones out of tune, this possibly disadvantaging their adaptability during group performance. This study aimed at empirically confirming an AP-specific auditory aversion. In order to measure aversive experiences, we recorded EEG in a sample of AP possessors and a sample of matched control musicians without AP while letting them perform an affective priming task. According to this paradigm, subjects were instructed to judge valenced pictures (selected from the International Affective Picture System) as quickly and accurately as possible while musical primes preceded these targets. These primes were bimodal, simultaneously presented as tones (i.e., auditory) and notes (i.e., visual) that either matched or mismatched (i.e., sharp or mistuned). Compared to controls, subjects with AP judged the targets less accurately and with greater delay when the preceding

musical primes were mismatched. In line with these behavioral findings, the N400 component peaked stronger in the mistuned conditions in subjects with AP. In addition, AP subjects exhibited increased P200 amplitudes in response to the primes. These findings (i.e., behavioral priming effect, N400 response) suggest that subjects with AP respond more aversively to tones when they are out-of-tune than control subjects do. Furthermore, the P200 responses indicate an early (undirected) affective integration, for which AP subjects appear to be accelerated. Taken together, these findings support an AP-specific auditory aversion and further suggest overall altered emotional responding to musical stimuli in subjects with AP.

Disclosures: L. Rogenmoser: None. H. Li: None. G. Schlaug: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.27/HH9

Topic: D.05. Audition

Support: Irish Research Council for Science, Engineering & Technology.

Guarantors of Brain travel grant

Title: Investigating the effect of perceptual enhancement on the cortical representation of speech using source space analysis with MEG

Authors: *G. M. DI LIBERTO[1], R. E. MILLMAN[2], E. C. LALOR[1];
[1]Trinity Ctr. for Bioengineering, Trinity Col., Dublin, Ireland; [2]Univ. of Manchester, Manchester, United Kingdom

Abstract: It has been suggested that speech perception in humans is underpinned by a hierarchical cortical system which constantly attempts to match incoming sensory inputs with top-down predictions (Friston & Kiebel 2009; Peelle et al., 2010; Clark, 2013). In this context, perception depends on both "external" sensory inputs and "internal" predictive mechanisms. A number of recent studies have identified effects consistent with this view by measuring cortical activation while modulating these internal predictions by providing prior-knowledge of an upcoming stimulus. In particular, enhanced cortical activation has been linked with perceived intelligibility (post. STG/MTG) while cortical suppression has been associated with repeated stimuli (ant. STG/STS; Tuennerhoff & Noppeney, 2016). Importantly, the timing of cortical activations has been shown to be crucial. Specifically, areas involved in higher order functions have been shown to be activated at very short latencies when prior-knowledge is available, while

suppression of lower level cortical areas emerged at longer latencies (Sohoglu et al., 2012). These findings advanced our current understanding of speech perception, however it has been suggested that counteracting effects may co-occur in particular cortical areas (Tuennerhoff & Noppeney, 2016). A recent EEG study derived a measure of speech encoding at the phonemic level that is sensitive to differences in the recognition of degraded speech stimuli (Di Liberto et al., in press). However the cortical areas involved in this phenomenon remain unclear.
Here, we used magnetoencephalography (MEG) to study the effect of perceptual enhancement on both cortical activation and entrainment measures in response to speech. Beamforming-based analysis was used to estimate cortical activity in brains areas of interest. Participants were presented with tone-vocoded speech sentences, which were unintelligible in the first part of the experiment. Subsequently, priming was used to enhance the perceived intelligibility of the same degraded sentences. The "pop-out" condition was compared with a control condition where no prime was provided. The effect of perceptual enhancement on cortical activation was consistent with previous findings. Additionally, cortical entrainment to the speech envelope revealed significant variations in STS and MTG in the pop-out condition.

Disclosures: G.M. Di Liberto: None. R.E. Millman: None. E.C. Lalor: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.28/HH10

Topic: D.05. Audition

Support: Irish Research Council Postgraduate Scholarship Scheme

Title: Decoding cocktail party attention using EEG signatures of hierarchical speech processing

Authors: *E. TEOH, E. C. LALOR;
Trinity Col. Dublin, Dublin, Ireland

Abstract: The cocktail party problem is a phenomenon that refers to the remarkable ability of humans to selectively focus on a single speaker in noisy environments. Exactly how the auditory system performs this feat remains incompletely understood. Recently, progress has been made with the realisation that cortical activity entrains to the amplitude envelope of speech. By employing a stimulus-reconstruction approach where neural activity is mapped back to the envelope, it has been shown that attentional selection can be decoded to a high degree of accuracy, even from single-trial (~60 s) electroencephalography (EEG) data (O'Sullivan et al., 2014).

While this approach has opened up new opportunities for studying cocktail party attention, the reliance on the amplitude envelope as the representation of speech is almost certainly suboptimal. This is because speech is processed by a hierarchical cortical network with multiple stages performing specialized, but interrelated roles. Thus, a mapping of EEG to the speech envelope may be to conflate the multi-stage processing of many speech features into a single aggregate measure. Indeed, recent research has shown that EEG responses to natural speech are best predicted when that speech is represented in terms of both its low-level spectrotemporal acoustics and a higher-level categorical labelling of phonetic features, rather than simply as its envelope (Di Liberto et al., 2015).
Here, we sought to explore the efficacy of decoding attentional selection when relating cortical activity to different low- and high-level representations of natural speech. EEG data were collected as subjects engaged in a listening task where they were cued to attend to a story in one ear whilst ignoring a different story in the other ear. We perform stimulus-reconstruction with speech represented in the form of a so-called AI-gram, which reflects the spectrotemporal features of speech necessary for articulation and intelligibility, as well as in terms of word categories and sentences. Finally, we leverage the complementary information provided by these various representations to further improve the decoding of attentional selection.

Disclosures: E. Teoh: None. E.C. Lalor: None.

Poster

237. Auditory Processing: Humans

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 237.29/HH11

Topic: D.05. Audition

Title: Scenereader: a high-level visual prosthesis

Authors: *Y. LIU, M. MEISTER;
Caltech, Pasadena, CA

Abstract: To achieve the goal of helping blind people to navigate, we translate information about the surrounding scene into sounds, using simple and intuitive rules that require minimal training. This technology may lead to non-invasive, cost-effective visual prostheses, as well as augmented reality for normally sighted subjects.
There are tens of millions of visually impaired people worldwide. Several approaches are being developed to directly restore visual function, but most are surgically invasive and not yet available for clinical use. As a noninvasive alternative, sensory substitution methods deliver visual information through other sensory modalities, such as sound (“sonification”). Current

devices in this domain encode low-level scene features, such as the distance to an obstacle, or the intensity pattern in a static image, and have met with limited success. We propose to take advantage of modern computer vision technology to extract high-level knowledge about the surrounding scene. For example, driverless cars already combine GPS, radar and real-time computer vision into a rich, high-level and actionable representation of the environment. Here we focus on the remaining challenge, namely to efficiently communicate this high-level knowledge to blind subjects.
We have created an experimental platform with which diverse sensory substitution methods can be implemented and compared, using sighted or blind human subjects. The subject wears virtual reality goggles and navigates in a 3D virtual environment, which provides visual experience as well as a sonification of the scene. After training we remove the visual input and the subject navigates based on acoustic signals alone. Any given method of sonification can be assessed by the subject's final performance and training time required. As an example, we have developed a strategy called SceneReader that describes the scene in natural language. It calls out the names of selected actionable objects with natural sound localization cues so that the user experiences the sound as coming from the object's location. Obstacles to be avoided emit a generic noise signal. Sighted naive subjects have learned to use SceneReader in minutes and successfully navigate in some common urban environments with ease. In ongoing studies we are also recruiting blind subjects to perform the same tasks.
Future directions for this project include support for the blind in tasks other than navigation, such as social encounters, as well as augmented reality benefits for sighted subjects. The system also serves as a valuable research tool into crossmodal perception, owing to the ease with which multisensory relationships can be manipulated.

Disclosures: Y. Liu: None. M. Meister: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.01/HH12

Topic: D.06. Vision

Support: Faculty for the Future fellowship

Title: Assessment of the effects of Schisandrin-B on apoptosis in the pde6c Zebrafish mutant retina

Authors: *P. VENKATRAMAN[1], Y. LEUNG[2];
[1]Biol. Sci., [2]Purdue Univ., West Lafayette, IN

Abstract: Loss of photoreceptors (PRs), rods and/or cones, in eye diseases such as retinitis pigmentosa, expedites loss of vision in humans. However, no proper chemical treatment has been developed to tackle PRs degeneration so far. In our lab we wish to address this by identifying naturally extracted compounds as potential therapeutic targets. One of the compounds we are currently testing is Schisandrin-B (SchB), which is one of the active components of a fruit used in traditional Chinese medicine for many years. We recently showed that that SchB treatment reduced the size of the abnormally enlarged rods found in *pde6c* mutants. We also observed an improvement in vision-mediated behavior in the SchB-treated mutant larvae when compared with the untreated mutants. Based on these results, ***we hypothesized that SchB could be interfering with the apoptosis of rods thus exerting a preserving effect on the degenerating rods in pde6c mutants.*** To test this hypothesis, I performed TUNEL staining to assess the level of apoptosis in the *pde6c* mutants. Taking together these results and our previous work, we observe cell death in the *pde6c* mutants. This will be corroborated with immunohistochemical (IHC) analysis of activated-caspase-3 labeling for apoptotic pathway combined with co-labelling of antibody markers for rods and cones. I will present some preliminary data on this work by comparing the cell death in untreated *pde6c* mutants SchB-treated *pde6c* mutants. Following IHC, I will quantify the number of apoptotic cells in both experimental conditions. I expect that the SchB-treated *pde6c* retinas will show fewer or delayed apoptosis in rods. This will give us more information about the pathway which the drug (SchB) is targeting.

Disclosures: P. Venkatraman: None. Y. Leung: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.02/HH13

Topic: D.06. Vision

Title: Determine the development basis of retina-SCN functional connectone

Authors: *P.-S. CHIN[1], Y. CHANG[2], S.-K. CHEN[2];
[1]Dept. of Life Sci., [2]Natl. Taiwan Univ., Taipei, Taiwan

Abstract: In mammals, suprachiasmatic nucleus (SCN) located at the hypothalamus is the master clock to control daily activity pattern and influence many physiological functions. In mammalian retina, intrinsically photosensitive retinal ganglion cell (ipRGC) contributes to many light-induced non-image forming functions. They directly project to suprachiasmatic nuclei (SCN) for circadian photoentrainment. The SCN can be separated into several regions, such as the ventral lateral SCN (vlSCN), which contains VIP and GRP expressing neuron, and the dorsal

medial SCN, which contains AVP neurons. Previous studies suggests that light input is only transmitted to VIP and GRP expressing neuron in the vlSCN, while the AVP expressing neurons in the dmSCN provide output from the SCN. However, our single cell tracing study indicated that ipRGCs innervate to whole SCN and make putative synaptic contact with VIP, GRP and AVP neuron. Furthermore, we found that each ipRGC preferentially innervates to a specific region of the SCN. In the retina, ipRGCs projecting to ventral part of the SCN are clustered together. To determine whether a specific chemo-attractive or repulsive cue is expressed in the SCN, we used immune-histochemistry method to stain axon guidance molecular in SCN coronal sections.

Disclosures: P. Chin: None. Y. Chang: None. S. Chen: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.03/HH14

Topic: D.06. Vision

Support: NRF-2013R1A2A2A01068457

Title: Possible role of zinc dyshomeostasis in matrix metalloproteinase-2, 9, endoplasmic reticulum stress, and photoreceptor cell death in experimental retinal detachment

Authors: *J. CHOI[1], Y. KIM[2], B.-R. SEO[1], J.-Y. KOH[1,3], Y. YOON[2];
[1]Neural Injury Res. Ctr., Asan Inst. For Life Sci., SEOUL, Korea, Republic of; [2]Ophthalmology, Asan Med. Center, Univ. of Ulsan Col. of Med., SEOUL, Korea, Republic of; [3]Neurol., Asan Med. Center, Univ. of Ulsan Col. of Med., Seoul, Korea, Republic of

Abstract: Purpose: To investigate the possibility that zinc dyshomeostasis has a role in activation of matrix metalloproteinase (MMP), induction of endoplasmic reticulum (ER) stress, and photoreceptor cell death in the retinal detachment (RD).
Methods: In order to induce retinal detachment in mice, we created by injecting 3 µl of 1% sodium hyaluronate through self-sealing scleral incision. Pathological endpoints examined in vivo included photoreceptor cell death, intracellular zinc level, ER stress induction, and MMP-2, 9 expressions in RD mice at 30minutes, 1 day, 3 days, and 7 days post RD. In addition, we examined whether expressions of ER stress markers and the degree of apoptotic cell deaths may change by modulating extracellular zinc concentration or MMP activation in subretinal fluid.
Results:. We observed that the intracellular zinc was markedly decreased after 30 min of RD induction. Concurrently, activities of MMP-2 and 9 increased in RD mouse retinal tissue,

followed by photoreceptor cell death. Photoreceptor cell death was peaked on day 3. Immunostaining for the ER stress marker GRP78 Bip, revealed that ER stress was also induced in our RD model. We examined that subretinal injection of CaEDTA reduced ER stress markers and photoreceptor cell death in RD eyes. Additionally, we observed that ER stress markers and photoreceptor cell death were decreased by treated with GM6001 (MMP inhibitor).
Conclusions: Our work showed that redistribution of zinc in retinal tissues may be a contributing factor in MMP activation, ER stress, and photoreceptor cell death in RD. Since MMP inhibition protected against ER stress in and death of photoreceptor cells, MMPs may be a reasonable target for cytoprotection in case of RD.
Keywords: Endoplasmic reticulum (ER) stress, Matrix metalloproteinase (MMP), Photoreceptor cell degeneration, Retinal detachment, Zinc dyshomeostasis

Disclosures: J. Choi: None. Y. Kim: None. B. Seo: None. J. Koh: None. Y. Yoon: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.04/HH15

Topic: D.06. Vision

Support: Macular Society

Title: Characterisation of a mouse model for testing the efficacy of transplanted human RPE cells

Authors: *A. LYNCH, M. SEMO, J. ASLAN, A. VUGLER;
ORBIT, UCL Inst. of Ophthalmology, London, United Kingdom

Abstract: Introduction: The RCS dystrophic rat has been used to test efficacy of human retinal pigment epithelium (RPE) cells for the treatment of dry age-related macular degeneration (AMD). However, unlike dry AMD, where geographic atrophy denudes Bruch's membrane of RPE, the dysfunctional RPE cells of RCS rats fail to degenerate. An alternative approach uses sodium iodate ($NaIO_3$) to selectively kill the host RPE, a procedure that facilitates survival of polarised human RPE cells along Bruch's membrane in mice (Carido et al., 2014). However, despite RPE phagocytosis of outer segments, no evidence of functional recovery could be found by electroretinography (ERG), possibly due to insufficient delivery of visual chromophore by the grafted cells. Here we characterise the functional and anatomical consequences of administering sodium iodate to mice and test whether the complete restoration of retinal chromophore supply by systemic 9-cis-retinal supplementation can restore retinal function to $NaIO_3$-treated mice.

Methods: Adult C57 mice received a single intravenous injection of $NaIO_3$ (40mg/Kg) and photoreceptor function was then assessed by ERG at 3 days, 7 days and 1-month post-$NaIO_3$ treatment. Mice were dark adapted and received intraperitoneal injections of 9-cis-retinal (1mg/mouse) or saline 16h prior to ERG recordings. Photoreceptor function was also assessed in $NaIO_3$-treated mice by measuring the pupillary light reflex (PLR) at 1 month post-injection. Mice were sacrificed after functional testing and Immunohistochemistry was performed in order to assess RPE ablation and photoreceptor integrity.
Results: In the absence of systemic chromophore, the ERG was flat or negative at all time points studied. Functional recovery of the ERG in $NaIO_3$-treated mice (restoration of A and B waves) could be produced following systemic delivery of 9-cis-retinal. However, this effect only occurred in 50% of animals at 3 days post-$NaIO_3$-treatment and at 7 days post-treatment the ERG was unrecoverable. The sensitivity of the PLR in $NaIO_3$-treated mice was reduced but appeared largely intact at high irradiances. Immunohistochemistry confirmed focal RPE degeneration, disruption to the outer nuclear layer and the persistence of melanopsin expression in $NaIO_3$-treated mice.
Conclusion: The ability of systemic chromophore supplementation to recover ERG function in $NaIO_3$-treated mice is lost between 3 and 7 days post-treatment. This suggests that photoreceptors may be irreversibly damaged by $NaIO_3$-treatment and may help to explain the failure of others to correlate functional improvement by ERG with the presence of grafted human RPE (Carido et al 2014).

Disclosures: A. Lynch: None. M. Semo: None. J. Aslan: None. A. Vugler: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.05/HH16

Topic: D.06. Vision

Title: Effects of prolonged treatment with uv-a radiations in arpe19 cells: expression of early and late cellular damage markers.

Authors: *G. TRINGALI[1], B. SAMPAOLESE[2], M. CLEMENTI[2];
[2]CNR-ICRM-Catholic Univ. Sch. Med., Roma, Italy, [1]Catholic Univ. Sch. Med., Roma, Italy

Abstract: Exposure to solar radiation has been implicated in many ocular pathologies and in particular in the macular degeneration: UV radiations can induce the production of reactive oxygen species (ROS), mitochondrial dysfunction, DNA damage, and an increasing of apoptotic activity. However the underlying mechanisms of UV-mediated retinal pigment epithelium (RPE)

cell death are controversial and remain unclear. Two hypotheses are the most accredited in literature: i) exposure to UV radiation causes the production of ROS, which may damage RPE cells through apoptotic mechanisms activation; ii) UV radiation stimulates an elevated production of ROS which can lead to an irreversible cellular necrosis. Within this framework, to better understand both molecular basis and temporal sequence of the degenerative processes underlain to RPE dead after UV radiation exposition, we investigated cell viability, ROS production and the expression of principal apoptotic genes (Bax, Bcl2, Caspase-3) on Arpe-19 cells after UV-A radiation for consecutive times (5/7 hours). We found that UV-A prolonged exposure induced: i) cell death: the decrease of cell viability was time-dependent and reached statistical significance after three hours; ii) a significant and substantial increase in ROS levels that remained constant for the duration of the experiment: this increase was a statistically significant result from one hour of exposure; iii) an activation of apoptotic genes (Bax and Caspase 3) after 1 h of treatment, which it is accompanied by a decrease of Bcl2, anti-apoptotic gene; iv) a loss of apoptotic signals and a rapid decrease in the cellular availability after three hours of consecutive treatment; these processes could trigger the necrosis observed in morphological traits of cells treated consecutively for five hours. To the best of our knowledge, this is the first report that describes the sequence of molecular events that occur after continuous exposure to UV-A radiation: it starts with ROS levels increase, which lead before to activation of apoptotic events and then to an irreversible cellular necrosis. These interesting findings, even if preliminary, may be useful for improving our understanding about oxidative damage which is underlain various UV-induced ocular cell damage.

Disclosures: G. Tringali: None. B. Sampaolese: None. M. Clementi: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.06/HH17

Topic: D.06. Vision

Support: 103-2628-B-002-001-MY3

Title: Dim light at night influence gut microbe and metabolic status through melanopsin

Authors: *C.-C. LEE[1], Y.-F. ZOU[1], S.-K. CHEN[1,2];
[1]Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan; [2]Genome and Systems Biol. Degree Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: In 20 th century, the invention of artificial light source has dramatically change the human society. In addition to sun light during the day time, artificial light prolongs the light exposure time at the night time. Furthermore, increasing number of shift workers are working at the night time with bright light exposure. Recent human studies suggested that exposure to the artificial light during nighttime (LAN) is strongly associated with obesity, which could lead to cardiovascular disease. In addition, studies in mice indicated that the LAN exposure induces metabolic disorder such as obesity and hyperglycemia independent of the total caloric intake. Therefore, it is likely that light exposure could have direct influence on metabolic function in both diurnal and nocturnal aniamls. However, the exact mechanism for LAN induced metabolic disorders remains unclear. It has been shown that intrinsically photosensitive retina ganglion cells (ipRGCs), which expressed novel photo pigments melanopsin, transmit the light signal to control many non-image forming physiological functions. Thus, we hypothesize that melanopsin photo-detection system is involved in the LAN induced metabolic disorders. Using genetic mouse model and metagenomics analysis, here we showed that dim light at night induced obesity, hyperglycemia and insulin resistance through melanopsin. Moreover, the LAN induced metabolic disorders are partially blocked by antibiotics treatment which eliminated gut microbiota. Together our data suggests that aberrant light dark cycle could modulate gut microbiota and affect the energy metabolism in mice through melanopsin photo detection pathway.

Disclosures: C. Lee: None. Y. Zou: None. S. Chen: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.07/II1

Topic: D.06. Vision

Title: Photoreceptor-glial signaling during neurovascular coupling underlying functional retinal imaging.

Authors: *M. BEGUM, D. Y. TS'O;
Neurosurg., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Light-evoked reflectance decreases in the retina, seen using intrinsic signal optical imaging, are likely to be of an outer retinal origin. Other studies have shown that such reflectance changes are dominated by hemodynamics. We have sought to dissect the role of several prospective signaling pathways in the observed stimulus-evoked neurovascular responses in vivo through intravitreal injections of selected agonists, antagonists and blockers. Of particular

interest are the possible mechanisms by which the light response that is transduced in the photoreceptors is then transmitted into neighboring Muller cells to ultimately effect a neurovascular response.
Adult cats were anesthetized and positioned in a stereotaxic. Using a modified fundus camera, the retina was stimulated with visible (550nm) patterned stimuli and illuminated in the near-infrared (700-900nm), while intrinsic optical signals were recorded with a CCD camera. Previous retinal imaging studies using intravitreal injections of blockers of inner retinal function (e.g. TTX, PDA, APB) yielded little impact on the retinal imaging signals. Those studies also indicate no role of glutamate receptors in the observed response. Among the pathways by which photoreceptors might signal Muller cells without the involvement of the inner retina, we considered 1) changes in K^+ concentration, 2) adenosine/ATP signaling, 3) glutamate via transporters (e.g. GLAST). We have therefore employed injections of Ba^{+2}, adenosine agonists and antagonists, and GLAST blockers, performing functional retinal imaging before and after these intravitreal injections. Each agent exhibited a profound impact on the observed light-evoked retinal imaging signals. In some cases, the sign of the observed retinal imaging signal changed from negative (vasodilation) to positive (vasoconstriction). Injections of Ba^{+2} abolished the imaging signal completely, while GLAST blockers inverted the sign of the reflectance change. Adenosine agonist and antagonists exhibited multiple effects with differing time courses, suggesting actions at several sites/receptors. These studies help establish the chain of retinal events from light absorption to observed changes in retinal reflectance in vivo.

Disclosures: M. Begum: None. D.Y. Ts'o: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.08/II2

Topic: D.06. Vision

Support: R01EY024982

Title: Transcriptomic and epigenetic profiling of cone photoreceptor generation from a restricted retinal progenitor population

Authors: *D. F. BUENAVENTURA[1], M. EMERSON[2];
[2]Biol., [1]City Col. of New York, New York, NY

Abstract: Every cell type in the retina is derived from multipotent retinal progenitor cells (RPCs). Current evidence suggests that some RPCs are molecularly restricted to produce only

certain cell types. Recently, a specific cis-regulatory module of the early cone photoreceptor marker Thrb was identified. The ThrbCRM1 regulatory element drives reporter expression in cells that normally express the thrb gene, including a subset of RPCs. Our data indicates that these RPCs are fate-restricted and preferentially generate cones and horizontal cells. Additionally, Onecut1 (OC1) is present in these RPCs, necessary for activation of the ThrbCRM1 element, and sufficient to induce early cone photoreceptor gene expression. We have used differential transcriptomic and epigenomic profiling of the ThrbCRM1 cell population to investigate the transcription factor networks and intergenic regulatory regions that are active during this phase of cone photoreceptor development. To further dissect the role of OC1, transcriptional changes will be identified through similar profiling and testing after the introduction of a OC1 dominant-negative construct. Currently, ex-vivo/in-vivo electroporations in chicken retinas are being used to functionally assess the roles of identified genes and cis-regulatory regions in cone fate determination. Finally, analysis of the ThrbCRM1-negative retinal population at this developmental stage led to the identification of a regulatory element that our current evidence suggests is active in the multipotent progenitor population. Together, these studies will investigate the gene regulatory events underlying the formation of restricted RPCs and the generation of cone photoreceptors.

Disclosures: D.F. Buenaventura: None. M. Emerson: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.09/II3

Topic: D.06. Vision

Support: R01-EY023581

R01-EY023591

P30-EY003039

Eyesight Foundation of Alabama

Title: Photoreceptor shape variation does not account for perceptual threshold variation in single cones

Authors: M. DEFENDERFER[1], A. MEADWAY[1], K. S. BRUCE[1], *L. C. SINCICH[2];
[2]Vision Sci., [1]Univ. of Alabama Birmingham, Birmingham, AL

Abstract: To perceive a spot of light at photopic threshold, a minimum number of photons must be absorbed by cone photopigments. The threshold depends on many factors, such as background luminance and distance from the fovea. Less well appreciated are two additional factors operating at the cellular scale: the waveguide properties of photoreceptors and their individual variation in synaptic weighting. We reported previously that thresholds for stimuli delivered to single cones can vary widely; neighboring cones differ in threshold by up to 80% (Bruce et al. 2015). Because the efficiency of light absorption depends on photoreceptor size and shape, we investigated whether variation in these dimensions explains the threshold variation found with adaptive optics (AO) microstimulation. To estimate how waveguiding contributes to threshold, the interaction of diffraction-limited light in cones was modeled using a finite difference beam propagation method which quantifies light coupling efficiency in idealized cones. Human and macaque cones located at retinal eccentricities matching sites studied psychophysically were measured from wholemount histological material. Image processing was used to fit an ellipse to the cone inner segments when viewed axially along the normal light path. These fits were used in the cone model to estimate light coupling efficiency in the outer segment. For the model, the initial electric field was for an unaberrated eye with a power of 59.9 D at wavelength=543 nm. Light coupling was compared to luminance increment thresholds from cone-targeted stimulation measured in humans using AO-based microstimulation. For these data, a ~3.6 micron square of 543±11 nm light was flashed for 130 microsec per trial on single cones at various intensities. Thresholds were found by a staircase procedure from subject responses. Measured from local patches of retina, cone inner segment dimensions varied by 6% SD, with a mean anisotropy ratio (major/minor axis) of 1.1±0.05. To see how this shape variation compared to the distribution of relative threshold increments observed perceptually, we used a Monte Carlo method to calculate the likely distribution of relative light coupling from randomly selected pairs of nearby cones. We found that the estimated light coupling in human and macaque cones varied by a factor of 10 less than the threshold variation observed psychophysically. Because shape differences yielded such minor variation in light coupling between cones, our results suggest that synaptic weighting is likely to account for most of the variation in perceptual thresholds to cone-targeted microstimulation.

Disclosures: M. Defenderfer: None. A. Meadway: None. K.S. Bruce: None. L.C. Sincich: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.10/II4

Topic: D.06. Vision

Support: National Institute of Health RO1EY019053

Title: C-terminal phosphorylation of mouse melanopsin plays a crucial role in the kinetics of non-image forming visual behavior

Authors: P. SOMASUNDARAM[1], D. FERNANDEZ[2], A. RUPP[2], T. BADEA[3], Z. WU[4], R. BROWN[5], S. HATTAR[2], *P. ROBINSON[1];
[1]Biol. Sci., UMBC, Baltimore, MD; [2]Dept. of Biol., Johns Hopkins Univ., Baltimore, MD; [3]Retinal Circuit Develop. and Genet., [4]Ocular Gene Therapy Core, Natl. Eye Inst., Bethesda, MD; [5]Dept. of Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: The melanopsin photopigment is expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) of the mammalian retina, which are specialized for functions such as circadian photo-entrainment, pupillary light reflex, and certain visual functions. Melanopsin's role in a broad spectrum of functions calls for a deeper understanding of the regulation of its activity. Melanopsin resembles visual pigments found in rhabdomeric photoreceptors and *in vitro* data suggest that its activity is terminated by light-dependent phosphorylation by a G-protein coupled receptor kinase (GRK). The working hypothesis of this study is that GRK mediated melanopsin phosphorylation controls the lifetime of the activated visual pigment by initiating deactivation of the receptor. In order to test the impact of this phosphorylation on non-image forming behavior and physiology, a phosphorylation-deficient melanopsin, in which putative GRK phosphorylation sites on the carboxy-terminal tail were mutated to alanines (phosphonull), were expressed in mice through a cre-dependent AAV2 gene delivery method. These mice show successful viral transduction as seen by the expression of reporter gene in their retinas. Behavioral analyses indicate that mice expressing the phosphonull mutant of melanopsin exhibit a prolonged constriction of pupillary light reflex following a 30 second stimulus of high intensity blue light. The AAV2 gene delivery approach was also used to transduce ipRGCs in melanopsin knockout mice (MKO) with wild type melanopsin gene. This method of re-introducing melanopsin into MKOs restored non-image forming behavioral deficits that have previously been reported in these animals. In this study, an AAV2 transduction method has been successfully used to generate targeted mutations in melanopsin. The data indicate that putative GRK phosphorylation sites on melanopsin C-terminus play a vital role in pupillary light reflex behavior.

Disclosures: P. Somasundaram: None. D. Fernandez: None. A. Rupp: None. T. Badea: None. Z. Wu: None. R. Brown: None. S. Hattar: None. P. Robinson: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

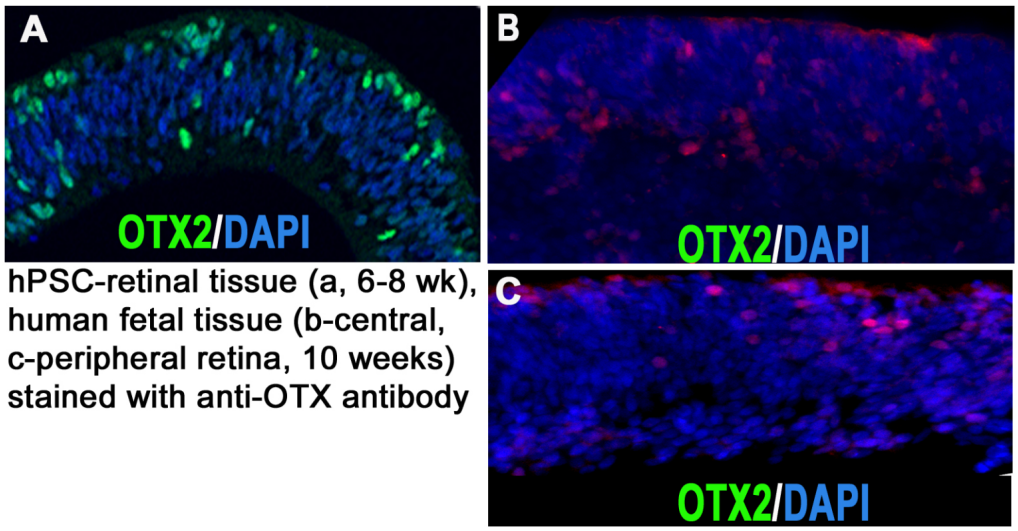
Program#/Poster#: 238.11/II5

Topic: D.06. Vision

Title: Comparison of developmental dynamics in human fetal retina and human stem cell-derived retinal tissue

Authors: R. K. SINGH, H. STERNBERG, O. CUZZANI, M. D. WEST, *I. O. NASONKIN; Biotime, Inc., Alameda, CA

Abstract: Retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are incurable blinding conditions, severely impacting quality of life and affecting millions of people. Finding efficient treatment for these devastating diseases is among the greatest unmet clinical needs. Retinal replacement strategy can bring a piece of healthy mutation-free human retina into a patient's eye to replace fully degenerated retinal tissue. The only donor tissue, which was demonstrated to work in animals with retinal degeneration as well as in RP patients is human fetal retinal tissue age 8-17 weeks, which cannot be used for routine therapies. 3D retinal tissue (retinal organoids) derived from human pluripotent stem cells (hPSCs) shares many similarities with human fetal retina and may be an excellent replacement of fetal retinal tissue in retinal transplantation experiments. To evaluate the optimal developmental point of hPSC-3D retinal tissue, which may replicate successful survival and differentiation of human fetal retina in subretinal grafts (Seiler, M.J., et al., Eur J Neurosci, 2010, **31**[3]: p. 508), we are characterizing a set (8-16 week old) of human embryonic and fetal retinal tissue samples. We are mapping RPE, retinal stem, progenitor and retinal cell fate markers in these samples to hPSC-3D retinal tissue (Singh et al., Stem Cells Dev. 2015 Dec 1;24[23]:2778) using histology, immunohistochemistry and RNA-seq methods. We present data on similarities and differences in distribution and expression of molecular markers in human developing embryonic and fetal retina and in hPSC-3D retinal tissue and suggest the optimal range of time points in hPSC-3D retinal tissue development, which may engraft successfully and develop into laminated human retina in subretinal grafts. The data will streamline the development of hPSC-3D retinal tissue (retinal organoid) technologies aimed at repairing and replacing human retina affected by degeneration and causing irreversible blindness.



hPSC-retinal tissue (a, 6-8 wk), human fetal tissue (b-central, c-peripheral retina, 10 weeks) stained with anti-OTX antibody

Disclosures: R.K. Singh: None. H. Sternberg: None. O. Cuzzani: None. M.D. West: None. I.O. Nasonkin: None.

Poster

238. Retina Photoreceptors

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 238.12/II6

Topic: D.06. Vision

Support: NIH Grant GM076430

NIH Grant EY024452

Title: A neural circuit uses distinct mechanisms to support transient and sustained pupil constriction

Authors: *W. T. KEENAN[1], A. C. RUPP[1], P. SOMASUNDARAM[2], S. HIRIYANNA[3], Z. WU[3], T. C. BADEA[3], P. R. ROBINSON[2], S. HATTAR[1];
[1]Biol., Johns Hopkins Univ., Baltimore, MD; [2]Biol. Sci., Univ. of Maryland, Baltimore County, MD; [3]Natl. Eye Inst., NIH, Bethesda, MD

Abstract: The fundamental goal of neuroscience is to understand how neural circuits drive behavior. Here, we investigated the neural circuit centered on intrinsically photosensitive retinal ganglion cells (ipRGCs), critical relays for light's effects on sleep, circadian rhythms, and pupil size. ipRGCs receive photosensory input from 3 sources (rods, cones, and melanopsin), and are

thought to transmit information to the brain with 2 neurotransmitters: glutamate and the neuropeptide PACAP. However, the functional contribution of each of these circuit components remains unclear. By measuring pupil size while silencing each ipRGC circuit component—photoreceptive inputs or neurotransmitters outputs—we show that transient and sustained behavioral responses are mediated by distinct inputs and outputs. Transient responses utilize input from rod photoreceptors and output by glutamate. In contrast, sustained responses are dominated by non-conventional signaling mechanisms: melanopsin phototransduction in ipRGCs and the neuropeptide PACAP. These results reveal a novel role for a neuropeptide in visual function and highlight a neuronal circuit transition that supports behavioral responses over time.

Disclosures: W.T. Keenan: None. A.C. Rupp: None. P. Somasundaram: None. S. Hiriyanna: None. Z. Wu: None. T.C. Badea: None. P.R. Robinson: None. S. Hattar: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.01/II7

Topic: D.06. Vision

Support: NSF Grant 1144469

NIH Project 5U01NS090562-02

Title: Circuits in the retina: A deep learning framework for algorithmic modeling.

Authors: *D. BAGHERIAN, M. MEISTER;
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Abstract: Retinal circuits, which take light stimuli as input and produce retinal ganglion cell (RGC) spike trains as output, are often modeled as layered networks of linear spatiotemporal filters and static nonlinearities, or “cascade models” (Meister and Berry, 1999). Such a model provides both a quantitative formulation of what a given RGC reports about the visual scene and a biologically interpretable hypothesis for the circuit mechanisms that implement the computation.
The network structure of the cascade is often selected based on the researcher’s intuition, but many networks may approximate a given RGC computation equally well, and the space of all such models is large and high dimensional. We develop a modeling paradigm that searches through this space comprehensively to produce a range of circuit models that are suitablecandidates for further circuit dissection experiments.

To solve this system identification problem, we employ feedforward convolutional neural networks (CNNs), which are mathematically equivalent to cascade models. In this machine learning setting, there is a direct mapping from CNN nodes and connections to biological neurons and synapses. Trains of action potentials are recorded from an RGC in response to a broad set of visual stimuli. Then, a CNN is used to fit this stimulus response relationship. We initialize a large, over connected CNN with a layered structure generally consistent with anatomy of the retina. Then we use backpropagation and gradient descent with L1 regularization to "prune" the network. Due to the highly degenerate nature of the model space, after many such initialization and training instances, the end result is often a family of candidate circuit mechanisms.
As a proof of concept for this method of circuit inference, we apply it to a canonical computation in the retina: direction selectivity. First, we simulate the neural circuitry that leads to the ON/OFF direction selective ganglion cell (DSGC) to produce large sets of stimulus response data (Vaney et al. 2012) . After training a deep learning model on these data, we find that the model can replicate the direction selective visual responses, but the learned circuit structure is somewhat dependent on the initialization and training parameters of the CNN. Second, we apply the same learning model to neural recordings from genetically targeted real DSGCs, We expect that this method for inferring neural circuits will serve to generate new hypotheses for circuit mechanisms beyond those currently considered that can then be evaluated with directed anatomical and physiological studies.

Disclosures: D. Bagherian: None. M. Meister: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.02/II8

Topic: D.06. Vision

Support: Penn State University College of Medicine

Plum Foundation

Title: ATP vesicular pools present in VNUT expressing zebrafish horizontal cells

Authors: *D. S. MCDEVITT, S. L. STELLA, Jr;
Neural and Behavioral Sci., Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: The discovery of a vesicular nucleotide transporter (VNUT) has established that ATP can be concentrated and stored in neuronal vesicles. VNUT expression has been localized to horizontal cells in the outer retina. Therefore, we hypothesize that an ATP pool is present in horizontal cells and contributes to ATP release which can serve as a precursor to adenosine during nocturnal conditions. Experiments were performed on both intact retinas and isolated horizontal cells from zebrafish retina. Confocal live cell imaging of quinacrine and molecules to label ATP stores in horizontal cells were used to monitor ATP levels and release. Immunohistochemical analysis using confocal microscopy of zebrafish retina was performed on vertical sections and wholemounts. Live cell imaging of vesicle cycling was monitored using a VNUT antibody targeted to the luminal face of the protein conjugated to CF488 or CF568 in order to characterize vesicle turnover in horizontal cells. Vesicles containing ATP labeled stores co-localized with VNUT in horizontal cells. VNUT labeled both horizontal cells and Muller cells in the zebrafish retina. Antibodies targeted to the luminal face of VNUT transporters labelled cycling vesicles within horizontal cell processes. Uptake was stimulation and Ca^{2+}-dependent arguing in favor of a vesicular mechanism, and reduced in the absence of Ca^{2+} and Ca^{2+} channel blockers. Synaptic vesicle recycling and retrieval was demonstrated with sequential Ca^{2+} labeling of vesicles with fluorescently labelled VNUT antibody followed by a secondary antibody targeted to the VNUT antibody. These findings support the hypothesis that horizontal cells contain a competent compartment containing VNUT expressing vesicles that have the capacity to release ATP from horizontal cells in the outer retina. Thus, during nocturnal conditions it is possible that outer retinal adenosine is derived from ATP present in horizontal cells.

Disclosures: D.S. McDevitt: None. S.L. Stella: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.03/II9

Topic: D.06. Vision

Support: NSF/IOS 1256782

Title: Immunohistochemical comparison of retinal cell populations in an aquatic predator species and a terrestrial prey species that share a common evolutionary ancestor

Authors: *N. Y. KHORSANDI, J. W. MADDOX, E. GLEASON;
Dept. of Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: The American alligator (*Alligator mississippiensis*) and domesticated chicken (*Gallus gallus domesticus*) are both archosaurs, derived from a common evolutionary ancestor about 240 Mya (Green et al. 2014). By comparing retinal cellular organization in these two species, we can test whether retinal structure has diverged in association with two different ecological niches: an aquatic predator (alligator) and a terrestrial prey (chicken). To investigate this, we have employed immunohistochemistry and confocal microscopy to identify key cell populations from frozen sections of paraformaldehyde-fixed retinas. We used the following primary antibodies known to recognize proteins expressed by specific cell types in the chicken retina: bipolar cells (α-PKCα, Caminos et al. 1999), Müller cells (α-glutamine synthetase, Norenberg et al. 1980), amacrine cells (α-syntaxin-1, Barnstable et al. 1985), target amacrine cells (α-parvalbumin, Weller et al. 2009), starburst amacrine cells (α-ChAT, Millar et al. 1985), and dopaminergic amacrine cells (α-tyrosine hydroxylase, Fauquet and Ziller 1989). The α-PKC antibody did not label bipolar cells in the alligator retina. The α-glutamine synthetase antibody labeled Müller cells in both species, but labeling in the inner plexiform layer (IPL) was less extensive in the alligator retina. Antibodies raised against syntaxin-1 gave a similar labeling pattern in both species with labeling of amacrine cell bodies in the inner nuclear layer (INL) and their processes in the IPL as well as labeling of presumed horizontal cell processes in the outer plexiform layer. Parvalbumin antibodies labeled target amacrine cells, which are confined to the ventral half of the chicken retina. In the alligator, these antibodies labeled morphologically distinct populations of amacrine cells in both the dorsal and ventral retina. The antibodies raised against ChAT labeled starburst amacrine cells in both species with labeling localized to cell bodies in the INL and in the ganglion cell layer and processes confined to two bands in the IPL. Interestingly, the density of α-ChAT-positive cell bodies was higher in the chicken retina. The α-tyrosine hydroxylase antibodies labeled morphologically distinct populations of amacrine cells in the chicken and alligator retinas. These results support the hypothesis that the chicken and alligator have evolved specializations that might tailor retinal signal processing to suit the lifestyle of each species.

Disclosures: N.Y. Khorsandi: None. J.W. Maddox: None. E. Gleason: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.04/II10

Topic: D.06. Vision

Support: NIH NEI R01 EY016435

NIH NEI R01 EY022342

NIH NEI R01 EY004864

NIH NEI P30 EY006360

Research to Prevent Blindness

Title: Effects of light luminance level on retinal dopamine metabolism and lens induced myopia susceptibility

Authors: *E. LANDIS[1], H. PARK[2], C. SIDHU[2], K. STOUT[3], G. MILLER[4], M. IUVONE[2], M. PARDUE[5];
[1]Neurosci., [2]Ophthalmology, [3]Mol. and Systems Pharmacol., [4]Envrn. Hlth., Emory Univ., Atlanta, GA; [5]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Purpose: The purpose of this study is to determine how varied ambient light impacts dopamine activity in the mouse retina and alters susceptibility to lens induced myopia. High and low light levels decrease myopia susceptibility but intermediate light increases susceptibility. We hypothesize that this occurs through different dopamine related mechanisms. To determine the role of dopamine, we analyzed dopamine signaling in the retinas of mice housed in varied ambient light for acute or chronic durations.
Methods: Wild-type C57BL/6J mice (Jackson Labs) were housed individually in scotopic (0.005 lux), mesopic (50 lux) and photopic (15,000 lux) light beginning at the start of a light phase. A subset of mice were housed under each light level for 3 hours and were sacrificed for retina collection 3 hours (acute response) after light onset (ZT 3). A second group of mice were housed under each light level for 10 days (chronic response) and sacrificed for retina collection 4 hours after light onset. Retinas were analyzed for dopamine (DA) and its primary metabolite DOPAC via high performance liquid chromatography.
Results: Results were normalized to mean levels under mesopic light to compare relative light responses across exposure periods. The acute response to scotopic light showed the highest levels of DA (1.11 normalized to mesopic) and the lowest level of DOPAC (0.48 normalized), relative to the other light levels. The acute response to photopic light had the lowest level of DA (0.86 normalized) and the highest of DOPAC (1.48 normalized). Chronic response retinas showed higher levels of DA and DOPAC in both scotopic (DA: 1.27, DOPAC: 1.18 normalized) and photopic (DA: 1.13, DOPAC: 1.50 normalized) housed mice. The DOPAC/DA ratio, a measure of DA turnover, increased with light level regardless of exposure period but was higher in acute exposure scotopic light in comparison to chronic exposure; the opposite was true under photopic light.
Conclusion: The acute response to various ambient light shows that early in light adaption DA metabolism is modified to varied ambient light. With chronic exposure to the different luminances, scotopic and photopic light lead to increased DA activity relative to mesopic light. DA metabolism increased with longer exposure to scotopic light but decreased with exposure to photopic light. This finding could indicate different DA mechanisms in the response to luminance levels, which could further alter lens induced myopia in mice. How these differences

lead to changes in myopia susceptibility will be investigated by measuring changes in retinal DA release and uptake by fast-scan cyclic voltammetry.

Disclosures: E. Landis: None. H. Park: None. C. Sidhu: None. K. Stout: None. G. Miller: None. M. Iuvone: None. M. Pardue: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.05/II11

Topic: D.06. Vision

Support: NS003145

Title: Inhibition within starburst amacrine cell network localizes SAC dendritic signaling and sharpens direction selectivity

Authors: *H. DING, J. S. DIAMOND;
NIH, Bethesda, MD

Abstract: Directionally-tuned inhibitory signals from starburst amacrine cells (SACs) play a key role in the direction selective circuit in the mammalian retina. Past studies have shown that different regions of the SAC dendritic arbor can respond independently to visual stimuli and exhibit distinct directional tuning. By using two-photon calcium imaging, we study how individual branches within a single SAC dendritic arbor transform excitatory and inhibitory inputs to produce directionally tuned output signals. We examined the angular tuning properties of individual presynaptic varicosities on SAC dendrites by measuring calcium signals evoked by bars moving across the visual field in eight different directions. We found that outputs could be separated into clusters based on their response correlations and that the clusters corresponded to outputs arising on the same parent dendrite. The correlation between outputs from the same parent dendrite was much higher than between outputs from other parent dendrites. We also mapped the visual receptive field properties of each varicosity to determine the spatial relationship between light-evoked inputs and outputs. As predicted by recent studies, we found that the centers of mapped receptive fields lay over parent dendrites located more proximal to the soma.
Blocking SAC-SAC inhibition with GABAzine broadened the tuning width of individual outputs and decreased the separability of responses among outputs from different parent dendrites. GABAzine decreased the difference in output correlations of branches within the same parent dendrite and branches from different parent dendrites. GABAzine expanded the receptive field

size of individual varicosities by increasing the overall amplitude of responses, but did not change the receptive field shape. This effect was primarily due to blockade of SAC-SAC inhibition rather than feedback inhibition onto bipolar cell terminals, because GABAzine did not dramatically increase responses or receptive field sizes in type 5 cone bipolar cells, which provide excitatory input to ON SACs. Taken together, these results indicate that SAC-SAC inhibition compartmentalizes DS signaling within smaller regions of SAC dendrites, enabling finer discrimination of motion direction.
This work was supported by the NINDS Intramural Research Program.

Disclosures: H. Ding: None. J.S. Diamond: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.06/II12

Topic: D.06. Vision

Support: NIH Grant EY014454

NIH Grant EY021372

NSF Graduate Research Fellowship

Gruber Science Fellowship

Title: Optogenetic interrogation of synaptic computation in the retina

Authors: *J. POTTACKAL[1], G. E. PERRIN[2], J. H. SINGER[2], J. B. DEMB[1];
[1]Dept. of Ophthalmology and Visual Sci., Yale Univ., New Haven, CT; [2]Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Synapses perform complex transformations of presynaptic voltage into postsynaptic current. However, the extent to which functional properties of synaptic transmission differ between specific cell types in the central nervous system remains largely unknown. The mouse retina presents a tractable system in which to address this problem due to significant recent progress in the identification and characterization of its ~100 types of neurons. Here, we describe a novel optogenetic approach that enables quantitative analysis of synaptic transmission between identified cell types in the retina. We use the Cre-Lox conditional expression system to drive expression of the light-gated ion channel channelrhodopsin-2 (ChR2) in genetically identified subsets of retinal interneurons, including bipolar cells and amacrine cells (ACs). Under

pharmacological blockade of photoreceptor-mediated visual responses, whole-cell current clamp recordings from ChR2-expressing ACs in the wholemount retina demonstrate that ChR2 stimulation provides rapid, faithful modulation of membrane potential in graded (i.e. non-spiking) neurons over a physiologically relevant range of temporal frequencies up to ≥30 Hz. We find that ChR2-mediated depolarization of CRH-1 ACs evokes robust inhibitory postsynaptic currents (IPSCs) in ON α retinal ganglion cells (RGCs) voltage-clamped at 0 mV. Subsequent linear-nonlinear cascade analysis of ChR2-evoked IPSCs in ON α RGCs rules out a gain control mechanism of variance adaptation at the CRH-1 AC—ON α RGC synapse. Finally, this approach complements existing techniques for interrogating synaptic transmission in the retina while offering improved stability and signal-to-noise ratio compared to paired whole-cell recording.

Disclosures: J. Pottackal: None. G.E. Perrin: None. J.H. Singer: None. J.B. Demb: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.07/II13

Topic: D.06. Vision

Support: NIH EY021855

NIH EY023341

Title: Mechanisms of surround inhibition in the object motion sensitive circuit of the mouse retina

Authors: *T. KIM, D. KERSCHENSTEINER;
Washington Univ. In St.Louis, Saint Louis, MO

Abstract: Object motion is first detected in the retina. In mice, W3-retinal ganglion cells (W3-RGCs) respond robustly to movements of small objects, but remain silent during global image motion. We previously showed that object motion sensitive responses of W3-RGCs are driven by excitatory input from vesicular glutamate transporter 3-expressing amacrine cells (VG3-ACs). Both VG3-ACs and W3-RGCs receive strong inhibitory input from their receptive field surrounds, which suppresses responses to global image motion. Here, we use transgenic mouse lines expressing Cre recombinase in a candidate cell type to identify and characterize the source of surround inhibition in the object motion sensitive circuit of the retina. In 2-photon guided patch clamp recordings, we characterize responses of the candidate cells, test their functional

connectivity with VG3-ACs and W3-RGCs using optogenetics, and evaluate the contribution of candidate cells to the feature-selective responses of VG3-ACs and W3-RGCs using conditional knockouts of the vesicular inhibitory amino acid transporter (VIAAT).

Disclosures: T. Kim: None. D. Kerschensteiner: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.08/II14

Topic: D.06. Vision

Support: Penn State University College of Medicine

Title: GABA-induced chloride gradients in cone bipolar cells of the mammalian retina

Authors: *S. L. STELLA, Jr., D. S. MCDEVITT;
Neural and Behavioral Sci., Penn State Univ. Hershey-College of Med., Hershey, PA

Abstract: In mammalian retina, photoreceptor signals are segregated into ON and OFF pathways, rods contact a single class of bipolar cell, the rod ON bipolar cell, whereas cones contact both ON and OFF cone bipolar cells. Little is known about GABA's action at cone bipolar cells, and the ability of GABA to regulate their excitability through intracellular chloride ion (Cl^-) changes. Ionotropic GABA receptor expression is well documented in rod bipolar cells, but the spatial distribution and pharmacological profile is not known in cone bipolar cells. The goal of this project was to characterize GABA-mediated $[Cl^-]_i$ changes in cone bipolar cells of the mouse retina using yellow fluorescent protein (YFP) as a Cl^- biosensor. Mice expressing YFP under the Thy-1.2 promoter were used for this study. YFP fluorescence is sensitive to quenching by many anions, of which Cl^- is the most physiologically relevant. This provides a way to monitor $[Cl^-]_i$ changes in real-time. To address ionotropic GABA receptor function on cone bipolar cells, two techniques were employed: real-time Cl^- imaging using YFP fluorescence confocal microscopy and immunocytochemistry. Currently, we have identified both ON and OFF cone bipolar cell types expressing YFP. We discovered that changes in YFP fluorescence were correlated with increasing GABA concentrations (0.001 to 1 mM). Changes in YFP fluorescence were observed at the dendrites and soma. These YFP fluorescence changes to GABA were suppressed by replacing $[Cl^-]_o$ with gluconate. In addition, the YFP fluorescence changes were independent of changes in pH, suggesting $[Cl^-]_i$ changes were induced by GABA. We also observed different responses in the soma and dendrite to both GABA and the Cl^- transport inhibitor, furosemide, suggesting that there is a unique spatial Cl^- gradient in some cone

bipolar cells. $GABA_A$ and $GABA_C$ receptors were expressed at the dendrites, soma and terminals of cone bipolar cells. This distribution of GABA receptors on cone bipolar cells is in agreement with the Cl^- imaging of GABA-induced changes in $[Cl^-]_i$, suggesting that YFP expressing neurons provide an elegant way to monitor spatial and temporal Cl^- gradients in real time.

Disclosures: S.L. Stella: None. D.S. McDevitt: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.09/II15

Topic: D.06. Vision

Support: NIHR01 EY04067

NIDDDK P30 DK41301

Title: VIP-expressing amacrine cells comprise different cell types in mouse retina

Authors: *L. PEREZ DE SEVILLA[1], H. TE HAPUKINO[2], N. BRECHA[2];
[1]Neurobio., [2]UCLA, Los Angeles, CA

Abstract: Amacrine cells consist of a heterogeneous group of inhibitory interneurons that form distinct microcircuits and participate in visual information processing in the inner retina. This study has focused on defining and characterizing the vasoactive intestinal peptide (VIP) expressing amacrine cells in the mouse retina using a VIP-Cre recombinase mouse line. **Methods:** VIP-tdTomato and VIP-Brainbow mouse lines were generated by crossing a VIP-Cre transgenic mouse line (JAX #10908) with a Cre-dependent tdTomato (JAX #7909) and a Brainbow2.1 (JAX #13731) reporter mouse line. Fluorescent cell bodies in retinal whole-mounts were imaged and reconstructed using a Zeiss LSM 710 confocal microscope. VIP-tdTomato fluorescent cell bodies in the INL and GCL were injected with Neurobiotin in retinal whole-mounts to define their general morphology, stratification patterns and to test their gap junction connectivity with other cells. **Results:** TdTomato fluorescent cell bodies in the INL and their processes were distributed to laminae 1, 3, 4 and 5 of the IPL. Brainbow2.1 fluorescence and tracer injections revealed three types in the INL and four types in the GCL, based on the ramification of their processes in the IPL, general morphology, dendritic field sizes and coupling patterns. Neurobiotin injections of tdTomato fluorescent cells in the INL revealed VIP-2 amacrine cells coupling to VIP-2 amacrine, non-VIP amacrine and ganglion cells. The remaining VIP amacrine cells in the INL and GCL exhibited no tracer coupling. **Conclusion:** We have

identified a novel amacrine cell population consisting of 4 main types and 3 other types; four of them located in the GCL. All types are characterized by VIP expression and they differ in their general morphology, stratification patterns and gap junction coupling.

Disclosures: L. Perez De Sevilla: None. H. Te Hapukino: None. N. Brecha: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.10/II16

Topic: D.06. Vision

Title: Spectral-spatial mapping by primary photoreceptors organized in a somatotopic array

Authors: T. K. H. GROVES[1], *J. A. JELLIES[2];
[1]Biol. Sci., [2]Western Michigan Univ., Kalamazoo, MI

Abstract: We wondered how animals with simple, non-image-forming eyes can extract and use image features (such as spectral contrast across a visual field). Our overarching hypothesis is that 2-dimensional pixel arrays of simple eyes can be used to extract low-resolution image features. The medicinal leech, *Hirudo verbana* is endowed with a visual system composed of "simple" eyes in 2 discrete arrays. *Hirudo* collects visual information using 5 pairs of pigmented cephalic eyecups at the margin of the anterior sucker, and a second distributed and segmentally iterated array of 7 pairs of dermal sensilla arranged dorsal to ventral along the central annulus of each mid-body segment (for a total of at least 294 sensilla). Because these eyespots sample different spatial domains of the external environment we propose that they comprise a type of low-resolution somatotopic retinal surface. *Hirudo* can discriminate green and near ultraviolet (UV) light (Jellies. 2014. J. Exp. Biol. 217:974), and they rotate away from ventrally, but not dorsally presented UV light. Furthermore, a visually-sensitive interneuron (the S-cell) integrates spectral contrast in that it responds best to UV only when presented ventrally, and best to green light when presented dorsally (Jellies. 2014. J. Comp. Physiol. A 200:923). We have suggested that the array of dermal sensilla can act as a “spectral statocyst” to inform 3-D body position. We predicted that the primary sensillar photoreceptors may be responsible for comparing wavelengths across the visual field, information that is then projected into the CNS. Adult leeches were dissected to expose discrete sensillar nerves from ventral, dorsal, and lateral sensilla. We used extracellular recording in combination with light stimulation (using previously developed LEDs, red, green, blue, UV) to characterize complex trains of light-evoked action potentials from sensilla across 4 wavelength ranges and luminosity, and under light-adapted conditions. Ventral sensilla were strikingly preferentially responsive to UV light, while dorsal

sensilla were more broadly responsive to green light. Furthermore, the unique ventral UV response had distinctly tonic components while the dorsal sensillar responses to green light resembled the phasi-tonic responses seen in posterior cephalic eyes. We suggest that spatially relevant spectral contrast information is established at the primary sensory level. Thus, synaptic interactions between sensillar axons and interneurons in the CNS may underlie previously described UV evoked behaviors and provide the substrates for synaptic computation for visual feature extraction by a system lacking image-forming eyes.

Disclosures: T.K.H. Groves: None. J.A. Jellies: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.11/II17

Topic: D.06. Vision

Support: NSF/IOS Grant 1256782

Title: Nitric oxide promotes neurotransmitter release by activating a voltage-independent Ca^{2+} influx pathway in amacrine cells

Authors: *J. W. MADDOX, E. GLEASON;
Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Nitric oxide (NO) synthase is localized to amacrine cell (AC) presynaptic terminals (Cao and Eldred, 2001) suggesting that NO synthesis might affect neurotransmitter release. We have previously shown that NO elevates cytosolic Ca^{2+} (Ca^{2+}_{Cyt}), enhances GABAergic evoked postsynaptic currents, and increases GABAergic spontaneous postsynaptic current frequency ($sPSC_{Fq}$). These effects are independent of soluble guanylate cyclase, and NO does not alter Ca_v currents. Here, we test the hypothesis that NO increases $sPSC_{Fq}$ by eliciting a voltage-independent Ca^{2+}_{Cyt} elevation. Cultured chick ACs (Gleason et al., 1993) contacting other ACs were voltage clamped at -70 mV and exposed to the NO donor *S*-Nitroso-*N*-Acetylpenicillamine (SNAP, 500 µM), which increased $sPSC_{Fq}$ by 63% ($p < 0.01$). To determine if the NO-dependent Ca^{2+} elevation is action potential-dependent, the Na_v channel blocker TTX (300 nM) was included in the external solution, but NO still increased $sPSC_{Fq}$ by 68% ($p = 0.03$). However, preloading the cells with the fast Ca^{2+} chelator BAPTA-AM (10 µM, 1 hr) prevented the NO-dependent increase in $sPSC_{Fq}$ ($p = 0.6$). To determine the contribution of Ca^{2+} stores, ACs were pre-treated with the SERCA pump inhibitor thapsigargin (TG, 2 µM, 1 hr) then loaded with the Ca^{2+} indicator Oregon Green BAPTA-AM (2 µM, 1 hr). In the presence of external Ca^{2+}, NO

increased Ca^{2+}_{Cyt} from baseline in control ($p < 0.01$) and TG treated cells ($p < 0.01$). In the absence of external Ca^{2+}, NO was unable to increase Ca^{2+}_{Cyt} from baseline in control and TG treated cells ($p = 0.06$). NO was still effective at increasing $sPSC_{Fq}$ after Ca^{2+} store depletion ($p < 0.01$). These results indicate that NO activates a Ca^{2+} influx pathway exclusively. The Ca^{2+} permeable cation channel TRPC5 can be activated by S-nitrosylation (Yoshida et al., 2005), and antibody labeling suggests that TRPC5 is expressed by these cells. To determine the involvement of TRPC5, the TRPC5 inhibitor clemizole (10 μM) was applied prior to and during application of SNAP. Clemizole prevented the NO-dependent increase of Ca^{2+}_{Cyt} ($p = 0.5$) and the increase in $sPSC_{Fq}$ ($p = 0.97$). Additionally, 2 mM La^{3+} prevented the NO-dependent increase in $sPSC_{Fq}$ ($p = 0.5$), whereas 10 μM La^{3+} potentiated the NO-dependent increase of $sPSC_{Fq}$ ($p = 0.02$), an effect consistent with TRPC5 activity. TRPC5 can be activated by PLC (Svobodova and Groschner, 2016). Consistent with this, the PLC inhibitor U-73122 (10 μM) reduced the NO-dependent increase in $sPSC_{Fq}$ ($p = 0.02$), but was not affected by the less active U-73343 ($p = 0.9$). These results suggest that presynaptic NO signals can increase inhibitory output of GABAergic ACs in the absence of depolarization.

Disclosures: J.W. Maddox: None. E. Gleason: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.12/JJ1

Topic: D.06. Vision

Support: NIH Grant EY15573 (NCB)

Stein-Oppenheimer Grant (AH, NCB)

NSERC Discovery Award 194640 (SB)

Plum Foundation (SB, NCB)

VA Career Scientist Award (NCB)

Title: Extrasynaptic $GABA_A$ receptor Gabra6 and Gabra4 are expressed in mouse outer retina

Authors: *A. A. HIRANO[1,2], A. SOLOMON[1], S. A. BARNES[3,1], N. C. BRECHA[1,2];
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[3]Dalhousie Univ., Halifax, NS, Canada

Abstract: Horizontal cells possess the molecular machinery for vesicular release, including VGAT/VIAAT, which accumulates inhibitory amino acids (IAA) into synaptic vesicles, and horizontal cell VGAT/VIAAT knockout eliminated inhibitory feedback to photoreceptors (Hirano et al., 2016). To determine the possible targets of horizontal cell GABA/IAA release, we investigated the expression of GABA receptors in the mouse retina. Immunohistochemistry on vertical sections of mouse retina with Gabra6 antibodies (Synaptic Systems) produced hotspots of labeling deep within the outer plexiform layer (OPL) and faint outlines of bipolar cell bodies. Staining with PNA to label cone pedicles demonstrated Gabra6-like immunoreactivity was concentrated beneath cone pedicles. Staining of Cx57-channelrhodopsin (ChR2)-tdTomato, which outlines the cell membrane of horizontal cells, retinas indicated that Gabra6-like immunoreactivity is on horizontal cell processes and runs up the stalks of horizontal cell endings innervating rods. Rod bipolar cells, identified by PKC immunostaining, showed Gabra6-like immunoreactivity concentrated on the dendrites running along the same horizontal cell stalk-like regions. ON bipolar cell dendrites, immunolabeled for $Galpha_o$, showed a similar pattern of Gabra6 immunoreactivity, which did not seem to reach the very tips. RT-PCR using multiple sets of primers designed from Gabra6 cDNA sequence(s) produced amplicons of the predicted sizes from retina, although the intensity was much less than those from cerebellum, indicating a low level of mRNA expression in retina. Immunolabeling with Gabra4 antibodies (PhosphoSolutions, Inc.) labeled putative cone photoreceptor and bipolar cell bodies. In addition, there was strong Gabra4-like immunolabeling of the distal calbindin band in the inner plexiform layer (IPL). Double label experiments with Gabra4 and vAChT antibodies confirmed this OFF ChAT band co-localization. Extrasynaptic $GABA_A$ receptor expression in the outer retina suggests tonic inhibition by GABA/IAA of bipolar cells and photoreceptors and that these receptors may be targets of horizontal cell signaling.

Disclosures: A.A. Hirano: None. A. Solomon: None. S.A. Barnes: None. N.C. Brecha: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.13/JJ2

Topic: D.06. Vision

Support: NSF 0924372

NSF 0924383

Sigma Xi Grants-In-Aid of Research

Title: ATP-induced changes in intracellular Ca^{2+} levels and extracellular H+ concentration from retinal Muller (glial) cells

Authors: *B. K. TCHERNOOKOVA[1], C. HEER[3], M. YOUNG[3], D. SWYGART[3], R. KAUFMAN[3], M. KREITZER[3], R. MALCHOW[2];
[1]Biol. Sci., [2]Univ. of Illinois at Chicago, Chicago, IL; [3]Indiana Wesleyan Univ., Marion, IN

Abstract: Adenosine 5'-triphosphate is believed to be co-released with neurotransmitters and can act as a signaling molecule via the activation of ATP-sensitive ionotropic and metabotropic receptors. In the present work, we examine changes in extracellular H^+ concentrations and intracellular Ca^{2+} rises triggered by ATP application onto isolated Müller cells and slices prepared from retinae of tiger salamanders. Recordings of H^+ concentration were obtained via the self-referencing technique and Ca^{2+} levels were imaged with the Ca^{2+} dye Oregon Green. All recordings were performed with 1mM HEPES as the extracellular pH buffer. ATP causes a robust extracellular acidification from isolated Muller cells. This acidification is reduced in the presence of the ATP receptor blockers suramin and PPADS, as well as by the anion transport blocker DIDS. ATP application also causes intracellular calcium rises in Müller cells. Both the calcium rise and the extracellular acidification are reduced when the reuptake of calcium into the endoplasmic reticulum is inhibited with thapsigargin and when PLC-IP3 signaling is disrupted with the inhibitors U-73122 and 2-APB. Bath-applied ATP causes a robust extracellular acidification from retinal slices when the H^+-selective microelectrode is positioned in close proximity to the outer plexiform layer. Suramin and PPADS reduce the acidification measured from slices. ATP-induced extracellular acidification is also observed from Müller cells of other species, including human, rat and lamprey. This work emphasizes the role of ATP as an intercellular signaling agent in the retina, which can induce intracellular calcium rises in Müller glia, which in turn lead to robust changes in extracellular acidity. The fact that the ATP-induced responses occur in Müller cells of a variety of species suggests a highly evolutionarily conserved response that is likely to play an important role in regulating responses of retinal neurons.

Disclosures: B.K. Tchernookova: None. C. Heer: None. M. Young: None. D. Swygart: None. R. Kaufman: None. M. Kreitzer: None. R. Malchow: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.14/JJ3

Topic: D.06. Vision

Support: a Grant-in-Aid for Scientific Research (C) from JSPS (Nos. 18500312 and 21500373) to M. Kaneda

Nippon Medical School Grant-in-Aid for Medical Research to M. Kaneda

Grant-in-Aid for Young Scientists (B) from JSPS (KAKENHI No. 26860150) to T. Ishii

Grant-in-Aid for Young Scientists (B) from JSPS (KAKENHI No. 26861473) to K. Homma

Title: Choline transport through P2X2-purinergic receptors in the mouse retina

Authors: *M. KANEDA[1], T. ISHII[2], H. KOHEI[2], A. MANO[1], Y. SHIGEMATSU[3], H. INOUE[4], Y. SHIMODA[3], Y. KAKINUMA[1];
[2]Dept. of Physiol., [1]Nippon Med. Sch., Tokyo, Japan; [3]Tokyo Women's Med. Univ., Tokyo, Japan; [4]Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: For acetylcholine synthesis in the presynaptic terminal of cholinergic neurons, choline uptake by the high affinity choline transporter is essential. In a previous study, we have reported that $P2X_2$-purinoceptors are densely expressed in OFF-cholinergic amacrine cells of the mouse retina. When $P2X_2$-purinoceptors are expressed at high density in the expression system, they acquire permeability to large cations such as N-methyl-D-glucamin. In the present study, we examined whether $P2X_2$-purinoceptors densely expressed in OFF-cholinergic amacrine cells can acquire a permeability choline in the mouse retina. An application of ATP produced detectable choline currents in OFF-cholinergic amacrine cells but not in ON-cholinergic amacrine cells in retinal slice preparations. In dissociated preparations of cholinergic amacrine cells, ATP-induced choline current were carried by $P2X_2$-purinoceptor-mediated cation channels. In HEK293T cells, ATP induced choline current and facilitated the choline uptake into the cell in the $P2X_2$-purinoceptor expressing cells. High-affinity choline transporters are distributed at higher levels in ON-cholinergic amacrine cells than in OFF-cholinergic amacrine cells. These findings support the hypothesis that $P2X_2$-purinoceptors function as a novel choline uptake pathway and may regulate the synthesis of acetylcholine in the OFF-cholinergic amacrine cells in the mouse retina.

Disclosures: M. Kaneda: None. T. Ishii: None. H. Kohei: None. A. Mano: None. Y. Shigematsu: None. H. Inoue: None. Y. Shimoda: None. Y. Kakinuma: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.15/JJ4

Topic: D.06. Vision

Support: NIH Grant EY026027-01

NIH Cardiovascular Training Grant 2T32HL749-36A1

JDRF Grant

Title: Retinal dopaminergic function after 6 weeks of diabetes.

Authors: *M. FLOOD;
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Abstract: Previous studies have shown dysfunction of amacrine cells in the rod pathway early in diabetes. Dopamine is involved in light adaptation, and several studies have also found a deficit in total retinal dopamine content and dopaminergic amacrine cell number in diabetes. This suggests a disruption in the dopaminergic system underlies the signaling changes we see in the diabetic retina. Here we examined the effects of dopamine Type 1 Receptor (D1R) activation on inhibition to rod bipolar cells in a mouse model of diabetes, and quantified total dopaminergic amacrine cell number, in order to determine whether the dopaminergic signaling pathway is compromised. Diabetes was induced in C57BL/6J mice at 5 weeks of age by i.p. injections of streptozotocin (STZ, 75 mg/kg) or citrate buffer control. Diabetes was confirmed by blood glucose levels > 200 mg/dL. Six weeks post injections, whole-cell voltage clamp recordings of light-evoked (L) and spontaneous (s) inhibitory post synaptic currents (IPSCs) were made from rod bipolar cells (RBCs) by holding at 0 mV, the reversal potential for Cl^- ions. D1Rs were activated with the selective agonist SKF-38393 (20 μM). Light responses were elicited at multiple intensities by a 30ms full field LED stimulus (λ = 525 nm). The peak, charge transfer (Q), rise time and 37% decay time (D37) were measured for all evoked responses. Amplitude, frequency and decay constant were measured for sIPSCs. All light response data was analyzed by 2-way repeated measures ANOVA. sIPSC data was analyzed by paired t-tests. Dopaminergic amacrine cells were identified by staining for tyrosine hydroxylase (TH). Retinas were costained with α-caspase to identify apoptotic cells. Cell number, soma size and mean intensity of TH immunoreactivity were measured in ImageJ and analyzed by one way ANOVA. We found that in STZ treated and control mice, SKF treatment significantly reduced L-IPSC Q and peak (n=7 cells, $p < 0.05$) with no significant changes in response rise time or D37. Although on average responses were larger in control than STZ mice, there were no significant differences between the two groups. In both control and STZ groups, sIPSCs showed significant decreases in

frequency ($p < 0.05$) but no significant differences in amplitude or decay constant. No significant differences were found in dopaminergic amacrine cell number, apoptotic cell number, soma size or mean intensity of TH immunoreactivity between STZ and control animals. Our data suggests that at 6 weeks of diabetes, there is no significant impairment of D1R modulation of RBC inhibition and no significant change in the number or viability of dopaminergic amacrine cells.

Disclosures: M. Flood: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.16/JJ5

Topic: D.06. Vision

Title: Simultaneous modeling of neural phenomena from multiple time scales

Authors: *R. RAMEZAN[1], D. WYNNE[2];
[1]Dept. of Mathematics, California State University, Fullerton, Fullerton, CA; [2]Dept. of Mathematics, California State Univ., Fullerton, CA

Abstract: A large portion of our knowledge about the brain relies on the statistical models developed for the analysis of neuronal data. In this research, we show how our conclusions based on neural spike trains depend on the time scales in which the data is analyzed. Two flexible models within the inhomogeneous Poisson process framework are discussed. Both of these formulations account for simultaneous biological phenomena, from multiple time scales, such as refractory period, bursting, brain rhythms, and learning.
Analyzing a data-set from LGN and retina, we compare the fit of these models with the Bayesian adaptive regression splines (BARS) method and discuss the strengths and limitations of the methodology. Computational efficiency, which is usually a challenge, is one of the highlights of these new models. The reconstruction quality of a complex intensity function demonstrates the power of this new multiscale methodology.

Disclosures: R. Ramezan: None. D. Wynne: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.17/JJ6

Topic: D.06. Vision

Support: NINDS Intramural Research Program

Title: Synaptic transfer between ON and OFF visual channels mediated by AII amacrine cells in mouse retina

Authors: *C. W. GRAYDON, J. S. DIAMOND;
Synaptic Physiol. Section, NINDS, Bethesda, MD

Abstract: The mammalian retina is a model system for studying how information travels through neural circuits. In vertebrate retinas, visual information is split into ON and OFF channels (signifying response to the onset and offset of light, respectively) at the first synapse between photoreceptors and bipolar cells. In the mammalian retina, an interneuron called the AII amacrine cell sits at the confluence of rod (night, ON only) and cone (day, ON and OFF) pathways. In the mouse, the AII receives ribbon synapse inputs from rod bipolar cells (RBCs, ON) and one type of OFF cone bipolar cell (CBCs), provides glycinergic output to OFF CBCs and ganglion cells, and connects to other AIIs and multiple types of ON CBCs through gap junctions. However, the characteristics of AII output synapses onto OFF CBCs and whether the AII processes incoming RBC information before passing it onto OFF CBCs are poorly understood topics. We made paired electrophysiological recordings in mouse retina slices to characterize the synaptic inputs and outputs of the AII (e.g. RBC -> AII and AII -> CBC), as well as the filtering that occurs as a signal flows through the AII (e.g. RBC -> CBC through unpatched AIIs). Similarly to ribbon synapses, the glycinergic output of the AII (AII -> CBC) was observed to exhibit a large transient release component following the onset of a stimulus (thought to encode contrast information), followed by a sustained component of ongoing release throughout the duration of the stimulus (thought to encode luminance). Curiously, we found that the RBC information flowing through the AII to OFF CBCs maintained the same synaptic transfer function as the immediately connected pairs within the circuit. In other words, there was no gain or loss in sensitivity when information flowed through the AII - responses in a CBC when stimulating an RBC exhibited the same transfer function as the responses of the AII when stimulating an RBC, despite traveling through an additional, sign-inverting (glycinergic inhibitory) synapse. While this transfer function was the same for the transient synaptic component occurring at the onset of a stimulus, the sustained component that persists throughout the duration of the stimulus was completely filtered out and not transmitted by the AII. This filtering can be explained by basic biophysical properties of the AII and synaptic release

probabilities. These results indicate that the AII acts as a temporal filter in the mammalian retina, passing transient signals with great sensitivity while eliminating sustained information.

Disclosures: C.W. Graydon: None. J.S. Diamond: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.18/JJ7

Topic: D.06. Vision

Support: Department of Veterans Affairs Rehabilitation R&D Service Merit Award E0951-R

NIH Grant P30 EY006360

NIH Grant R01 EY004864

Research Career Scientist Award C9257

Research to Prevent Blindness (Departmental Award)

Foundation Fighting Blindness

Department of Veterans Affairs Rehabilitation R&D Service Career Development Award

Title: Temporal progression of retinal, cognitive, and motor deficits in a rat model of Type II diabetes

Authors: *R. E. ALLEN[1], A. FEOLA[3], C. MOTZ[2], A. L. OTTENSMEYER[2], L. HE[4], P. IUVONE[4], P. M. THULE[1], M. T. PARDUE[2,5];
[2]Ctr. for Visual and Neurocognitive Rehabil., [1]Atlanta VA Med. Ctr., Decatur, GA; [3]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA; [4]Dept. of Ophthalmology, Emory Univ., Atlanta, GA; [5]Biomed. Engin., Georgia Inst. of Techonology, Atlanta, GA

Abstract: Diabetic retinopathy is the leading cause of blindness in working age adults and is likely closely related to other diabetic complications, such as cognitive deficits and vascular changes in the brain. The Goto-Kakizaki (GK) rat is a spontaneously occurring, polygenic, non-insulin-dependent, non-obese model of Type II diabetes that develops impaired glucose-induced insulin secretion at 2 weeks and fasting hyperglycemia at 4 weeks. Retinal, cognitive, and motor deficits have been reported in the GK rat. However, the temporal appearance of these deficits

and the relationship between them has not been studied. We hypothesize that Goto-Kakizaki rats exhibit retinal dysfunction prior to cognitive and motor dysfunction. The following assessments were performed at 4 and 8 weeks of age in male and female GK rats and Wistar (W) controls: electoretinogram (ERG, retinal function), rotarod (motor function), and glucose and insulin tolerance tests (hyperglycemia and insulin resistance). Spatial alternation (cognitive function) and exploratory behavior were assessed using a Y-maze task at 5, 6, 7, and 8 weeks. By 4 weeks of age, GK rats exhibited significant glucose intolerance ($p < 0.001$), insulin resistance ($p < 0.001$), and retinal deficits, including delays in ERG implicit times (flicker, $p < 0.01$; b-wave, $p < 0.001$; oscillatory potentials, $p < 0.001$). Additionally, greater ERG amplitudes for a-wave, b-wave, oscillatory potential, and flicker were observed in GK rats at 4 weeks ($p < 0.001$ for each), though amplitudes began to approach Wistar levels by 8 weeks. Beginning at 6 weeks, GK rats showed cognitive function deficits ($p < 0.05$) and exploratory behavior deficits ($p < 0.05$). However, no motor function deficits were observed using rotarod, even by 8 weeks. Interestingly, male GK rats had higher levels of hyperglycemia ($p < 0.05$), but female GK rats showed greater delays in flicker ERG implicit time ($p < 0.001$). Retinal function deficits in GK rats presented by 4 weeks, with deficits in cognitive and exploratory behavior presenting at 6 weeks and motor deficits not presenting during the 8 week testing period. Future studies will investigate dopamine loss as a common mechanistic link, assess long term changes in function and retinal vasculature, and determine if retinal deficits can predict cognitive dysfunction and late stage retinal disease.

Disclosures: R.E. Allen: None. A. Feola: None. C. Motz: None. A.L. Ottensmeyer: None. L. He: None. P. Iuvone: None. P.M. Thule: None. M.T. Pardue: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.19/JJ8

Topic: D.06. Vision

Support: NIH Grant EY24826

Title: Revisiting cannabinoid receptor 2 expression and function in murine retina

Authors: N. MURATAEVA[1], J. BOROWSKA-FIELDING[2], A.-M. SZCZESNIAK[2], B. SMITH[2], C. HILLARD[3], J. ROMERO[4], M. KELLY[2], *A. STRAIKER[1];
[1]Indiana Univ., Bloomington, IN; [2]Dalhousie Univ., Halifax, NS, Canada; [3]Med. Col. of Wisconsin, Milwaukee, WI; [4]Cajal Inst., Madrid, Spain

Abstract: Cannabinoid receptor 2 (CB_2) plays a significant role in the regulation of the immune function, and is mainly expressed by cells of the immune system. CB_2 expression has been reported on activated microglial cells and astrocytes, while neuronal expression remains a subject of contention. CB_2 has been reported in retina along with evidence that CB_2 contributes to retinal visual processing. The purpose of this study was to revisit CB_2 expression using a CB_2 reporter model and to examine in detail the consequence of CB_2 deactivation on the retinal responses to light using both transgenic and pharmacological approaches.
In CB_2 receptor knockout mice we observed increases in the a-wave of the ERG in scotopic conditions, as well as dark adapted cone-driven ON bipolar cells and to a lesser extent cone-driven ON bipolar cells early in light adaptation. Significantly however, acute block of CB_2 with the antagonist AM630 did not mimic the results observed in the CB_2 KO mice while chronic (7 days) block did. Consistent with this we find that the staining for the CB_2 antibody previously used to show retinal CB_2 protein expression is also present in CB_2 knockout tissue. Separately, using CB_2 reporter mice we do not observe CB_2 reporter (GFP) expression under baseline conditions, though we do see ocular expression under inflammatory conditions.
We conclude that chronic but not acute loss of CB_2 function modulates retinal visual processing. However the lack of retinal CB_2 expression or response to acute CB_2 blockade suggests that this occurs as a result of developmental or adaptive changes rather than acute CB_2 deactivation.

Disclosures: N. Murataeva: None. J. Borowska-Fielding: None. A. Szczesniak: None. B. Smith: None. C. Hillard: None. J. Romero: None. M. Kelly: None. A. Straiker: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.20/JJ9

Topic: D.06. Vision

Support: NIH EY023020 – 01

Burroughs Wellcome Fund Career Award

Pew Charitable Trusts Scholarship

Title: Dendritic plasticity of deafferented cone bipolar cells

Authors: *C. BEIER[1], A. HOVHANNISYAN[1], D. V. PALANKER[2], A. SHER[1];
[1]UC Santa Cruz, Santa Cruz, CA; [2]Ophthalmology, Stanford Univ., Stanford, CA

Abstract: The adult retina is well known to be destructively plastic in response to injury or disease. However, recently we showed that deafferented adult rod bipolar cells in rabbit retina actively restructure their dendrites to make connections with healthy photoreceptors. Here we test if the deafferented cone bipolar cells in the ground squirrel retina undergo similar restructuring. We also investigate if the deafferented S-on bipolar cells make new connections exclusive to S-cones in both ground squirrel and rabbit retina.
Line-shaped lesions were produced in ground squirrels and rabbits with a 532nm laser, using beam diameter of 100μm, scanned at speeds and powers titrated to destroy photoreceptors, leaving bipolar cells intact. Ground squirrel blue bipolar cells were labeled with the HCN4 antibody (Puller, et al. J Comp Neurology, 2011). Additional immunohistochemistry (IHC) was performed to identify photoreceptors and synaptic proteins found at outer plexiform layer (OPL) synapses and other cone bipolar cell types (PNA, S-Opsin, CtBP2, iGluR5, CD15, secretagogin). Subsequent analyses were performed on Z-stacks of IHC data. To selectively label blue bipolar cells in the rabbit retina we injected biocytin intravitreously and enucleated 40-50 hours later (MacNeil and Gaul, J Comp Neurology, 2008).
In the ground squirrel PNA and S-Opsin staining is lost in acute lesions, matched by the reduction in the number of photoreceptor ribbons. Dendrites of HCN4, CD15, and secretagogin stained cone bipolar cells are not noticeably altered in acute lesions though iGluR5 staining is reduced. 1 month after photoreceptor ablation, dendritic tree changes in deafferented bipolar cells are apparent but differ between cell types. CD15 stained cells prune their dendritic trees in response to deafferentation. Dendrites of secretagogin stained cells remain with no signs of drastic restructuring. Deafferented HCN4 stained cells maintain a vertical dendritic stalk that either terminates near the OPL or turns at the OPL and then continues laterally within the plexiform layer.
Cone bipolar cells types in the ground squirrel retina respond uniquely to deafferentation suggesting the structural plasticity mechanisms available to adult cone bipolar cell types are different.

Disclosures: C. Beier: None. A. Hovhannisyan: None. D.V. Palanker: None. A. Sher: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.21/JJ10

Topic: D.06. Vision

Title: Probing selectivity and connectivity in *Drosophila* visual projection neurons

Authors: *M. MORIMOTO, A. NERN, A. M. WONG, M. B. REISER;
Howard Hughes Med. Inst. Janelia Farm Res. Campus, Ashburn, VA

Abstract: What information of the external world is extracted by the sensory system and used to produce behavior? To approach this question, we are studying the visual projection neurons in the *Drosophila* visual system. These neurons project from the third optic neuropil (lobula) to the central brain, and have been proposed to carry behaviorally relevant visual features to higher brain regions. It was recently shown that optogenetic activation of individual visual projection neuron types could induce distinct behaviors such as takeoff and backward walking, linking these visual neurons to specific behavioral programs downstream.
Using *in vivo* two-photon GCaMP imaging, I recorded visually evoked Ca^{2+} responses from several of these cell types. Cell types that showed induced takeoff and backward walking preferentially responded to dark looming stimuli or fragmented expanding local features, which suggests their role in behaviors triggered by object approach. I plan to determine whether directional selectivity (DS) underlies the mechanism for this approach sensitivity.
To explore how this visual information is transformed in the downstream circuit, we identified several candidate neurons that receive input from this cell type by anatomical overlap, then validated their connections using optogenetic activation and GCaMP imaging. I am currently investigating the response properties of these downstream neurons, with the hypothesis that visual information becomes more selective downstream. We hope this work will detail how behaviorally relevant information is extracted across several layers of a neural circuit.

Disclosures: M. Morimoto: None. A. Nern: None. A.M. Wong: None. M.B. Reiser: None.

Poster

239. Retina Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 239.22/JJ11

Topic: D.06. Vision

Support: McNair Scholar's Program - GVSU

Title: Real-time characterization of dmp 543-mediated elevations in acetylcholine release in porcine retina

Authors: B. SINCOX, D. LINN, *E. RAMSSON;
Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Glaucoma, among other retinal diseases, is believed to be caused by excitotoxicity in the nervous system of the eye. Evidence suggests that acetylcholine is linked to neuroprotection, and inducing the release of acetylcholine can possibly reduce the excitotoxicity caused by elevated intraocular pressure. Therefore, the regulation of the release of acetylcholine may prevent the loss of eyesight associated with glaucoma. Using Fast-Scan Cyclic Voltammetry and an acetylcholine biosensor, acetylcholine release in response to both light and DMP 543 was identified and characterized. Since acetylcholine does not participate in carbon-based redox reactions, the electrodes could not directly detect it. Instead, we measured the release of hydrogen peroxide which is a product of the breakdown of acetylcholine and subsequent choline breakdown. A recording electrode was placed within the inner plexiform layer of pig retina, where acetylcholine is released, and optimized according to release profiles. Using light stimulation as a control, preliminary results indicate both light and DMP 543 caused an increase in the release of acetylcholine. Since acetylcholine release was found to be elevated in response to DMP 543, it may be a useful drug in the treatment of glaucoma.

Disclosures: B. Sincox: None. D. Linn: None. E. Ramsson: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.01/JJ12

Topic: D.06. Vision

Support: NSF CAREER Award IIS-1254123

NSF Grant IOS-1556388

NEI Grant 1R01EY025087

Salk Women and Science Award 2016

Title: The impact of sensory uncertainty on maximally informative adaptive dynamics in neural populations

Authors: *W.-M. HSU[1,2], D. B. KASTNER[3], S. A. BACCUS[4], T. O. SHARPEE[1]; [1]CNL-T, Salk Inst., La Jolla, CA; [2]Physics, UCSD, La Jolla, CA; [3]Psychiatry, UCSF, San Francisco, CA; [4]Neurobio., Stanford Univ., Stanford, CA

Abstract: Sensory neural populations are optimized to transmit information about the sensory environment. This optimization has occurred through the course of evolution in the creation of

different cell types, and also occurs dynamically to adapt neural responses to the current stimulus environment. Recent experimental and theoretical work has shown that when encoding a particular stimulus feature, the existence of multiple neuronal types with different thresholds increases information transmission when sensory noise drops below a certain level. This prediction across an evolutionary timescale simultaneously explains the existence of adapting and sensitizing Off retinal ganglion cells (RGCs), which have high and low thresholds for spiking, respectively, as well as the absence of comparable types among On that have higher effective noise level. However, the difference in thresholds between adapting and sensitizing cells is systematically lower than the one that would yield maximal information in an environment of stationary contrast. Yet, to achieve the optimal threshold for the current environment, ganglion cells must dynamically measure the contrast. Here we show that smaller differences in thresholds are optimal in the case where the stimulus contrast is not known but is estimated from sensory inputs. In some cases, we find evidence of a hysteresis where coordination between thresholds lags behind changes in sensitivity that are induced by contrast adaptation. This causes neural populations to follow locally optimal but globally suboptimal solutions in terms of information transmitted. Further, we find that sensory uncertainty increases both the average firing rate and information transmission, but only in the regime of low firing rates. At high firing rates, sensory uncertainty increases the average firing rate but decreases information. Our findings reveal the relationship between sensory uncertainty and maximal information transmission in neural populations, and provide additional arguments for sparse coding in the brain.

Disclosures: W. Hsu: None. D.B. Kastner: None. S.A. Baccus: None. T.O. Sharpee: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.02/JJ13

Topic: D.06. Vision

Support: NIH Grant R15EY026255

Title: Asymmetric distribution of melanopsin ganglion cell outer retinal dendrites in the mouse retina

Authors: J. R. ONYAK, S. W. ISLAM, *J. M. RENNA;
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Abstract: There are 6 different subtypes of melanopsin ganglion cells that express the photopigment melanopsin and function as autonomous photoreceptors. These melanopsin ganglion cells are photosensitive prior to birth and are critical for a variety of non-image forming visual functions. Somas of M1 melanopsin ganglion cells are asymmetrically distribution in the retina, inverse to the asymmetrically distributed S-opsin expressing cones. We have previously found that a percentage of M1-type melanopsin ganglion cells extend dendrites into the outer retina. Here we examine the topographical distribution of these Outer Retinal Dendrites (ORDs) relative to S-opsin expressing cones at various stages of development.
Retinal whole-mounts of C57BL/6 mice at postnatal days 4, 8, 12, and 30 were double-labeled for S-opsin and melanopsin using immunohistochemistry. They were imaged via an epifluorescence microscope at 4x to capture the S-opsin gradient and to create a digital retinal map. Each region of the retina was then systematically examined at 40x magnification to identify and plot the topographical distribution of all ORDs. In house software (C#) was used to determine the area of highest S-opsin concentration. Retinal maps were aligned accordingly and divided into quadrants for quantitative analysis.
At all ages examined, S-opsin expression was confirmed to be most concentrated in the ventral half of the retina and ganglion cell ORDs were found to be more numerous in the dorsal half. Throughout development, ORDs show an asymmetric dorsal distribution. At P12 71% of ORDs were located in the dorsal retina and 29% were found in the ventral retina. In the mature retina (P30), 77% of ORDs were located in the dorsal retina and 23% were found in the ventral retina. While these data parallel previous reports of the asymmetric distribution of M1 ganglion cell somas, our data suggest that ORDs are even more asymmetrically distributed with a very strong preference for the dorsal retina. These data suggest a functional connection with cones in the dorsal retina, most likely M-opsin expressing cones.

Disclosures: J.R. Onyak: None. S.W. Islam: None. J.M. Renna: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.03/JJ14

Topic: D.06. Vision

Support: Academy of Finland grant 253314

Academy of Finland grant 256156

Academy of Finland grant 283268

Emil Aaltonen Foundation

Sigrid Juselius Foundation

Ella and Georg Ehrnrooth Foundation

Oskar Öflund Foundation

Title: The diurnal control of visual sensitivity in the mouse retina and visually guided behavior

Authors: *S. KOSKELA[1], T. TURUNEN[2], P. ALA-LAURILA[1,2];
[1]Dept. of Biosci., Univ. of Helsinki, Helsinki, Finland; [2]Dept. of Neurosci. and Biomed. Engin., Aalto Univ., Espoo, Finland

Abstract: The retina is a rhythmic tissue, with its independent circadian clock and several circadian rhythms in its morphology and biochemistry. This has led to the hypothesis that visual functions are under circadian control. However, it has never been fully assessed whether visual sensitivity depends on the circadian clock. We determined if the sensitivity limit of light detection in mouse retinal ganglion cells (RGCs) and visually guided behavior depend on the diurnal rhythm. We used both melatonin proficient (CBA/CaJ) and melatonin deficient (C57BL/6J) mouse strains to compare the visual sensitivity of mice in their subjective night with those measured in their subjective day. We used flat-mounted retinas and cell-attached patch clamp technique to measure the sensitivity limit of alpha-like RGCs: On sustained RGCs (OnS) and Off sustained RGCs (OffS). We determined the visual threshold and detection strategies of mice in a water-maze test by using a novel fully-automated tracking system of mouse behavior. We show that the behaviorally measured visual performance near the detection threshold is better during the night than during the day, on both mouse strains. However, the sensitivity limit of RGCs does not show strong dependence on the diurnal rhythm. We have evidence that the difference in behavioral performance is, at least partly, due to diurnal differences downstream from retina, i.e. differences in cognitive functions.

Disclosures: S. Koskela: None. T. Turunen: None. P. Ala-Laurila: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.04/JJ15

Topic: D.06. Vision

Support: NIH Grant EY007360

Title: Gap junctional coupling between retinal ganglion and amacrine cells underlies reduced global object perception in connexin 36 knockout mice

Authors: *K. ROY, S. A. BLOOMFIELD;
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Abstract: Widely separated retinal ganglion cells (RGCs) exhibit coherent spiking in response to global stimuli as opposed to local stimuli (Neuenschwander and Singer, 1996), which has been proposed to underlie global object perception. Since the large spatial separation between the RGCs eliminates common bipolar cell input, the mechanism responsible for this coherent activity remains unclear. Here we demonstrate that wide field amacrine cells (WFACs) and/or polyaxonal amacrine cells (PACs), electrically coupled to RGCs are responsible for correlated firing of RGCs in the mouse retina. Pharmacological blockade of gap junctions or genetic ablation of connexin 36 (Cx36) eradicates this coherent activity. Since, such coherence is proposed to underlie global object perception, we tested whether $Cx36^{-/-}$ mice will have poor global object perception compared to $Cx36^{+/+}$. $Cx36^{+/+}$ and $Cx36^{-/-}$ littermates were trained to perform a two-alternate forced choice task in a Y water maze. Each of the monitors adjacent to the arms of the Y maze pseudorandomly displayed two separate rectangles and a contiguous rectangle representing discreet vs. global objects, respectively. Mice were trained to swim towards the contiguous rectangle. The gap between the discreet rectangles was decreased in increments of 20, 10, 7, 5, 2, 1.5 and 1 degree of visual angle. Responses from both $Cx36^{+/+}$ and $Cx36^{-/-}$ mice were recorded and all the trials were concatenated together for both the genotypes individually. $Cx36^{+/+}$ and $Cx36^{-/-}$ mice were also tested for spatial acuity to determine whether any difference in global object perception might be due to attenuated spatial acuity. All the experiments were performed in photopic condition as $Cx36^{-/-}$ mice had been shown to have deficiencies in scotopic vision. The threshold was set at 75% accuracy. The percentage of correct responses of $Cx36^{+/+}$ mice indicated that they could distinguish rectangles separated by as close as 2 degree of visual angle. However, the $Cx36^{-/-}$ mice demonstrated a significantly inferior performance at 2 degree. The $Cx36^{-/-}$ mice could not distinguish rectangles separated by less than 7 degree of visual angle. Additionally, $Cx36^{+/+}$ and $Cx36^{-/-}$ did not show any significant difference in spatial acuity up to 0.5 cycles per degree, which is the threshold for mouse vision. Taken together the results indicate that concerted activity between distant RGCs is mediated via electrical coupling with the WFACs/PACs. Loss of this coherent spike activity results in an impairment of global object perception. Our findings suggest a novel role of retinal neuron gap junctions in stimulus encoding that plays a key role the perception of contiguous, global objects.

Disclosures: K. Roy: None. S.A. Bloomfield: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.05/JJ16

Topic: D.06. Vision

Support: JSPS KAKENHI, Grant-in-Aid for Scientific Research (B), 25282130

Title: Cell-attached recordings of light-induced postsynaptic potentials in mouse retinal ganglion cells.

Authors: S. KITORA, T. YAGI, *Y. HAYASHIDA;
Grad.Eng., Osaka Univ., Suita-Shi, Osaka, Japan

Abstract: Previous studies showed that the cell-attached mode of the patch-clamp techniques is useful for recording, not only single-channel currents on the patch membrane, but also supra-threshold action potential firings in retinal ganglion cells while preserving cytoplasmic integrity. However, little has been reported on the applicability of this method to record sub-threshold postsynaptic potentials. Thus, in the present, we made recordings of light-induced responses of the ganglion cells under the cell-attached (CA) as well as the ruptured-patch whole-cell (rpWC) configurations in mouse retinas. The isolated retinas were prepared as whole-mounts from juvenile C57/BL6 mice (3-8 week-old, n=18), and were kept under dark conditions (~0.5-1 nW/cm^2) while being perfused with oxygenated saline at ~30 ^oC. Na^+-based and K^+-based solutions were filled in the pipettes (1-2 μm and 3-7 MΩ at the tip) for CA and rpWC recordings, respectively. As similar to previous studies, relatively large amplitudes of the action-potential currents (60-600 pA in peak-to-peak) were recorded under voltage clamp at 0 mV in CA mode from the ganglion cells responding to light steps (~1 $x10^7$ Rh^* /rod/sec, ~525 nm). Not only that, light-induced graded changes of the holding currents (less than 10 pA in peak) were discernible when the current traces were averaged over 10-30 trials and low-pass filtered off-line (15 Hz cut-off, 3-order Bessel). These graded currents remained under blockade of the action-potential currents with 1-μM tetrodotoxin. Moreover, time courses of these currents were overlapped with those of the light-induced subthreshold potentials that were recorded from the same cells under current-clamp in rpWC mode within 6 minutes post-break-in; the peak coefficients of the cross-correlations between time courses of the graded currents and the subthreshold potentials were 0.82±0.07 and 0.87±0.04 (mean±SEM, *n*=5) in hyperpolarizing and depolarizing directions, respectively. These results suggested that the cell-attached voltage-clamp recording is usable for monitoring, not only the spike firings, but also the postsynaptic subthreshold activities in the retinal ganglion cells.

Disclosures: S. Kitora: None. T. Yagi: None. Y. Hayashida: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.06/JJ17

Topic: D.06. Vision

Support: NIH Grant 5T32EY025202-02

Title: Receptive field analysis using whole-cell spatiotemporal maps

Authors: *S. COOLER, G. W. SCHWARTZ;
Northwestern Univ., Chicago, IL

Abstract: Retinal neurons generate their particular response characteristics by nonlinearly combining spatially and temporally localized inputs. We measured the receptive field (RF) maps of several types of retinal ganglion cells (RGCs) and classified the patterns we observed, using whole-cell voltage-clamp recordings and flashed-spot visual stimuli. Using a closed-loop experimental system, we mapped the spatial extent of the contrast adaptation effect to identify nonlinear RF subunit locations.

RGCs varied in the spatial extent of their excitatory and inhibitory RFs. By fitting the RFs to a Gaussian approximation, we were able to quantify their sizes and positions. While the RFs generally had centers near the somas, a subset of RGCs had RFs centered some distance away, typically in alignment with the dendrites of the cell. A further subset of those RGCs had excitatory and inhibitory inputs spatially offset from each other. We were able to model the effects of these offsets, and predict qualities of the feature selectivity of the RGCs.

Using a novel contrast adaptation stimulus, we identified the spatial extent of input subunit adaptation. We studied how a high-contrast region shifted the light intensity response profile in the space around it. Using this protocol, we anticipate identifying the locations of individual subunits, and matching them to bipolar cell receptive field locations using immunohistochemistry and genetic labeling. Accurately mapping RF subunits online physiologically would be a major step in the field, allowing us to predict RGC responses to complex spatiotemporal patterns of light.

Disclosures: S. Cooler: None. G.W. Schwartz: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.07/KK1

Topic: D.06. Vision

Support: NIH Grant EY009256

Title: The effects of D1 and D4 dopamine receptor knockouts on retinal ganglion cell receptive fields and contrast sensitivity

Authors: *D. SPRINZEN, H. DAI, A. KHANDHADIA, D. MCMAHON;
Vanderbilt, Nashville, TN

Abstract: Within the mammalian retina, it is believed dopamine acts as a circadian-regulated neuromodulator to reconfigure retinal processing during light adaption. Decreased retinal dopamine causes various deficits in light adapted visual responses including loss of visual acuity and decreased contrast sensitivity during optokinetic tracking. Furthermore, these deficits are independently mediated by dopamine receptors classes, with D1-like and D2-like receptors involved with visual acuity with contrast sensitivity, respectively. Here, we used multi-electrode array (MEA) recordings of D1 and D4 specific dopamine receptor knock out mouse models to elucidate the role of dopamine receptor activity on retinal function. Retinal Ganglion Cell (RGC) physiological responses are classified and compared based on spontaneous activity, on-off characteristics, response duration, directional selectivity, receptive field profile, center-surround antagonism, response latency, and contrast sensitivity. Analysis by K-means clustering found 5 broad RGC classes: ON-sustained, ON-transient, OFF, ON direction selective, and ON-OFF direction selective. Preliminary data for both D1 and D4 receptor knockouts showed an increase in the size of the receptive field center of ON-sustained and OFF cell classes of 30-50% as compared to control animals. Interestingly, contrast sensitivity profiles for D1 receptor knockout RGCs was similar to wildtype, however D4 receptor knockout RGCs exhibited a shift in spatial tuning such that ON-sustained cells preferred higher spatial frequencies and OFF cells preferred lower spatial frequencies compared to wildtype RGCs. To further study the control of dopamine receptors within the retinal circuit, we will record from retinas with targeted deletion of D1 receptors on horizontal cells.

Disclosures: D. Sprinzen: None. H. Dai: None. A. Khandhadia: None. D. McMahon: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.08/KK2

Topic: D.06. Vision

Support: NEI grant R01EY024567

the Karl Kirchgessner Foundation

Title: Light adaptation across diverse retinal ganglion cell types in the mouse retina

Authors: *K. RUDA, G. D. FIELD;
Neurobio., Duke Univ., Durham, NC

Abstract: Purpose: Adaptive coding is a fundamental feature of sensory systems. In vision, the retina must adapt to the 9 orders of light intensities between a starry night and a sunny day. How do retinal ganglion cells (RGCs), the sole output of the retina, encode this dynamic visual signal? A crucial framework for understanding this question is the organization of RGCs into distinct cell types, because light adaptation may impact RGC types differently. Here we determine how light adaptation changes the stimulus encoding of several types of RGCs in mouse.
Methods: We performed multi-electrode array recordings on isolated mouse retinas in response to visual stimuli. The multi-electrode array consisted of 512 electrodes. Spikes were identified and sorted by custom software, and 300-400 RGCs were identified in each recording. We measured differences in general spiking dynamics such as latency, interspike intervals distributions and spontaneous activity in several cell types. Next, we measured changes in spatiotemporal receptive fields (RFs) and gain. These quantities estimate the stimulus features that drive spiking and the relationship between these features and spike number; they correspond to the linear and nonlinear parts of a linear-nonlinear-poisson (LNP) model, respectively. We used measured RFs and gain in LNP models to determine which aspects of RGC encoding can account for the observed changes in spiking dynamics (RF changes, gain changes, or both).
Results: We observed changes in spiking activity in many RGC types across light levels, including background firing rates and interspike intervals. Similar to previous work, we found that response latency to stimulus features was shorter with increased light intensity across RGC types. We also observed that RF properties of different RGC types were altered in distinct ways, and that these changes contributed to differences in spiking dynamics across light levels.
Conclusions: These results suggest that RGC types have different input-output functions during light adaptation, and provide a starting point for elucidating how populations of specialized neurons can adaptively encode dynamic stimuli.

Disclosures: K. Ruda: None. G.D. Field: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.09/KK3

Topic: D.06. Vision

Support: NSC-102-2321-B-002-081

Title: Intra-retinal feedback connections composed of melanopsin expressing retinal ganglion cells and amacrine cells

Authors: *P.-T. YEH, S.-K. CHEN;
Dept. Life Sci., Natl. Taiwan Univ., Taipei City, Taiwan

Abstract: Retinal structure and functional circuit have been study for several decades. It is well known that the information flow of retinal circuit starts form light reception by rods and cones, to horizontal cells, amacrine cells and bipolar cells, and transduces to brain by retinal ganglion cells. However, our previous studies indicated that a group of melanopsin containing retinal ganglion cells, intrinsically photosensitive retinal ganglion cells (ipRGCs), connect to dopaminergic amacrine cells (DACs) by intra-retinal axon collaterals. By randomly genetic labeling of ipRGCs in mice and immunochemical methods, our study shows morphologically distinct groups of ipRGC intra-retinal axon collaterals, innervate varies layers in inner plexiform layer. At the same time, we also found those collaterals connect to different cells. The feedback connections via ipRGC intra-retinal collaterals may change our comprehension to visual and non-visual retinal circuitries.

Disclosures: P. Yeh: None. S. Chen: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.10/KK4

Topic: D.06. Vision

Title: Adaptive and predictive coding by spatiotemporal dynamics in the retina

Authors: *K. S. CHEN[1,2], C.-K. CHAN[1], C.-T. YEN[2];
[1]Inst. of Physics, Academia Sinica, Nankang, Taipei 11529, Taiwan; [2]Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Adaptation to time-dependent stimulations in order to make predictions is a crucial task for sensory neural systems. It is known that the retina can detect periodic patterns and produce temporally precise omitted stimulus response (OSR) to violation of periodicity. When the simulation is consisted of multiple periods, it is not clear if a predictive phenomenon similar to OSR can still occur. In our experiments with bullfrog retina under simulations of multiple periods, an OSR-like response is found. In this second-order OSR phenomenon, the main output of the retina ganglion cells will follow the effective period of the stimulations. Even for spatial-uniform stimulations, heterogeneous spatiotemporal firing patterns can be observed during the adaptive process. Presumably, such adaptive heterogeneity enables the retina to make prediction to the upcoming stimuli. Distributing multiple temporal information spatially might be a general strategy for predictive coding in sensory systems under complex temporal stimulations.

Disclosures: K.S. Chen: None. C. Chan: None. C. Yen: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.11/KK5

Topic: D.06. Vision

Title: Congenital nystagmus gene FRMD7 is required to establish a neuronal circuit asymmetry for retinal direction selectivity

Authors: *A. DRINNENBERG[1], K. YONEHARA[2,3], M. FISCELLA[4], F. ESPOSTI[1], S. TRENHOLM[1], J. KROL[1], F. FRANKE[4,5], B. GROSS-SCHERF[1], A. KUSNYERIK[5], J. MUELLER[4], A. SZABO[5], J. JUETTNER[1], F. CORDOBA[3], A. REDDY[1], J. NEMETH[5], Z. NAGY[5], F. MUNIER[6], A. HIERLEMANN[4], B. ROSKA[1];
[1]Friedrich Miescher Inst. For Biomed. Res., Basel, Switzerland; [2]Danish Res. Inst. of Translational Neurosci. – DANDRITE, Aarhus, Denmark; [3]Novartis Inst. for Biomed. Res., Basel, Switzerland; [4]Biosystems Sci. and Engin. of ETH Zurich, Bio Engin. Lab., Basel, Switzerland; [5]Semmelweis Univ., Budapest, Hungary; [6]Jules-Gonin Eye Hosp., Lausanne, Switzerland

Abstract: Neuronal circuit asymmetries underlie many circuit computations in the central nervous system. However, the molecular pathways controlling their establishment are poorly

understood. A principle feature of the retinal direction-selective circuit is asymmetric inhibitory input from starburst amacrine cells to direction-selective retinal ganglion cells (DS cells). Using a combination of molecular, genetic, physiological and behavioral strategies, we show that *FRMD7* - a gene that is defective in human congenital nystagmus - is required for proper functioning of the directionally selective retinal circuit. Remarkably, we find that the mutation of *FRMD7* in mice leads to the selective loss of the horizontal optokinetic reflex, as it does in humans. Microelectrode array recordings from *FRMD7* mutant mouse retina reveal that the loss of the horizontal optokinetic reflex can be ascribed to the selective loss of horizontal direction selectivity in retinal ganglion cells. Two-photon targeted patch-clamp recordings of cells with the genetic identity of wild-type horizontal DS cells show that the inhibitory input to horizontal DS cells switches from asymmetric to symmetric when *FRMD7* is mutated. To assess the differential impact of *FRMD7* mutation on ON vs ON-OFF DS cells we targeted these cell types using transgenic mouse lines (*Hoxd10-GFP* and *Drd4-GFP*). Indeed we found differential effects of *FRMD7* mutation on these different populations of DS cells. Finally, we pinpointed the source of the effect of the *FRMD7* mutation to starburst amacrine cells, which are the only cells in the mouse retina to express this gene. Interestingly, *FRMD7* is also expressed in ChAT expressing, starburst-like amacrine cells in the primate retina. We conclude that in the retina *FRMD7* is required to establish spatially asymmetric inhibitory inputs from starburst cells to DS cells along the horizontal axis, and is thus required for horizontal direction selectivity. This study indicates that FRMD7 is a key regulator for establishing a neuronal circuit asymmetry, and these results reveal the involvement of a specific inhibitory neuron type in the pathophysiology of a neurological disease.

Disclosures: A. Drinnenberg: None. K. Yonehara: None. M. Fiscella: None. F. Esposti: None. S. Trenholm: None. J. Krol: None. F. Franke: None. B. Gross-Scherf: None. A. Kusnyerik: None. J. Mueller: None. A. Szabo: None. J. Juettner: None. F. Cordoba: None. A. Reddy: None. J. Nemeth: None. Z. Nagy: None. F. Munier: None. A. Hierlemann: None. B. Roska: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.12/KK6

Topic: D.06. Vision

Support: SUDA Glaucoma Grant 2015

Title: High resolution *In vivo* imaging tracks progressive retinal degeneration in aged marmosets

Authors: *T. NORO[1,2], K. NAMEKATA[1], A. KIMURA[1], T. NAKANO[2], H. TSUNEOKA[2], T. HARADA[1];
[1]Visual Res., Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Ophthalmology, The Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: The population of elderly people is growing worldwide as life expectancy is drastically increased, and this creates new challenges as aging is a risk factor for many chronic conditions including neurodegenerative diseases such as glaucoma. Glaucoma is one of the major causes of blindness. It is a progressive optic neuropathy characterized by axonal injury at lamina cribrosa, retinal ganglion cell (RGC) loss, and visual field defects.
The common marmoset (*Callithrix jacchus*), a small New World monkey, is becoming increasingly attractive as an experimental animal model amenable to gene manipulation with transgenic technologies, particularly in neuroscience research.
The purpose of this study was to monitor age-associated changes in the common marmoset by evaluating retinal structure and function using non-invasive methods: spectral-domain optical coherence tomography (OCT) and multifocal electroretinogram (mfERG). We also examined the lateral geniculate nucleus (LGN) and visual cortex in young and aged marmosets.
Experiments were performed in 12 adult common marmosets. Aged 1 year 6 months - 2 years 6 months (n=6, 23.1±1.4 months, 357±9.7 g) for the young group, 7 years 11 months - 12 years (n=6, 117.8±5.4 months, 372±21.3 g) for the aged group.
The analyses from the scanned images using OCT showed that the thickness of the inner and outer retinal layers were significantly reduced in the aged marmoset compared with the young. Consistently, when we measured visual function *in vivo* using mfERG, the responses were significantly decreased in the aged marmoset compared with the young marmoset. Interestingly, we found one marmoset that presented with naturally occurring normal tension glaucoma (NTG) and observed disease progression after 1 year. As with glaucoma patients, reduction in the thickness of the lamina cribrosa and inner retinal layer was observed by using OCT in this NTG marmoset. Consistent with data from NTG patients, the glutathione level in the blood, brain-derived neurotrophic factor level in the cerebrospinal fluid (CSF), CSF pressure, the number of cells in the LGN and visual cortex were significantly decreased.
In summary, we demonstrate that the marmoset is a good model for studying aging and retinal degeneration such as glaucoma and that our *in vivo* imaging systems enable mapping and tracking of changes in retinal structure and function during aging or disease. This experimental system will provide useful information for development of novel treatment for retinal diseases.

Disclosures: T. Noro: None. K. Namekata: None. A. Kimura: None. T. nakano: None. H. tsuneoka: None. T. harada: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.13/KK7

Topic: D.06. Vision

Support: BBSRC grant (BB/M000664/1) to R.H.

MRC PhD studentship (1413592) to P.A.

Title: Circuit mechanisms underlying orientation selectivity in the zebrafish retina

Authors: *P. ANTINUCCI, R. HINDGES;
Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: In the vertebrate retina, visual stimuli are converted into electrochemical signals and the processed information is then sent to higher visual centres through parallel feature-specific neuronal pathways. How retinal circuits process and extract the orientation of elongated visual stimuli and how the underlying circuitry is established is, however, poorly understood. In this study, we dissect an orientation-selective circuit in the larval zebrafish retina and describe its underlying synaptic, cellular and molecular mechanisms. Using bacterial artificial chromosome transgenesis, we genetically identify a class of GABAergic amacrine cells (ACs) that express the cell-adhesion molecule Teneurin-3 (Tenm3) and possess elongated dendritic arbours. By performing two-photon calcium imaging in the intact retina we reveal that these cells show orientation-selective responses to drifting gratings. Notably, these orientation-tuned Tenm3^{+} ACs respond maximally when the orientation of elongated visual stimuli coincides with the orientation of their dendritic fields. Both selective optogenetic ablation of Tenm3^{+} ACs using KillerRed and pharmacological interference with their function through picrotoxin show that these cells generate orientation selectivity in retinal ganglion cells (RGCs) by being a source of tuned GABAergic inhibition. Furthermore, our morphological analyses suggest that Tenm3^{+} ACs connect to orientation-selective RGCs and that they require Tenm3 to correctly develop their dendritic morphology. Finally, we reveal that orientation tuning is present also among a fraction of bipolar cell presynaptic terminals. We propose a circuit model based on our findings whereby orientation-selective RGCs integrate orthogonal orientation-tuned inhibitory input from Tenm3^{+} ACs and preferred orientation-tuned excitatory input from bipolar cells to acquire their selectivity. In conclusion, our results outline how defined retinal cell types form a circuit capable of detecting elongated stimuli in visual scenes and provide orientation-specific information to the brain.

Disclosures: P. Antinucci: None. R. Hindges: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.14/KK8

Topic: D.06. Vision

Support: NIH Grant EY024567

Whitehall Foundation

Title: Direction selective and intrinsically light sensitive retinal ganglion cells of the tree shrew

Authors: E. N. JOHNSON[1], R. SHAYESTEH[1], E. CHEN[1], J. SCHUMACHER[2], D. FITZPATRICK[2], *G. D. FIELD[1];
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Abstract: The tree shrew (Tupaia belangeri) is a diurnal, evolutionary intermediate between primates and rodents. Classified as a "living fossil," the tree shrew offers a potential view to the evolutionary origins of the primate visual system. While much is known about the functional and structural organization of the dorsal lateral geniculate nucleus (dLGN) and primary visual cortex (V1) in tree shrews, relatively little is known about the diversity of retinal ganglion cells (RGCs). The purpose of this study is to elucidate three structural elements of the tree shrew retina: (1) determine the organization and prevalence of direction selective RGCs and associated circuitry; (2) determine the density and diversity of melanopsin expressing, intrinsically light sensitive, RGCs, and (3) determine the density of RGCs across retinal eccentricity.
To determine the density of putative direction selective RGCs, we used CART antibodies. We show that CART positive cells are RGCs and not displaced amacrine cells. They are bistratified and approximately co-stratify with starburst (ChAT positive) amacrine cells. Thus, CART positive RGCs are likely direction selective, similar to mouse. Counter labeling with anti-RBPMS, revealed that CART positive RGCs are at least 20% of all RGCs. This prevalence is similar to that observed in mice and rabbits and distinct from that observed in primates. We further show that ChAT positive starburst amacrine cells express GABA, consistent with studies in other mammals, but contrary to a previous report in the tree shrew.
Similar to other mammals, anti-melanopsin revealed the presence of at least two distinct populations of intrinsically light-sensitive RGCs, one with cell bodies in the RGC layer and one with cell bodies in the inner nuclear layer. Each type formed an independent and complete mosaic of cells with dendrites that approximately tiled space. The pattern of melanopsin expression closely resembles previous reports of melanopsin expression in the marmoset retina, but differs from that observed in mouse. Finally, we show that RGCs are approximately half of all cells in the RGC layer.

These results point to elements of retinal organization that are similar to rodents, with other elements more similar to primates. An important direction for future studies is to determine the prevalence of a parvocellular-like pathway in the tree shrew retina.

Disclosures: E.N. Johnson: None. R. Shayesteh: None. E. Chen: None. J. Schumacher: None. D. Fitzpatrick: None. G.D. Field: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.15/KK9

Topic: D.06. Vision

Support: German Research Foundation (DFG: JO-886/1-3)

Title: S100 alone or combined with HSP27 leads to specific retinal ganglion cell loss in a glaucoma model

Authors: *S. C. JOACHIM[1], S. REINEHR[2], C. CASOLA[2], S. KUEHN[2], H. B. DICK[2]; [1]Exptl. Eye Res. Insitute, Bochum, Germany; [2]Ruhr-University Bochum, Bochum, Germany

Abstract: Purpose: As previously shown, immunization with ocular antigens, like heat shock protein 27 (HSP27), leads to retinal ganglion cell (RGC) loss in an autoimmune glaucoma model. Aim of this study was to investigate how immunization with S100 alone and in combination with HSP27 affects neuronal and glial cells in the retina at late stages. **Methods:** Rats were immunized with S100 or S100 plus HSP27 (S100+HSP) and compared to controls (n=5/group). 4 weeks after immunization retinas were processed for immunehistology and Western blot analysis. Retinal ganglion cells (Brn-3a), amacrine cells (ChAT), and photoreceptors (rhodopsin, opsin) were quantified. Additionally, macroglia (GFAP, Vimentin) and microglia (Iba1, ED1) were analyzed. Groups were compared via ANOVA with Dunnett's post-hoc test. **Results:** No IOP alterations were noted in both immunized groups throughout the study ($p>0.05$). About a 30% RGC loss was observed in both immunized groups at 4 weeks (S100: $p=0.005$; S100+HSP: $p=0.004$). Cholinergic amacrine cells were also affected (S100: $p=0.02$; S100+HSP: $p=0.05$), while cone (S100: $p=0.5$; S100+HSP: $p=0.2$) and rod photoreceptors (S100: $p=0.9$; S100+HSP: $p=0.1$) remained intact in both immunized groups. An increase in $Iba1^+$ microglia cells was noted in both antigen groups at this late point in time ($p<0.001$), especially in the RGC layer. Also, the number of activated microglia was increased ($p<0.02$). A slight increase in astrocyte reactivity was noted in both immunized groups (S100: $p=0.05$; S100+HSP: $p=0.04$), while Müller glia remained unaltered ($p=0.9$). **Discussion:**

Immunization with ocular antigens rather damages RGCs and amacrine cells than photoreceptors. Also, an increase of activated microglia was seen. Surprisingly, combining S100 with HSP27 did not lead to additional retinal damage or more severe cell loss. Both antigens might interact, possibly having inhibitory effects on each other and thus preventing additional damage to the retina. This IOP-independent glaucoma model can serve as a tool to study specific neuroprotective agents in the future.

Disclosures: S.C. Joachim: None. S. Reinehr: None. C. Casola: None. S. Kuehn: None. H.B. Dick: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.16/KK10

Topic: D.06. Vision

Support: NIH Grant RR00166

NEI Grant EY06678

NEI Grant EY01730

NEI Grant EY07556

Title: Connectomics and color tuning of S-OFF midget ganglion cells

Authors: *L. E. WOOL[1], O. PACKER[2], Q. ZAIDI[1], D. M. DACEY[2];
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Abstract: The primate midget ganglion cell circuit has historically been considered selective for L- and M-cone inputs, but studies suggest S cones contribute to midget OFF cells. The anatomical and physiological origin of this input is unclear. In this study, we physiologically quantified the sign and magnitude of S cone input to midget OFF cells and identified the S-cone synaptic connection at the electron microscopic level. We also used a spatial receptive field model to predict the frequency and magnitude of S input across the visual field. Midget ganglion cells in macaque monkey retina were recorded extracellulary *in vitro* using a full-field stimulus whose chromaticity was modulated around a color circle in L-M versus S cone-opponent space (pure +L-M at 0°; pure +S at 90°; pure -L+M at 180°; pure -S at 270°). von Mises distributions were fitted to peristimulus time histograms; the cell's preferred color vector was the angle of

maximum response, from which signs and relative magnitudes of L, M, and S cone inputs were computed. The majority of midget ON and OFF cells had preferred vectors clustered along the 0-180° L-M axis and showed no appreciable S-cone input (*n*=93, mean +4.32±1.83%). A separate population of OFF midgets showed intermediate color vectors departing from the L-M axis toward the -S 270° pole, showing OFF S input (*n*=14, mean -30.89±7.28%). A previous study suggested that in macaque retina, S cones synapse with OFF but not ON midget bipolar cells (Klug et al., J. Neurosci. 2003 23:9881), but this result is controversial. Using serial block-face scanning electron microscopy of foveal retina, we identified S cones (*n*=17) by their exclusive input to S-cone ON bipolar cells. OFF bipolar cell reconstructions revealed that each S cone was linked to an OFF midget bipolar cell. We used a random-wiring receptive field model to predict S cone input to OFF midgets. We used cone mosaics randomly populated with realistic L:M cone ratios and 10% S cones. Cone density and midget receptive field size changed as a function of eccentricity. A difference-of-Gaussians kernel was applied to all cones in the receptive field, and relative L, M, and S inputs were evaluated for 5000 model cells. Our model predicts strong OFF S cone input to OFF midget cells in the perifovea, and weaker input to a larger number of OFF midgets in the periphery, where OFF midgets receive convergent inputs from L, M and S cones to the receptive field center. Our results confirm that S cones synapse with OFF midget bipolar cells and that this excitatory S OFF pathway contributes to the color tuning of a subpopulation of OFF midget ganglion cells. Our modeling results predict that these S OFF midget cells are present across the retina.

Disclosures: L.E. Wool: None. O. Packer: None. Q. Zaidi: None. D.M. Dacey: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.17/KK11

Topic: D.06. Vision

Support: NIH Grant EY24997-01

Title: Altered physiological function and dendritic morphology of retinal ganglion cells in optic neuropathy

Authors: *S. PASINI, M. L. RISNER, D. J. CALKINS;
Ophthalmology and Visual Sci., Vanderbilt Univ., Nashville, TN

Abstract: Developing effective therapeutic strategies for neurodegenerative diseases requires dissecting how different types of neurons are affected during early progression. The retina is a

well-characterized circuit that provides an accessible model to study how different neuronal compartments respond to stress in disease. Glaucoma is a neurodegenerative disease that selectively targets retinal ganglion cells (RGC) through sensitivity to intraocular pressure. Roughly 30 types of RGC have been identified. Studies indicate particular RGC types may be more vulnerable. Here we assessed how elevated ocular pressure affects the physiology and morphology of RGCs and if AMPA receptors are implicated.
To model glaucoma in C57 mice, we increased ocular pressure unilaterally by microbead occlusion of the anterior chamber; an equivalent saline injection of the opposing eye served as control. After 4 weeks of elevated pressure (+30%) we compared anterograde transport along RGC axons, current- and voltage-clamp responses of single RGCs to light and pharmacological agents, dendritic length, area, and complexity.
Microbead-induced elevation in ocular pressure led to a 55% deficit in the anterograde transport of cholera toxin b to the superior colliculus (saline n=5; microbead n=5, $p < 0.05$). Concurrently, we found that light-evoked AMPA-mediated currents were significantly reduced along with dendritic parameters in αOFF transient RGCs compared to control retinas (saline n=14; microbead n=8, $p < 0.05$). The αOFF sustained RGCs showed an increase in the OFF component of light-evoked AMPA-mediated currents along with a significant increase in dendritic parameters with elevated pressure (saline n=7; microbead n=5, $p < 0.05$). ON-OFF RGCs showed a significant reduction in both light onset and offset of AMPA-mediated light responses. Morphological analysis of ON-OFF RGC dendrites revealed a decrease in dendritic parameters in the ON sublamina of the inner plexiform layer, but no change in the morphology of dendrites that stratify in the OFF sublamina (saline n=17; microbead n=9, $p < 0.05$). Finally, we did not observe any differences in either physiology or dendritic morphology of αON sustained RGCs with elevated ocular pressure (saline n=11; microbead n=10).
Our study suggests that while ocular pressure has a profound effect on anterograde axonal transport to central brain structures, the effect on RGC physiology and morphology is highly specific. While the αOFF transient RGC is most dramatically influenced, αON sustained RGCs are the least vulnerable to elevated pressure. RGC-type specific modifications appear to arise at least in part by changes in AMPA receptor activation.

Disclosures: S. Pasini: None. M.L. Risner: None. D.J. Calkins: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.18/KK12

Topic: D.06. Vision

Support: Core Research for Evolutional Science and Technology, Japan Science and Technology Agency (CREST, JST) to MT

Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) Fellows to AM

Title: Rapid processing of global motion images by locally coordinated retinal ganglion cells

Authors: *A. MATSUMOTO[1], M. TACHIBANA[1,2];
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Abstract: Our eyes repeat fixation and rapid shift (saccade), and thus the retinal image is always in global motion induced by eye movements. Accumulating evidence indicates that the brain is equipped with specific mechanisms for processing global motion images. However, it is not yet known how the retina processes visual information during eye movements. Here we show that the global motion images evoke novel coordinated firing patterns among local groups of retinal ganglion cells (GCs), retinal output neurons to the brain. We simultaneously recorded the firing of GCs in the goldfish isolated retina using a multi-electrode array. GC subtypes were classified based on the temporal profile of classical receptive fields. Global jitter motion of background (simulated fixational eye movements) shortened the integration time of stimulus inputs and increased the spike sensitivity of specific GC subtypes. Subsequent global rapid motion (simulated saccades) evoked synchronous firings and temporally correlated firings to the moving target in local groups of specific GC subtypes. This response modulation was evoked under stimulus conditions comparable to those found for *in vivo* goldfish eye movements. The modulation was mediated by electrical and GABAergic synaptic transmission in the retina. These fast and temporally coordinated firings transmit afferent information during saccade to the brain and may contribute to visual computation.

Disclosures: A. Matsumoto: None. M. Tachibana: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.19/KK13

Topic: D.06. Vision

Support: Boehringer Ingelheim Fonds

International Max Planck Research School

Max Planck Society

Title: FLRT3 a new retinal ganglion cell marker and attempts towards a functional analysis

Authors: *T. RUFF, D. DEL TORO, R. KLEIN;
Molecules - Signaling - Develop., Max Planck Inst. For Neurobio., Martinsried, Germany

Abstract: In the vertebrate retina different subsets of retinal ganglion cells (RGC) code for distinct features of the visual scene, such as direction of motion or contrast. Dense retinal recordings of mouse RGCs suggest, that each feature is encoded by a defined subgroup of RGCs. However, so far only a few genetic markers are known to specifically label each RGC subpopulation.
Here we show the fibronectin leucine-rich transmembrane 3 (FLRT3) receptor as a genetic marker for a specific RGC subpopulation. Co-labeling of FLRT3-positive cells with the pan-RGC marker RBPMS revealed, that in adult mice the majority (90%) of FLRT3-positive cells in the ganglion cell layer are RGCs. FLRT3-positive RGCs distribute homogeneously across the whole retina and represent about 20% of all RGCs. Nearest neighbor distance analysis indicates that FLRT3-positive RGCs arrange in a non-random mosaic like pattern. These results raise the possibility that FLRT3-RGCs represent a specific subpopulation. To further characterize the function and circuit of FLRT3-RGCs, we generated a FLRT3-CreERT2 knock-in mouse. We will use virus-mediated approaches to map FLRT3-RGC projections, connectivity and investigate their morphology. We will also study their physiological responses to different visual stimuli by two-photon calcium imaging.
Using a FLRT3-LacZ reporter knock-in mouse, we also show that FLRT3 retinal expression starts postnatally and persists during adulthood. Hence, FLRT3 is expressed during a developmental timeframe when processes such as inner nuclear layer stratification, regular RGC distribution and dendritic arbor formation take place. Thus, we generated a RGC specific FLRT3 knock-out mouse to further investigate the role of FLRT3 in retina development. With these experiments we hope to reveal the role of a new genetically defined RGC subpopulation that contributes to our understanding of visual signal processing in distinct retinal circuits.

Disclosures: T. Ruff: None. D. del Toro: None. R. Klein: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.20/KK14

Topic: D.06. Vision

Support: 2011CB707501

81100669

81470656

S2012010008874

Title: Lycium barbarum polysaccharides delay the functional decay of retinal ganglion cells during photoreceptors degeneration

Authors: *F. LIU, J. ZHANG, Z. XIANG, D. XU, Y. XU;
GHM Inst. of CNS Regeneration, Jinan Univ., Guangzhou City, China

Abstract: Aims: To investigate whether lycium barbarum polysaccharides (LBPs) rescue the morphology and function of retina in rd1 mouse, a fast photoreceptor degenerated animal model, and how LBP affect the function of retinal ganglion cells (RGCs) during photoreceptors degeneration. **Methods:**LBP or PBS was i.p. injected in rd1 mouse everyday from P4 to P14, P20, P24 or P28 when photoreceptors totally degenerate. DAPI staining, electroretinogram, visual behavior and multi-electrode-array (MEA) recording were applied to check the structure and function of retina after treatment. **Results:** Our study showed that LBP treatment preserved the photoreceptors, enhanced the electroretinogram responses and improved the visual behavior significantly in rd1 mice compared to the non-treated group. For the function of RGCs, LBP treatment significantly enhanced the percentage of light-responsive RGCs and increased both the average and the peak responses during photoreceptors degeneration till P24. The spontaneous spiking, which usually increases during retinal degeneration, was overall inhibited by LBP; while the changes of the light-evoked responses and spontaneous spiking of various types RGCs in treated rd1 mice varied at different stages. Intensity profile tested with a series of increasing flash intensities also showed improved light sensitivity in most RGCs after LBP treatment. **Conclusion:** LBP recue the retinal morphology and function in rd1 mice, which is consistent with the reported results on rd10 mice, a slow photoreceptor degenerated animal model. Furthermore, LBP delay the functional decay of retinal ganglion cells during photoreceptor degeneration, which therefore, may help to elongate the effective time window for treatment of retinal degenerative diseases such as retinitis pigmentosa. **Keyword:**Retinal degenerative diseases; Retinal Ganglion Cells; Multi-electrode arrays; Lycium barbarum polysaccharides

Disclosures: F. Liu: None. J. Zhang: None. Z. Xiang: None. D. Xu: None. Y. Xu: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.21/KK15

Topic: D.06. Vision

Support: NIH Grant EY023441

NIH Grant EY021855

Title: Temporal facilitation of retinal ganglion cell responses to drifting gratings

Authors: *A. R. GOEL[1,2], D. KERSCHENSTEINER[2,3,4,5];
[1]Dept. of Biol., Washington Univ. In St. Louis, Saint Louis, MO; [2]Dept. of Ophthalmology and Visual Sci., [3]Dept. of Anat. and Neurobio., [4]Dept. of Biomed. Engin., [5]Hope Ctr. for Neurolog. Disorders, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Visual perception in mammals is modulated by past visual experience. A recent study identified pattern-specific flash facilitation in neurons in the primary visual cortex (V1). In this temporal facilitation, prior experience of a visual stimulus enhances subsequent responses, particularly to stimuli that share key features. This interaction appears to be conserved from rodents to human both physiologically and behaviorally. While the feedforward pathway of visual information to the V1 is responsible for hierarchical processing of features of the visual world, such as motion detection, many of these features are encoded by neurons as upstream as retinal ganglion cells (RGCs). The exact point along the visual processing pathway at which this facilitation phenomenon arises is unclear. In the present experiment, we test to see if the mechanism behind the observed priming effect based on stimulus features originates in the retina. We presented visual stimuli to an *ex vivo* retina and analyzed how RGCs respond to different combinations of stimuli to determine whether or not they use recent previous visual inputs to enhance their response to a new input. We created a stimulus set in which we displayed brief flashes of a solitary grating with no subsequent stimulus, drifting gratings with no subsequent stimulus, and a pair of a solitary grating followed by a drifting grating stimulus in either the same or a different orientation. Using multi-electrode array (MEA) technology, we recorded simultaneously from individual ganglion cells to see if the population response kinetics implicate early processing giving rise to this facilitation effect in the specific case of grating orientation. In addition, we characterized RGCs by typical response characteristics such as response polarity and direction selectivity to determine how the presence and strength of the temporal facilitation varied among these subpopulations of RGCs. While we know the retinal circuitry is capable of isolating salient features of visual stimuli, our experiment seeks to determine whether or not it compares the respective features to those of a preceding stimulus.

Disclosures: A.R. Goel: None. D. Kerschensteiner: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.22/KK16

Topic: D.06. Vision

Support: FP7 EC grant agreement no 600847

Newcastle University, Faculty of Medical Sciences

Title: The effect of retinal GABA depletion on mouse retinal ganglion cell receptive fields

Authors: *E. SERNAGOR[1], R. B. M. AMIN[2], S. SOFTLEY[2], G. HILGEN[2];
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Abstract: GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the vertebrate retina. It is metabolized by glutamic acid decarboxylase (GAD) which exists in two isoforms in the mature CNS, GAD65 and GAD67. Allylglycine, a glycine derivative, is the precursor to 2-keto-4-pentanoic acid, a non-specific inhibitor of both GAD isoforms. Exposure to allylglycine therefore results in GABA synthesis inhibition, leading to the depletion of endogenous GABA over time (Orlowski *et al*, 1977; Chabrol et al., 2012). Here we applied Allylglycine *in vitro* over several hours to gradually deplete GABA in the adult mouse retina. We used immunohistochemistry to monitor the depletion of endogenous retinal GABA. A high-density large-scale MEA (Biocam, 3Brain) featuring 4096 electrodes (42 μm spacing) with an active area of 7.12 mm^2 was used to study light-evoked responses from hundreds to thousands of individual retinal ganglion cells (RGCs) simultaneously at pan- retinal level. We used a set of stimuli that allowed us to compare RGC receptive field organization, contrast sensitivity and direction selectivity. We compared the effects of GABA depletion on RGC receptive fields with those obtained by simultaneously blocking all GABAergic receptors (type A, B and C). Immunohistochemistry revealed that GABA was depleted from the retina after 6-8 hours of incubation in allylglycine, except for residual expression in inner retinal neurons, most likely reflecting starburst amacrine cells. Longer incubation times with allylglycine did not have any further effect on GABA expression. MEA recordings revealed that although allylglycine had several similar effects on RGC receptive fields as $GABA_{A+B+c}$ receptors blockade, there were nevertheless also some striking differences. Indeed, the overall RGC firing rate, loss of direction selectivity and the non-homogeneity of receptive fields is much more pronounced in GABA-

depleted retinas than during $GABA_{A+B+C}$ receptor blockade. In summary, pharmacological depletion of endogenous retinal GABA reveals profound changes in receptive field properties and suggest that in addition to activating its three classical types of receptors, GABA may have some other trophic roles that influence how RGCs respond to light.

Disclosures: E. Sernagor: None. R.B.M. Amin: None. S. Softley: None. G. Hilgen: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.23/KK17

Topic: D.06. Vision

Support: NEI grant R01EY024567

Title: Genetic disruption of amacrine cell spacing alters the tuning of direction selective retinal ganglion cells

Authors: *S. ROY, J. CAFARO, J. N. KAY, G. D. FIELD;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Genes play major roles in establishing the locations and connectivity of neurons throughout the brain. Determining the consequences of altering these genetic programs is critical to understanding their role in shaping brain function. To gain insight into this problem, we leverage the recent discovery that a single gene, Megf10, is necessary for the regular spacing of starburst amacrine cells (SACs). Constitutive deletion of Megf10 causes SACs to be randomly spaced, introducing gaps and gluts in dendritic coverage across the retina. The purpose of this study is to determine the functional consequences of altering the spatial arrangement of SACs. SACs largely determine the tuning of direction selective retinal ganglion cells (DS-RGCs) by providing GABAergic inhibition to stimuli moving in the null direction. We tested for altered function among DS-RGCs by recording from the retinas of wild-type (WT) and Megf10 knockout (KO) mice using a multielectrode array (MEA). The MEA consisted of 512 electrodes with 30 μm pitch, allowing for simultaneous recording from 300-500 RGCs. Spikes were identified and sorted with custom software. Drifting gratings were used to identify ds-RGCs. Moving bars of different contrasts (10 to 100%) were used to measure the prefered direction, tuning width, and response sensitivity of ON and ON-OFF DS-RGCs. DS-RGCs were observed in Megf10 KO animals with a frequency similar to WT, indicating that the randomization of SAC locations did not eliminate direction selectivity. However, DS-RGCs from Megf10 KOs exhibited broader tuning to low contrast moving bars than those from WT animals. Furthermore,

while the preferred direction of the ON and OFF responses were closely aligned in WTs, they exhibited greater scatter in KOs. This is consistent with the observation that the spacing of ON and OFF SACs are randomized independently in Megf10 KO retinas. These results demonstrate that the mosaic arrangement of SACs produces sensitive and highly tuned responses among ds-RGCs. Furthermore, the function of a single gene can tightly regulate neural development to tune a complex computation. Finally, these results suggest that regular spacing of neurons in the retina reduces noise in computations in addition to generating uniform sensitivity across space.

Disclosures: S. Roy: None. J. Cafaro: None. J.N. Kay: None. G.D. Field: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.24/KK18

Topic: D.06. Vision

Support: The Macular Society, UK

Title: Chromophore acts on the retina to restore the intrinsic pupillary light reflex to isolated rd12 mouse eyes

Authors: *A. A. VUGLER, A. LYNCH;
Inst. of Ophthalmology, London, United Kingdom

Abstract: Introduction: The intrinsic pupillary light reflex (iPLR) allows the constriction of the pupil independently of the brain in nocturnal rodents. In mice, this response sustains pupil constriction under daylight conditions and requires the photopigment melanopsin, which is expressed in the iris sphincter muscle (Xue et al 2011). Functional and anatomical data also suggests a direct contribution to the iPLR from the ciliary body / neural retina retina (Semo et al., 2014). Here we examine the iPLR in rd12 mice, which lack retinal function due to a mutation in the RPE65 gene.
Methods: The iPLR was examined in dark-adapted rd12 mice in the presence or absence of systemic chromophore supplementation with 9 cis retinal (1mg/mouse i.p.). After cervical dislocation and eye removal, a bright white light was used to elicit the iPLR in isolated eyes *in vitro*, with recordings lasting for 3 minutes (30S baseline, 60S light stimulus, 90S recovery). In some rd12 mice the anterior chamber of the eyes was removed by either cutting along the limbus to disrupt the ciliary marginal zone (CMZ) or 1 mm below the limbus to include the CMZ and neural retina.
Results: The iPLR response was severely compromised in rd12 mice but could be restored by

systemic administration of 9-cis-retinal, which increased the iPLR 5-fold, pushing the constriction to surpass that of wild types. Chromophore administration also reliably restored the iPLR in rd12 mice when the preparation included retina (cuts made below the limbus). Here there was significant constriction from 6 seconds post-illumination with no clear recovery phase. However, no restoration of iPLR was seen in 9-cis-retinal-treated rd12 mouse anterior chamber preparations that had received cuts along the limbus which disrupt CMZ.
Conclusion: Here we show that RPE65 is necessary for the development of the iPLR in mice and provide functional evidence that systemic chromophore supplementation acts within the retina to restore the iPLR, presumably via a retino-iridial pathway.

Disclosures: A.A. Vugler: None. A. Lynch: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.25/LL1

Topic: D.06. Vision

Support: NIH Grant EY013528

Title: Electrical coupling between ganglion cell photoreceptors regulates light sensitivity of the developing retina

Authors: *F. HOLME, M. FELLER;
Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

Abstract: Prior to the maturation of rod and cone photoreceptors, intrinsically photosensitive retinal ganglion cells (ipRGCs) in the mouse retina exhibit robust light responses. Using two-photon calcium imaging of ganglion cells, we found that the number of light responsive cells decreased in the presence of gap junction antagonists. This indicates that in the developing retina, electrical coupling between ipRGCs regulates the extent and amplitude of light responses. Additionally, disrupting type-1 dopamine receptor signaling increases the number of light responsive cells. We report on recent progress to determine whether this modulation of gap junction coupling alters photo-aversion, an early ipRGC driven behavior described in mouse pups (Johnson et al. 2010). Taken together, our results suggest that electrical coupling between ipRGCs regulates light sensitivity of the developing retina.

Disclosures: F. Holme: None. M. Feller: None.

Poster

240. Retina Ganglion Cells and Circuitry

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 240.26/LL2

Topic: E.05. Brain-Machine Interface

Support: NIH/NEI Grant R01 EY021818

NIH/NEI Grant R21 EY025056

Research grant from Qualcomm Institute

UCSD Calit2 Strategic Research Opportunity

UCSD Core Grant P30EY022589

CNPq Fellowship 233829/2014-8

Title: A portable steady-state visual evoked potential-based method for assessment of visual impairment in glaucoma

Authors: *M. NAKANISHI[1], T.-P. JUNG[1], Y.-T. WANG[1], Y.-Y. CHIEN[1], J. K. ZAO[2], A. DINIZ-FILHO[1], Z. WU[1], F. A. MEDEIROS[1];
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Abstract: Glaucoma is the leading cause of irreversible visual impairment. Functional losses in the disease have traditionally been evaluated by visual field testing with standard automated perimetry (SAP). However, SAP testing is limited by the subjectivity and large variability of patient responses, which hamper diagnosis and detection of progressive visual field losses. In addition, SAP testing is costly and lacks portability, making it unsuitable for at-home testing or for disease screening in remote locations. In recent studies, we have demonstrated the feasibility of using steady-state visual evoked potentials (SSVEPs) for objective assessment of glaucomatous visual deficits. We subsequently developed a portable head-mount AR/VR display (HMD) with noninvasive, wireless, dry electroencephalogram (EEG) sensors, which made it possible to measure SSVEP signals in response to different visual stimuli. This study shows the feasibility of the this portable device to evaluate visual field losses in glaucoma in comparison to SAP (10-2).
The study included 14 eyes of 8 glaucoma patients and 20 eyes of 10 age-matched control healthy subjects (mean age: 66.7±9.3 and 64.9±10.1, respectively; P=0.790). The visual stimuli consisted of a flashing light flickering at 9.2 Hz presented on a smartphone-based HMD display. In the experiment, the visual stimuli were presented for 1-minute in total as the subjects maintained fixation on a central red dot. 10 5-s long data epochs comprising 6-channels SSVEPs

were extracted from the recorded EEG data after band-pass filtering from 5 Hz to 50 Hz. Spatial filtering based on a canonical correlation analysis (CCA) was applied to the data epochs and discrete Fourier transform (DFT) was applied to the data to obtain the signal-to-noise ratio (SNR) of SSVEPs. All subjects were also tested with SAP and had measurements of retinal nerve fiber layer thickness acquired with spectral-domain optical coherence tomography (SDOCT).
The average SNR of SSVEP signals obtained by the portable device was significantly lower in glaucoma than in control eyes (9.92±4.63 vs. 22.95±13.10; P=0.001). SSVEP SNR values were significantly associated with the SAP mean deviation (r=0.462, P=0.006) and with SDOCT average RNFL thickness (r=0.539, P=0.001). The results showed that SSVEP signals acquired by a portable device were significantly related to structural and functional damage in glaucoma and hold promise as a method for diagnosing and detecting disease progression.

Disclosures: M. Nakanishi: None. T. Jung: None. Y. Wang: None. Y. Chien: None. J.K. Zao: None. A. Diniz-Filho: None. Z. Wu: None. F.A. Medeiros: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.01/LL3

Topic: D.06. Vision

Support: NIH Grant F32 EY025523

NIH Grant R01 EY011379

NIH Grant T32 NS007484

Title: Motion prediction in V1 is facilitated by cortico-cortical feedback.

Authors: *T. S. HARTMANN[1], S. G. LOMBER[2], R. T. BORN[1];
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Abstract: Our brains routinely predict the trajectories of moving objects in order to, for example, catch a thrown ball. While it is clear that the motor commands sent to the muscles compensate for neural delays and anticipate the future location of an object, it is not known where along the neural pathways from vision to action that these functions begin.
We asked whether V1 responses to drifting bars have a predictive component. To do this, we recorded neuronal activity with a multi-electrode array in V1 of fixating monkeys and compared

responses under two conditions: 1) a white bar (75% contrast) drifted smoothly across the receptive fields (drifting-bar condition) or 2) an identical bar flashed at random locations along the same motion trajectory (space-time receptive field, or STRF). By convolving the STRF with the representation of the drifting bar stimulus we produced a feedforward model prediction of each unit's response profile. If V1 neurons respond linearly (i.e. without a predictive component), the feedforward model prediction should be identical to the drifting-bar response; however, we found that it significantly *lagged* the drifting-bar response (median difference 14 ms, $p<<0.001$, Wilcoxon signed-rank test). A second model, which included a negative feedback component, did a much better job of predicting the drifting-bar response profiles than the feedforward model (median improvement 11 ms, $p<<0.001$, Wilcoxon signed-rank test).
To gain insight into the underlying neural mechanism, we inactivated feedback by cooling areas V2 and V3, and repeated the drifting-bar experiment before, during inactivation, and after recovery. The average drifting bar response in V1 when V2/V3 feedback was inactivated significantly *lagged* the drifting bar response during the control periods ($p<<0.001$, Wilcoxon signed-rank test). The V1 response in the absence of feedback more closely resembled the response predicted by the purely feedforward model. Taken together these findings highlight an important role of feedback in shaping the temporal response profiles of V1 neurons.

Disclosures: T.S. Hartmann: None. S.G. Lomber: None. R.T. Born: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.02/LL4

Topic: D.06. Vision

Support: NIH Grant EY11747

NIH Grant EY016454

Title: Primate V1 population responses to local properties of natural backgrounds predict behavioral target detection thresholds

Authors: *S. C.-Y. CHEN, Y. BAI, S. SEBASTIAN, Y. CHEN, W. S. GEISLER, III, E. SEIDEMANN;
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Abstract: A central goal of systems neuroscience is to understand the neural mechanisms that mediate behavioral performance in natural, day-to-day, perceptual tasks. A fundamental natural

task is detection of targets in natural backgrounds. The goals of the current study are to examine how local properties of natural visual scenes affect neural representations in the primate primary visual cortex (V1), and to determine the possible contributions of these neural effects to behavioral detection sensitivity. To study the neural and behavioral masking effects of local background features, we cataloged and binned a variety of natural scene patches by their level of luminance (L), contrast (C) and similarity (S) to the detection target. We find that the masking effects of these scene properties in natural backgrounds on the neural responses in V1 are highly correlated with their behavioral masking effects. A rhesus monkey was trained to detect a 0.84 deg, 4 cpd additive Gabor target on a 3 deg natural scene patch at 2.5-3 deg eccentricity. While the monkey performed the task, voltage-sensitive-dye (VSD) optical imaging captured the neural population response from the corresponding location in V1. Thirty seven VSD detection experiments were conducted in 7 selected LCS bins (each repeated at least 7 times). The matched templates for the Gabor target at the retinotopic and orientation column scales were obtained in the same experiments. From these, we constructed neural response decoders in order to estimate the retinotopic- and columnar-scale neural response thresholds. Behavioral and neural target detection thresholds measured on natural scene backgrounds were significantly higher than those obtained on uniform backgrounds (background contrast = 0). The monkey's behavioral thresholds increased with background luminance, background contrast and target-background similarity. These trends are consistent with previously reported human psychophysical thresholds assessed with the same LCS natural scene binning paradigm (Sebastian et al., JoV 15:761, 2015). The monkey's neural thresholds on natural scene backgrounds were higher than the behavioral thresholds. Nonetheless, the behavioral and neural threshold values were highly correlated across the LCS bins, at both the retinotopic and columnar scales. The strong correlation between neural and behavior thresholds suggests that behavioral detectability of a visual target is largely determined by neural masking at, or prior to, V1.

Disclosures: S.C. Chen: None. Y. Bai: None. S. Sebastian: None. Y. Chen: None. W.S. Geisler: None. E. Seidemann: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.03/LL5

Topic: D.06. Vision

Support: Intramural Program of NIMH

Title: Mean luminance responsive cells in Primary Visual Cortex of Rhesus macaque

Authors: *S. CHANDRA, R. FALCONE, B. J. RICHMOND;
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Abstract: The cells in Primary Visual Cortex (V1) are known to be responsive to contrast. In their classic work, Hubel and Wiesel investigated V1 cells with bars, and Movshon et al used gratings as stimuli. The responses are thought to be dependent on changes in contrast across space, such in edges of bars or gratings. There are some studies of transient response to abrupt changes in mean luminance.
While recording from parafoveal V1 using an array of chronic electrodes (Utah array), we have found cells whose firing rate depends on the mean luminance even after adaptation. We present gratings at 4 orientations, and two phases, at a single mean luminance for several hundred trials, followed by another set of similar gratings but with different mean luminance for next several hundred trials, and so on. One of the stimuli is blank, that is, no stimulus comes on. We do all of these stimuli at four different mean luminances (1,3,10 and 50 cd/m^2) and then repeat the first mean luminance again at the end. In examining the activity for the blank stimulus, we find neurons with firing rate dependent on the mean luminance even after the excluding the transients. About half of the observed neurons show a significant variation in firing rate with change in mean-luminance. Out of these mean-luminance responsive neurons, approximately 60 per cent show an increase in firing rate with increasing luminance, another third show a decrease while for the remaining neurons the effect is idiosyncratic. There seem to be two populations of neurons, those with high background (17 Hz or greater) and those with low background (below 8 Hz). The high background neurons might be from layer 4.

Disclosures: S. Chandra: None. R. Falcone: None. B.J. Richmond: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.04/LL6

Topic: D.06. Vision

Support: NIH grant EY022928

NIH grant P30EY008098

NIH Grant EY05864

NIH grant EY15958

NIH grant EY02017

Title: Altered functional circuitry in the primary visual cortex of amblyopic macaque monkeys

Authors: ***K. CLEMENS**[1,2], L. KIORPES[3], J. A. MOVSHON[3], M. A. SMITH[2,4,5];
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Abstract: Amblyopia is a developmental disorder of spatial vision. In amblyopia, early, imbalanced binocular inputs during the critical period for visual plasticity result in weakened inputs to neurons driven by one eye, and reduce their spatial resolution and contrast sensitivity. Single-unit studies in amblyopic monkeys have documented altered responses in primary visual cortex (V1) and extrastriate visual cortex, but these effects do not completely account for the behavioral deficits observed in the same animals. Given that cortical circuits in V1 are the first to integrate information from the two eyes, abnormalities in the functional circuitry of amblyopic cortex may help explain deficits in spatial vision in amblyopia. Here, we examined the correlation structure between pairs of neurons in V1 of amblyopic monkeys in order to investigate amblyopia's effect on the functional structure of cortex. We recorded from V1 of three anesthetized, paralyzed adult *Macaca nemestrina* using 100-electrode arrays. The animals had surgically-induced strabismus in infancy, which resulted in deep amblyopia. We collected control data from visually-normal animals. The arrays were implanted 0.6 mm into a region of V1 representing the parafovea, yielding superficial layer recordings. We presented identical sinusoidal grating stimuli of fixed, moderate spatial and temporal frequency separately to each eye; on different presentations the gratings drifted in one of twelve directions with one of three contrast levels (1, 0.5, 0.12). From each electrode, we recorded all responses that exceeded a threshold and sorted these waveforms offline. We analyzed data only from well-isolated single neurons from a total of seven array implants in the amblyopic monkeys. High contrast stimuli presented to the amblyopic eye evoked lower firing rates and higher correlated trial-to-trial variability (spike count correlation) compared to the fellow eye. Lowering stimulus contrast in the fellow eye resulted in a gradual increase in correlation, consistent with our previous results. In the amblyopic eye, however, lowering stimulus contrast produced only a small increase in correlation. In other words, the differential correlation between the amblyopic and fellow eye grew with increasing contrast. These results suggest that a stimulus presented to the amblyopic eye is processed as if of low contrast, irrespective of its actual contrast. We found no evidence for such an asymmetry between the eyes in normal controls. These findings suggest that the abnormalities in amblyopic vision may in part be explained by changes in V1 functional circuitry.

Disclosures: K. Clemens: None. L. Kiorpes: None. J.A. Movshon: None. M.A. Smith: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.05/LL7

Topic: D.06. Vision

Support: R01 EY006821

Title: Synaptic organization of ON and OFF inputs within the dendritic field of individual layer 2/3 neurons in tree shrew primary visual cortex

Authors: *K.-S. LEE[1,2], D. E. WILSON[1,2], D. FITZPATRICK[1];
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Abstract: Each pyramidal neuron in visual cortex receives excitatory synaptic inputs from thousands of presynaptic neurons. Establishing the functional properties and the organization of these inputs at the level of single neurons is essential for understanding cortical circuits. Recent published work from our lab shows that simple cells in tree shrew visual cortex orderly integrate the ON and OFF inputs from separate regions in the visual space to construct a smooth representation of orientation and spatial phase. However, the spatial arrangement of ON and OFF inputs in the dendritic field of individual neurons that is responsible for simple cell receptive field (RF) properties remains unknown. In this study, we sparsely labeled neurons in visual cortex with GCaMP6s calcium indicator, and applied *in vivo* two-photon imaging to measure the receptive fields, and the ON and OFF visuotopic organization of individual dendritic spines from single pyramidal neurons. Consistent with previous studies, the aggregate RF structure of the synaptic inputs reliably predicted the somatic RF structure for individual neurons. An ON & OFF overlap index, defined by the proportion of spine RFs overlapping with the somatic RF (same – opposite polarity), was computed to quantify the functional specificity of the synaptic inputs. We found high agreement between the ON and OFF organization of the synaptic inputs and the somatic response (mean overlap index 0.36). Surprisingly, when we calculated a spatial phase-based overlap index (comparing synaptic input to the optimal spatial phase of the somatic RF), the relationship of synaptic inputs to the somatic response was even stronger (mean overlap index 0.51), indicating that synaptic inputs display high specificity in spatial phase. We noted considerable diversity in the spatial properties of the ON and OFF response fields of nearby spines. However, by comparing the ON and OFF inputs potentially contributing to somatic RF (ON & OFF overlap index > 0.5), the distribution of OFF inputs was biased to the proximal dendritic field while the distribution of ON inputs was broader and centered at more distal locations in the dendritic field. This observation supports the recent finding that OFF inputs in the primary visual cortex anchor the fine map of visual space and ON inputs exhibit orientation specific visuotopic displacement consistent with the map of orientation preference.

Disclosures: K. Lee: None. D.E. Wilson: None. D. Fitzpatrick: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.06/LL8

Topic: D.06. Vision

Title: An optogenetic study of feedback circuitry in macaque visual cortex

Authors: *S. S. SOLOMON, A. ASCHNER, A. KOHN;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Feedback pathways, which project from higher to lower cortex, make up roughly half the inter-areal connections in the cerebral cortex. Feedback has been proposed to mediate critical functions that include providing information about perceptual and cognitive context, relaying learned information and implementing attention. Although implicated in such diverse and important functions, the physiological properties of feedback circuits remain poorly understood. Prior studies in macaque visual cortex have shown that disrupting feedback modulates how spatial context affects single neuron responses. While important, this work has two important limitations. First, feedback was silenced with coarse manipulations, such as lesions and cooling. Second, the effects of disrupting feedback were only evaluated in single neurons, whereas feedback could function to alter network coordination. To address these limitations, we used an optogenetic approach to evaluate how feedback arising in area V2 influences neuronal populations recorded in primary visual cortex (V1) of an anesthetized macaque monkey. We injected AAV1 viral vectors (hSyn promoter) encoding channelrhodopsin and eYFP in V2, near its border with V1. After 11 weeks, we targeted a small fiber optic into V2 and were able to modulate V2 neuronal responses with laser light. Histology confirmed robust and widespread expression in V2 and V1, particularly in the deep layers. We measured the effect of V2 stimulation on V1 spontaneous activity and responses evoked by drifting gratings of different orientations, sizes, and contrasts. V2 stimulation modulated the firing rates of about half the neurons in V1; most of these neurons showed increased firing rates, but some were inhibited. The effect of V2 stimulation was evident nearly immediately after laser onset, but V1 responses grew slowly over time and persisted ~0.25 s after laser offset. Firing rates of V1 neurons were least modulated by V2 stimulation when driven by gratings of optimal size. While firing rates were only modulated in a subset of V1 neurons, nearly all V1 neurons showed a decrease in response variability during V2 stimulation. Finally, V2 stimulation caused a substantial and widespread decrease in V1 pairwise ‘noise correlations’. Decorrelation was evident even in V1

pairs whose firing rates were not modulated by V2 stimulation. We conclude that local recruitment of feedback signals can induce robust changes in firing of lower cortex, including substantial changes in network coordination.

Disclosures: S.S. Solomon: None. A. Aschner: None. A. Kohn: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.07/MM1

Topic: D.06. Vision

Title: Stimulus dependent laminar microcircuit interactions in primate V1

Authors: *M. A. GIESELMANN, A. THIELE;
Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Changing stimulus sizes has profound effects on neuronal firing rates and oscillatory activity in primary visual cortex. Stimuli that extend beyond the classical receptive field generally result in suppression of neuronal firing rates and increased gamma oscillatory power. RF center responses are assumed to be generated predominantly by feedforward inputs from the thalamus into the thalamorecipient layers, which is then further processed in the superficial layers (2/3), while suppression from the far surround in V1 is believed to be mediated by feedback from higher visual areas, which predominantly terminates in layer 1 and layer 5. We used 16-channel laminar electrodes, inserted perpendicular into V1, to analyse the flow of information across V1 cortical layers when different sized square wave grating stimuli were presented in the neuronal receptive fields. 24 recordings were obtained (17 in monkey, 7 in monkey 2). Recording channels were aligned in depth to the channel showing an early (40-50 ms) sink in the current-source density analysis (CSD) of the visual potential evoked by the stimulus onset, likely representing the thalamic input into layer 4c. Analysis was done based on locally bipolar LFP referenced signals. Activity in the gamma frequency power showed the most profound changes with stimulus size across cortical layers, even if differences existed in terms of its size dependent modulation across layers (Gieselmann et al., SFN 2014). To determine functional connectivity between cortical laminae we computed the non-conditional and conditional granger-causality-indices (GCI) between the bipolar LFP signals recorded from all pairs of electrode contacts for the gamma frequency band (30-55 Hz) interactions. Two connections showed high causality indices while being positively modulated by the stimulus size at the same time. The upper infra-granular (IG) channels granger caused gamma in lower IG channels (non-cond. GCI small: 0.06 large: 0.38), which in turn granger caused gamma in lower

supra-granular (SG) channels (non-cond. GCI small: 0.07 large: 0.33). These results were confirmed by gamma phase-triggered averaging analysis of individual channels/layers. The gamma oscillations in the lower IG layers lagged the upper IG layers by 4.5 ms and preceded the lower SG layers by 6.1 ms at 44.4 Hz. These results suggest that gamma oscillations induced by surround suppression are transmitted to upper IG layers by feedback connections (or at least are generated by upper IG layers), and the flow of gamma entrainment then entails lower IG, followed by SG layers.

Disclosures: M.A. Gieselmann: None. A. Thiele: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.08/MM2

Topic: D.06. Vision

Support: 5T32 EY007135

Title: Structural evolution of high-acuity circuitry in the primate primary visual cortex

Authors: *D. J. MILLER, J. H. KAAS;
Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Discovering the cellular composition of neurobiological tissues informs our understanding of brain evolution, and is a first step towards generating the computational models necessary to probe sensory system function. In the mammalian visual system, information from the retina is routed through the thalamus and on to the cerebral cortex. In particular, previous work has shown that the neurons in the lateral geniculate nucleus (LGN) of the thalamus that support high-acuity vision predominantly project to neurons in layer four (Layer 4A, 4B of Hassler, 1967; or Brodmann's 4C alpha, beta) of the primary visual cortical field (V1) (Casagrande & Kaas, 1994). Comparative anatomical studies of the primate visual system suggest differences between diurnal and nocturnal species, although clear evolutionary trends are difficult to establish. In this project, we investigated the evolution of visual acuity circuitry in primates by measuring the volume and counting the number of cells in layer 4 relative to the rest of the cortical mantle in V1. Our sample includes humans, chimpanzees, baboons, macaques, squirrel monkeys, owl monkeys, tamarins, marmosets, tarsiers and galagos. Our preliminary data show that V1 in diurnal catarrhine primates contains fewer cells per unit volume than in diurnal platyrrhines. In addition, V1 in nocturnal species exhibits fewer cells per unit volume than in closely related diurnal taxa. Our volumetric comparisons indicate an expansion of layer 4 in

haplorrhine relative to non-haplorrhine taxa. However, these data also show an expansion of layer 4 in the nocturnal owl monkey relative to diurnal platyrrhines and nocturnal prosimians. Together, these observations provide evidence of major evolutionary shifts in the structure of the primate visual system that are correlated with changes in behavioral foraging ecology. Furthermore, our analysis suggests that specific functional pathways, such as circuits serving high-acuity vision, may drive structural changes in the opposite direction of larger phylogenetic trends.

Disclosures: D.J. Miller: None. J.H. Kaas: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.09/MM3

Topic: D.06. Vision

Support: Research grant from the Whitehall Foundation

Career starter grant from the Knights Templar Eye Foundation

National Science Foundation Graduate Research Fellowship DGE-0909667

NEI Training Grant 2T32 EY007135-21

Title: Spatiotemporal profile of dichoptic cross-orientation suppression across the layers of primate V1

Authors: ***M. A. COX**, K. DOUGHERTY, A. MAIER;
Vanderbilt Univ., Nashville, TN

Abstract: Dichoptic cross-orientation suppression (dCOS)—a core mechanism in models of binocular rivalry and amblyopia—is defined as the reduction in a neuron's response to an optimally oriented stimulus in the neuron's preferred eye when an orthogonally oriented stimulus appears in the other eye. Determining when and where dCOS is instantiated in the primate visual stream is critical for a comprehensive understanding of binocular vision, yet these questions remain unresolved. Anatomically, V1's main input layer (L4) is the primary cortical site where signals from the two eyes converge onto single neurons. Consistent with this structural layout, virtually all V1 neurons are sensitive to both eyes, even though stimulation of one eye often evokes stronger responses compared to stimulation of the other eye. Neurons downstream of V1 are largely invariant to ocularity, and the vast majority of LGN neurons upstream of V1 are

sensitive to only one eye and indifferent to orientation. Taken together, these functional and anatomical findings render V1's L4 a prime candidate for the instantiation of dCOS. This study aimed to test this hypothesis. Using intracranial linear microelectrode arrays, we recorded spiking responses across the layers of V1 in two macaque monkeys. L4 was defined functionally using current-source density (CSD) analysis of visually evoked potentials. Stimulus conditions included monocular gratings, matching binocular gratings (dioptic), and orthogonally oriented dichoptic gratings intended to evoke dCOS. In order to quantify the stimulus drive required for dCOS, we parametrically varied stimulus contrast and stimulus onset asynchrony for all stimulus conditions. We found that most V1 neurons respond less to binocular stimulation than to preferred eye stimulation alone. In order to localize dCOS within V1's layers, CSD for dichoptic stimuli was compared to dioptic stimuli. We found that dioptic non-preferred stimuli elicited greater net depolarization than dichoptic stimuli within L4, suggesting that dCOS cannot be explained by a decrease in excitatory drive. Taken together, these findings support the hypothesis that orientation-specific interocular suppression first emerges within V1's retinogeniculate input layer.

Disclosures: M.A. Cox: None. K. Dougherty: None. A. Maier: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.10/MM4

Topic: D.06. Vision

Support: NIH Grant 2T32MH096331-06

Title: The origins of spatial frequency tuning in macaque visual cortex

Authors: *P. LEVY, C. ZIEMBA, J. MOVSHON, E. SIMONCELLI, R. GORIS;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Neurons in primary visual cortex (V1) are selective for the spatial frequency of image features. This selectivity first arises in the retina, is modified by the LGN, and is further sculpted by cortical mechanisms. To identify the contributions of these elements, we measured responses of V1 neurons in anesthetized macaques to mixtures of sinusoidal gratings dispersed in spatial frequency. For a fixed total contrast, we varied the frequency dispersion and studied selectivity for the modal frequency of the mixtures. Many neurons exhibited contrast-dependent frequency tuning: At low contrast, neurons had narrower octave bandwidths and preferred lower spatial frequencies. These contrast dependencies were more pronounced for responses to more dispersed

stimuli.
To explain these results, we developed a cascaded model of cortical computation. In the first stage of the model, visual stimuli are processed in parallel by two linear-nonlinear channels, each containing the half-wave rectified responses of center-surround filters whose properties are based on those of parvocellular and magnocellular LGN cells that project to cortex. In the second stage, these channel responses are combined linearly, scaled by a contrast gain control, and passed through a nonlinearity to obtain a firing rate. The model simulates cortical responses to mixture stimuli well, and suggests that contrast-dependent spatial frequency tuning might emerge from combinations of inputs with different contrast sensitivity and spatial frequency selectivity.

Disclosures: P. Levy: None. C. Ziemba: None. J. Movshon: None. E. Simoncelli: None. R. Goris: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.11/MM5

Topic: D.06. Vision

Support: NSC 101-2321-B-002-078

NSC 102-2321-B-002-059

MOST 103-2321-B-002-028

MOST 104-2320-B-002-065-MY3

Title: Contextual effects of spatial receptive fields in macaque monkey V1

Authors: *H.-Y. WU[1], C.-P. LIN[2], C.-I. YEH[2,3,4];
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Abstract: Using natural stimuli to study receptive field properties has become an important branch of visual research. In contrast with artificial stimuli that have simple and well characterized properties, natural stimuli have very complex dynamics. Some studies use both natural and artificial stimuli to study V1 properties (Ringach et al 2002; Sharpee et al 2006), but a systematic investigation of the contextual effect of receptive fields with both stimulus types has not been accomplished. It therefore remains unclear whether receptive fields measured with

natural and artificial stimuli are comparable. To address this question, we measured receptive fields with both the natural stimulus (a movie recorded with a camera mounted on the head of a cat walking in the wood, Einhäuser et al 2002) and the artificial stimulus (a binary white noise generated by m-sequence, Reid et al 1997). In addition, we investigated the context effect by changing the parameters of the natural movie: the orientation (a 90-degree rotation) and the spatiotemporal correlation (a randomized frame sequence). Natural-scene receptive fields were calculated by regularized pseudoinverse reverse correlation to deconvolve with the natural scene (Smyth et al 2003), and white-noise maps were calculated by reverse correlation. A multi-electrode matrix (8x8 array, 200 um spacing, Neuronexus) was used to simultaneously record from multiple neurons in different layers of macaque V1. Here we report some of the main findings. First, the average receptive field sizes measured with natural and artificial stimuli are comparable. Second, a stimulus-dependent change in the preferred orientation is found in some V1 neurons -- the preferred orientations can be very different (more than 45 degree) between the natural-scene map and the white-noise map. Third, the signal-to-noise ratio of the natural-scene map tends to be higher than that of the white-noise map and the random-natural-scene map (no significant difference in the average firing rate under these conditions). Overall, these results indicate that the primary visual cortex is highly adaptive and dynamic: V1 neuron's receptive field may change accordingly with the statistics of the visual scene.

Disclosures: H. Wu: None. **C. Lin:** None. **C. Yeh:** None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.12/MM6

Topic: D.06. Vision

Support: CNPQ

Capes

Faperj

Title: Response properties in cytochrome oxidase modules of capuchin monkey visual area V2.

Authors: *R. PERES, J. GM SOARES, B. LIMA, M. FIORANI, M. CHIORRI, M. FLORENTINO, R. GATTASS;
UFRJ, Rio DE Janeiro, Brazil

Abstract: Cortical columns are considered a basic building block of cortical organization. Within these structures, nearby neurons encode similar stimulus features, such as selectivity for orientation, direction or color contrast. The second visual area (V2) of primates exhibits a compartmental organization based on stripes that run along its surface. These compartments can be revealed using histochemical staining for the mitochondrial enzyme cytochrome-c oxidase (CO), and are comprised of CO-rich (thin and thick stripes) and CO-poor (inter-stripe) bands. Notably, it has been hypothesized that these bands have different neuronal properties, and participate in functionally distinct streams of visual information processing. However, there is still controversy whether the different V2 compartments indeed correlate with specialized neuronal response properties.
In this study, we use multiple-electrode arrays (16-32 tungsten microelectrodes per array, ~ 250 μm inter-electrode spacing) to record spiking activity along the dorsal-ventral extent of area V2 in the anesthetized and paralyzed capuchin monkey (Sapajus apella formerly Cebus apella). As visual stimulus, we presented moving bars and gratings with different contrasts, orientations, directions of motion, spatial frequency, velocity and color contrast. Receptive field position was mapped for each recording site. The individual V2 stripes were identified by means of CO histochemistry.
We recorded from 1080 V2 sites in 6 hemispheres of 3 monkeys. Overall, our preliminary results show that neurons recorded from nearby electrodes share similar functional response properties. Specifically, we found a correlation between orientation selectivity, as measured by circular variance, and electrode position relative to the distinct V2 stripes. Sites with stronger orientation selectivity were more frequently located on thick and inter-stripe bands in V2, as compared to those on thin stripes. We also observed that the responses for neurons on thick stripes showed a significantly shorter latency compared to neurons located on the thin stripes. We are currently studying the neuronal responses to the other stimulus features and correlating them to V2 modular organization.

Disclosures: R. Peres: None. J. GM Soares: None. B. Lima: None. M. Fiorani: None. M. Chiorri: None. M. Florentino: None. R. Gattass: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.13/MM7

Topic: D.06. Vision

Support: Max Planck Society

Title: Tracking orientation map and neural response dynamics during discrimination and perceptual learning in tree shrew V1 with chronic calcium imaging

Authors: *J. W. SCHUMACHER, T. WALKER, V. K. HOKE, S. FRELING, R. J. CORLEW, D. FITZPATRICK;
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Abstract: Neurons in primary sensory regions of neocortex encode stimulus features that are important for representing the external world. In some sensory circuits, neuronal feature preference is mapped in a highly organized fashion. Orientation preference is mapped across the surface of primary visual cortex (V1) of highly visual mammals, but whether these structured orientation maps contribute to fine orientation discriminations in visually guided behavior is unclear. To assess how orientation maps are engaged during active behavior, we first trained adult tree shrews (*Tupaia belangeri*) to perform a simple go/no go discrimination task. Licking in response to a grating stimulus at a target orientation yielded liquid rewards, while licking for a distractor orientation caused a timeout. We found that tree shrews typically learn the easiest form of their task within two to three weeks. Psychometric analysis shows that unrestrained tree shrews easily discriminate targets from distractors at an offset of 25 degrees, and can have discrimination thresholds as low as 10 degrees. Shrews that expertly learned an easy form of the task were imaged using 2 photon microscopy with the genetically encoded calcium indicator GCaMP6s in V1 layer 2/3. Neurons in layer 2/3 of tree shrew V1 represent the earliest stage of cortical processing to encode orientation. To assess if this stage of visual processing was sensitive to behavioral context we tracked the activity of relatively large populations of neurons during visually guided behavior across several days. Following each behavioral imaging session, we presented the shrews with oriented gratings for passive viewing to assess how well the neural population could discriminate orientations in the absence task-related attention. In some cases, neurons exhibited task-related response modulation, with somatic fluorescence magnitudes differing for the active discrimination and passive viewing contexts. When headfixed animals achieved a criterion level of discrimination performance ($d' > 1.5$), we increased the difficulty of the task by replacing the no go grating with a more similar orientation to the target. Increased task difficulty was accompanied by a decrease in behavioral performance and increased response latencies. To determine whether the layer 2/3 orientation map is the target of learning-related plasticity, we tracked the neural population during the subsequent perceptual learning of the more difficult task. This allowed us to ask whether the learning of fine orientation discriminations improves neural discrimination performance, and where in the orientation map the corresponding changes in neural responses occurred.

Disclosures: J.W. Schumacher: None. T. Walker: None. V.K. Hoke: None. S. Freling: None. R.J. Corlew: None. D. Fitzpatrick: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.14/MM8

Topic: D.06. Vision

Support: NIH Grant EY11379

NIH Grant EY12196

Title: Emergence of structured neuronal correlations during the learning of a perceptual discrimination task

Authors: *C. GOMEZ-LABERGE[1], R. M. HAEFNER[2], R. T. BORN[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Univ. of Rochester, Rochester, NY

Abstract: The senses are our gateway to the world, yet the information they provide is insufficient to unequivocally determine what is "out there." How then are competing interpretations of sensory input resolved to form a stable percept? This problem of inferring the state of the world from limited sensory information is mathematically ill-posed and could be resolved by combining incoming sensory input with prior information about the possible states. We hypothesize that structured correlated activity in the cerebral cortex is a manifestation of this prior information, encoded by top-down inputs that prime neuronal populations whose stimulus preferences are most compatible with one of the expected states. Studies have demonstrated decision-related activity in the visual cortex of animal subjects trained to discriminate noisy visual stimuli, which is consistent with an underlying correlation structure that could be encoding the task-related alternatives. To test this hypothesis, we implanted a chronic multi-electrode array to record from single cells and multi-unit clusters in the primary visual cortex of a rhesus monkey, while it was trained to perform a coarse orientation discrimination of bandpass-filtered noise images. We monitored behavioral performance and neuronal activity over daily sessions, spanning approximately one month, during which the subject first performed the task at a chance level and gradually approached psychophysical threshold. We found that the average spike-count correlation coefficient was small (daily mean 0.03, s.d. 0.08) and was comparable in magnitude throughout the study. As predicted by theory, correlation structure reflecting task contingencies emerged over the course of training. In later sessions when the monkey was performing at a stable threshold, the mean correlation between pairs of units with preferred orientations corresponding to the *same* contingency were larger than in pairs with preferences corresponding to *opposite* contingencies. And this correlation structure was absent in early sessions when the animal was performing at or near chance. Prior information about expected perceptual states may be reflected in the correlation structure of populations of sensory cortical neurons. Because

perceptual expectations can change rapidly with context, this structure may be under the control of top-down input such as feedback from higher sensory cortical areas.

Disclosures: C. Gomez-Laberge: None. R.M. Haefner: None. R.T. Born: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.15/MM9

Topic: D.06. Vision

Title: Comparing the time course of adaptation in V1 neurons with perception.

Authors: *P. L. APARICIO, B. G. CUMMING;
Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: Adaptation, or changes in response due to stimulus history, have been observed in both the responses of neurons and perceptual behavior. In the visual cortex, adaptation occurs on several time scales, which complicates direct quantitative comparisons between the neural and psychophysical effects of adaptation. Here we examine the temporal structure of adaptation recorded from neurons in the striate cortex of two monkeys with the timecourse of psychophysical adaptation using identical adaptation protocols in both a human and monkey subject. Subjects were presented with 3s trials, consisting of a constant disparity (the adaptor) followed by a sequence of correlated random dot stereograms (RDS). Psychophysical subjects reported whether the sequence of RDS appeared far or near. To ensure that the psychophysical effects could not be caused by subjects' awareness of the adapting stimulus, we used an anticorrelated random dot stereogram (aRDS) to induce adaptation because aRDS of opposite disparity are indistinguishable, yet produce adaptation in V1 neurons that depends upon the sign of the adapting disparity. Our protocol produced changes in the reported depth of RDS in a direction that matched the changes in rate observed in adapted V1 neurons. To quantify the timecourse of adaptation, we pooled data across blocks according to the trial position within a block. This generated a neuronal tuning curve for the first trial after a switch in adapting disparity, and separate curves for each subsequent trial position. Each of these was compared with the mean response for all data using a type II regression. We used the regression slope to quantify adaptation magnitude. Adaptation was clearly evident in the first trial of a block, but strengthened over 2-3 trials. The data were well described by an exponential function with a timecourse of 0.54 trials (mean of data from 16 neurons). Psychophysical data was similarly grouped and fit with cumulative Gaussian functions to the response data for each trial within a block. We characterized psychophysical adaptation as the difference in the fitted mean between

trials adapted with near and far aRDS. The extent of adaptation at each trial in a block was fit with an exponential function. The fitted time-constant (0.7 trials) was not significantly different from that observed for the neuronal data, suggesting that the perceptual effects of adaptation in our disparity task can be explained by the effects of the adaptor on neurons at the one of the earliest stages of the visual representation.

Disclosures: P.L. Aparicio: None. B.G. Cumming: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.16/MM10

Topic: D.06. Vision

Support: NIH Grant MH 93567

Title: Cortical areas with low dopamine content represent prospective regions for voltammetric detection of norepinephrine *In vivo*

Authors: *N. J. WARD, A. A. DISNEY;
Psychology Dept., Vanderbilt Univ., Nashville, TN

Abstract: Selectivity is an important consideration when detecting catecholamines in neural tissue. Structural similarities between dopamine and norepinephrine result in electrochemical signatures that are challenging to distinguish from one another. One solution is to accept a catecholamine signal that represents contributions of both dopamine and norepinephrine, but this does not allow for differentiation of the individual signals. Another solution is to conduct studies in brain regions with privileged innervation or content, such that signal measured can be attributed to one catecholamine rather than another. Prior studies involving subsecond detection of catecholamines in awake, behaving non-human primates have involved measurement of dopamine in the striatum. In the present study, we sought to identify candidate brain regions in non-human primate where norepinephrine can be detected using subsecond voltammetric recordings. We used high performance liquid chromatography with electrochemical detection on unfixed, frozen tissue samples from macaques to identify prospective neocortical regions for norepinephrine voltammetry. These results indicate that primary visual cortex (V1) and potentially primary somatosensory cortex exhibit low enough levels of dopamine that would allow for voltammetric detection of norepinephrine. Our V1 results support previous studies that have indicated low contribution of dopaminergic axons to the catecholaminergic innervation of

V1. Altogether, these results indicate promising cortical regions for subsecond detection of norepinephrine in awake, behaving macaques.

Disclosures: N.J. Ward: None. A.A. Disney: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.17/MM11

Topic: D.06. Vision

Support: National Basic Research Program of China (2014CB846101)

National Natural Science Foundation of China (91432102, 31125014)

111 Project (B07008)

Title: Interactions between feedback and lateral connections in the primary visual cortex

Authors: H. LIANG[1], X. GONG[1], M. CHEN[2], Y. YAN[2], C. D. GILBERT[3], *W. LI[2]; [1]Sch. of Biomed. Engin., Drexel Univ., Philadelphia, PA; [2]State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China; [3]The Rockefeller Univ., New York, NY

Abstract: Grouping of visual contours to form coherent percept has been thought to be mediated in part by long-range horizontal connectivity intrinsic to the primary visual cortex (V1), with a contribution by top-down feedback projections. In a recent study (Chen et al., Neuron 82:682-694, 2014), by simultaneous recording with microelectrode arrays implanted in monkey V1 and V4, we have shown that information about global contours within a complex background emerges initially in V4 and then rapidly builds up in both cortical areas. Bidirectional inter-areal interactions not only facilitate V1 neurons encoding the contour elements but also suppress those responding to the background. Since the onset of contour-related signals in V1 is much delayed relative to that in V4, an unsolved important question is whether the contour signals in V1 are derived from feedback inputs alone, or whether they are mediated by an intimate interaction between feedback and horizontal connections. In the current study we applied conditional Granger causality analysis of spike-train data to dissect the contributions of intra- and inter-areal connections. Our results showed that discounting the influences from V4 markedly reduced V1 lateral interactions, indicating dependence on feedback signals of the effective connectivity within V1. On the other hand, the feedback influences were reciprocally dependent on V1 lateral interactions, as the modulation strengths from V4 to V1 were greatly reduced after discounting the influences from other V1 neurons. By separating the data from trials in which the monkeys

made correct or erroneous choices in the contour detection task, we found that both the strengths of V4 to V1 feedback modulation and V1 intrinsic lateral interactions correlated with behavioral performance. Our findings suggest that feedback and lateral connections closely interact to mediate image grouping and segmentation.

Disclosures: H. Liang: None. X. Gong: None. M. Chen: None. Y. Yan: None. C.D. Gilbert: None. W. Li: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.18/MM12

Topic: D.06. Vision

Support: NEI Grant 1R01EY016715

Title: Coordinated neuronal activity reflects correct perceptual reports

Authors: *N. SHAHIDI[1], M. HU[2], A. R. ANDREI[3], V. DRAGOI[3];
[1]Univ. of Texas, Med. Sch. At Houston, Houston, TX; [2]MIT, Cambridge, MA; [3]Univ. of Texas, Med. Sch. at Houston, Houston, TX

Abstract: Visual perception is an active process of extracting relevant information for decision making. The degree to which spiking activity reflects perceptual choices vary widely across visual areas. We recorded simultaneously from small populations of neurons in V1 and V4 areas of monkeys while animals were engaged in a delayed match-to-sample orientation discrimination task. To our surprise, firing rates of single neurons and pairwise spike-time correlations were independent of behavioral choices. However, in area V4, but not V1, we found that coordination across three or more neurons was able to predict whether the monkey correctly detected the change in stimulus orientation (Figure 1). These results demonstrate that neuronal ensembles in visual cortex carry behaviorally relevant information that individual neurons or pairs do not. We also examined the spike time coordination between V1 and V4 by analyzing multiple neurons simultaneously in both areas. Interestingly, we found a significant inter-areal coordination when V4 lags V1 by 30 ms only when the animal was able to detect the orientation change correctly. This indicates that effective feed-forward connectivity plays a crucial role for perceptual discrimination.

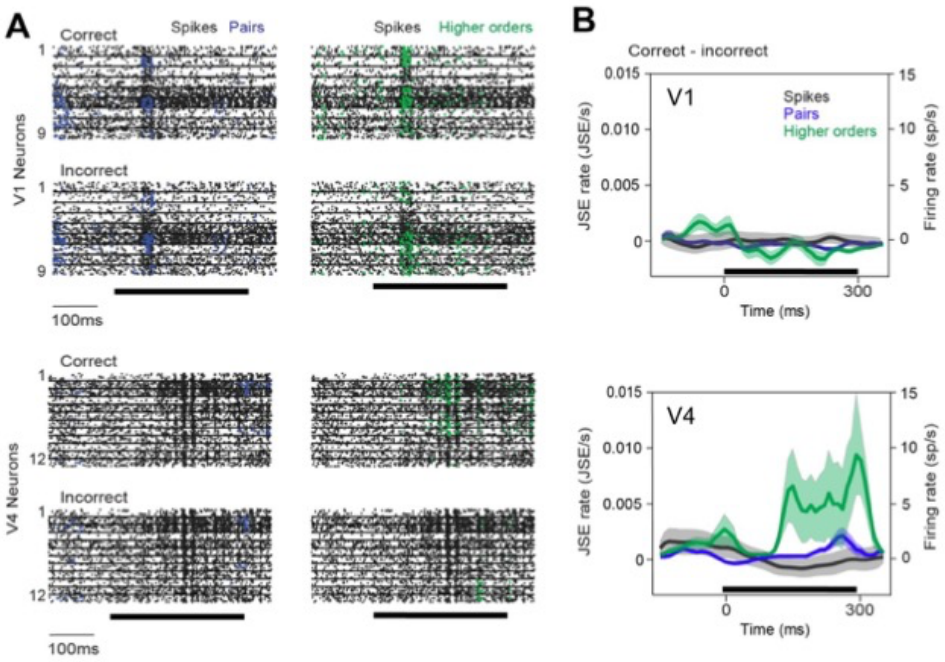

Figure 1| In V4, coordinated spikes occur more often in correct trials. (A) Raster plots of 9 cells in V1 and 12 cells in V4 for correct and incorrect trials overlapped with combinations of 2 (blue) or more (green) coordinated spikes (time bin of 5 ms). For better representation, the same number of random trials were selected from correct and incorrect set and only patterns for which the frequency of occurrences are significantly different between correct and incorrect sets (Wilcoxon rank sum, $p<0.01$) are shown. The horizontal lines show the presentation time of the test stimulus. **(B)** The frequencies of occurrences of coordinated spikes (JSE rates) were calculated for a 300ms window sliding with steps of 10ms (blue and green). Firing rates of all neurons were also calculated in the same time window and the difference between correct and incorrect firing rates were averaged across all neurons (gray).

Disclosures: N. Shahidi: None. M. Hu: None. A.R. Andrei: None. V. Dragoi: None.

Poster

241. Primate Visual Cortex

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 241.19/NN1

Topic: D.06. Vision

Support: NIH Grant EY016774

Title: The effects of adaptation on normalization signals within the receptive field

Authors: *A. ASCHNER[1], S. G. SOLOMON[2], M. S. LANDY[3], D. J. HEEGER[3], A. KOHN[1]; [1]Dominick P Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Dept. of Exptl. Psychology, Inst. for Behavioral Neurosci., London, United Kingdom; [3]Dept. of Psychology & Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Adaptation alters the responsivity of most sensory neurons. Although we have a rich description of adaptation effects in single neurons, we lack a conceptual framework that integrates this phenomenology and can predict how a neuron will adapt to different stimuli. We and others have proposed that explaining adaptation effects through altered normalization signals may provide such a framework. Normalization is a 'canonical' computation whereby a neuron's responses are suppressed by the pooled activity of other neurons (normalization pool). Previous work has shown that adaptation can weaken normalization from the receptive field (RF) surround, which can explain some facilitory effects of adaptation. Here we ask how adaptation alters normalization within the RF.

We performed extracellular recordings in primary visual cortex (V1) of anesthetized monkeys. We measured responses to plaid stimuli composed of two orthogonal, sinusoidal gratings of varying contrast, before and after adaptation with two high contrast gratings presented asynchronously ('component' adaptation). The strength of normalization was estimated by (1) fitting a standard descriptive model to the full set of plaid responses; and (2) comparing the area under the contrast-response function for the preferred grating alone and when paired with a mask. Both metrics revealed that normalization was strongly weakened after component adaptation.

We next tested whether adaptation can strengthen normalization, needed in this framework to explain the suppressive effects of adaptation. Recent theoretical work proposes that normalization is strengthened when a neuron and its normalization pool are consistently co-activated. Thus, we adapted neurons with two gratings presented synchronously ('contingent' adaptation). Using the same test stimuli and metrics, we found normalization was only slightly weakened by contingent adaptation. Further, when we controlled for changes in response magnitude, we found that signal summation was more sublinear after adaptation than before, consistent with a stronger interaction between a neuron and its normalization pool.

Our results indicate that normalization within the RF is shaped by adaptation. As a result, the diverse effects of adaptation may be explained in part by the manner in which a particular adapter and test stimulus recruit and alter those normalization signals.

Disclosures: A. Aschner: None. S.G. Solomon: None. M.S. Landy: None. D.J. Heeger: None. A. Kohn: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.01/NN2

Topic: D.06. Vision

Support: JPB fellowship

Title: Neurogranin gates experience dependent synaptic pruning and maturation in visual cortex

Authors: *K. HAN, S. COOKE, M. F. BEAR, W. XU;
MIT, Cambridge, MA

Abstract: Modification of synaptic connections by sensory experience during post-natal development is essential for functional maturation of cortical circuits. Here we show that neurogranin (Ng), a postsynaptic calmodulin (CaM)-binding protein in cortical principal neurons, regulates experience-dependent synapse maturation and synapse pruning in the primary visual cortex. In Ng KO mice, primary visual cortex exhibits a dramatically impaired response to visual stimuli and experience-dependent synaptic potentiation within primary visual cortex, known as stimulus-selective response potentiation (SRP), is also deficient. Additionally, loss of Ng in primary visual cortex impairs visually guided behavior, consistent with a profound impairment in cortical circuitry. At the synaptic level, Ng is required for experience-dependent maturation of AMPAR-silent synapses, stabilization of AMPAR-transmitting synapses, and regulation of spine loss. Knockdown of Ng in primary visual cortex during the commonly defined postnatal critical period leads to a significant decrease in spine density, AMPARergic synapse numbers and an abnormal retention of AMPAR-silent synapses. These functional deficits can be prevented by dark exposure during the molecular manipulation, suggesting that Ng levels gate experience-dependent modification of glutamatergic synaptic transmission in visual cortex. Decrease of Ng levels lowered threshold for LTD expression, providing a potential cellular substrate for experience-dependent damping of AMPAR-transmitting synapses. Taken together, our studies show that neurogranin levels control experience-dependent synapse

stabilization and synapse pruning for functional optimization of the cortical circuit during critical in primary visual cortex.

Disclosures: **K. Han:** A. Employment/Salary (full or part-time): MIT. **S. Cooke:** None. **M.F. Bear:** None. **W. Xu:** None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.02/NN3

Topic: D.06. Vision

Support: NIH Grant EY023871

Title: Network coding strategies underlying perceptual learning in primary visual cortex

Authors: ***N. D. OLIVAS**, L. O. JIMENEZ, D. L. RINGACH, J. T. TRACHTENBERG; Neurobio., UCLA, Los Angeles, CA

Abstract: We imaged large ensembles of pyramidal (PYR) neurons in L2/3 primary visual cortex (V1) of alert mice using resonant scanning 2-photon excitation of neurons expressing GCaMP6. Responses were recorded over 30 minute epochs as mice viewed continuous natural scenes taken from various BBC Nature episodes followed by 50 identical repeats of a 6-second long natural scene. Locomotion and pupil diameter were measured throughout the experiments. We find that cortical processing of naturalistic scenes is represented by sparse network level coding. Network sparseness is achieved as a consequence of the selectivity of V1 neurons for specific scenes. Sparse coding is modulated by the behavioral state where network sparseness is diminished during periods of locomotive activity. Whether network spareness is computationally advantageous for perception is under active investigation.

Disclosures: N.D. Olivas: None. L.O. Jimenez: None. D.L. Ringach: None. J.T. Trachtenberg: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.03/NN4

Topic: D.06. Vision

Support: PF

IUAP

FWO Flanders

HBP

Title: Visual perceptual learning and visual cortical plasticity in monkeys caused by VTA microstimulation in the absence of attention.

Authors: *J. T. ARSENAULT[1,2], W. VANDUFFEL[1,2,3];
[1]K.U. Leuven, Leuven, Belgium; [2]Mass. Gen. Hosp., Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA; [3]Dept. of Radiology, Harvard Med. Sch., Boston, MA

Abstract: Visual perceptual learning (VPL) is influenced by the interaction of attention and reinforcement. Interestingly, VPL can occur in absence of stimulus-directed attention (Seitz et al., 2009). Such task-irrelevant perceptual learning (TIPL) has been hypothesized to result from the temporal coincidence of a stimulus and neuromodulatory signals triggered by reinforcement. Consequently, TIPL offers an opportunity to isolate the role of neuromodulatory signals in the regulation of VPL and plasticity while excluding the role of attention. Although several neuromodulatory centers may be involved in VPL, the ventral tegmental area (VTA) is a good candidate because its neurons are activated by reinforcement, have widespread connections and release dopamine that facilitates plasticity. Therefore, we microstimulated monkey VTA (VTA-EM) to determine its causal role in VPL and cortical plasticity.
In Exp 1 we looked for plasticity. To do this we performed fMRI mapping (pre-association) to determine the response to 4 different grating stimuli [(L/R visual field) x (45°/135°)]. During all phases, the animals performed a difficult, orthogonal color discrimination task (80% correct performance) during grating presentation to avoid grating-directed attention. Next came a cue-VTA-EM association phase in which all gratings were shown but one was temporally associated with VTA-EM (20 sessions). Again, the color task was used to control attention. Finally, post-association fMRI mapping was identical to phase 1. The analysis demonstrated representation-specific enhancements (post vs. pre) in fMRI activity for the stimulated grating orientation within the ‘trained’ visual field, especially pronounced in PIT. These changes were not found for gratings displayed in the ‘untrained’ visual field.

In Exp 2 we looked for VPL effects. Exp 2 also consisted of pre- and post-association phases bookending a cue-VTA-EM association phase (6 - 20 sessions). Within the pre- and post-association phases the animals discriminated between upward and downward random dot motion stimuli presented in the left or right visual field at 6 motion coherences (0 - 50%). The cue-VTA-EM association phase consisted of bilateral presentations of 0 and 2% motion stimuli with only one 2% coherence stimulus being associated with VTA-EM. The color task was again used to avoid directed attention to the motion stimuli. Enhanced performance for lower motion coherences (2 - 12%) were consistently observed in the test phase for the 'trained' visual field. These results provide causal evidence for the role of primate VTA in VPL and stimulus-specific plasticity in the absence of stimulus-oriented attention.

Disclosures: J.T. Arsenault: None. W. Vanduffel: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.04/NN5

Topic: D.06. Vision

Support: China NSF Grant 31571079

Title: Modulation of visual processing by projection from the orbitofrontal cortex to the primary visual cortex

Authors: *L. DECHEN, J. DENG, Y.-G. SUN, H. YAO;
Inst. of Neuroscience,CAS, Shanghai, China

Abstract: Information processing in the primary visual cortex (V1) can be modulated by long-range corticocortical projections (Zhang et al, Science, 2014). A recent study showed that the orbitofrontal cortex (OFC) is reciprocally connected with V1 in mice (Zingg et al, Cell, 2014). Although many studies have shown that the OFC plays an important role in learning, the functional role of the OFC projection to V1 remains unclear. Here, we examined how this projection modulates visual processing in V1 and impacts learning of visual discrimination in mice. Using *in vivo* extracellular recordings from V1, we found that optogenetic activation of the OFC fibers in V1 decreased the response amplitude of V1 neurons without changing the orientation selectivity. In acute brain slices, optogenetic activation of the OFC fibers in V1 produced EPSPs and monosynaptic EPSCs in a large fraction of somatostatin (SOM)-expressing neurons but rarely elicited responses in parvalbumin (PV)- or vasoactive intestinal peptide (VIP)-expressing neurons in V1. Our behavioral experiments showed that neurotoxic lesion or

optogenetic inactivation of the ventral OFC could improve the learning rate of visual discrimination in mice. We are currently investigating how the OFC-V1 projection modulates responses of V1 neurons in different layers and how inactivation of the OFC-V1 projection influences the learning of visual discrimination behavior. Since the OFC receives dopaminergic projections from the ventral tegmental area (VTA) (Berger et al, TINS, 1991), we are also examining the effect of activating the VTA fibers in OFC on V1 responses and visual behavior.

Disclosures: L. Dechen: None. J. Deng: None. Y. Sun: None. H. Yao: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.05/NN6

Topic: D.06. Vision

Title: Gamma-band synchronization in primate visual cortex can increase or decrease with stimulus repetition

Authors: *A. PETER[1], J. DOWDALL[1], L. KLEIN[2], J. KLON-LIPOK[2], K. KOUROUPAKI[1], M. SCHOELVINCK[1], J. SCHMIEDT[1], K. SHAPCOTT[1], M. SCHMID[1], W. SINGER[2], P. FRIES[1];
[1]Ernst Struengmann Inst. For Neurosci. In Cooperation With Max Planck Soci, Frankfurt Am Main, Germany; [2]Max Planck Inst. for Brain Res., Frankfurt, Germany

Abstract: Repeated encounters with visual stimuli often lead to reduced firing rate responses throughout the visual system. This phenomenon is known as adaptation or, especially in higher-order cortical areas, as ‘repetition suppression’. However, behavioral performance typically remains stable or improves with repeated encounters despite reduced visual responses. To explain this apparent discrepancy, it has recently been proposed that stimulus repetition results in a more efficient stimulus representation by means of increased neuronal synchronization (Gotts et al., 2012).
Brunet et al. (2014) indeed showed that over the course of several hundred repetitions of grating stimuli, there was an increase in V1 and V4 LFP gamma-band power and V1-V4 gamma-band coherence. In V4, MUA and putative interneurons showed firing rate decreases, yet increases in gamma synchronization. Putative pyramidal cells showed no firing rate change, yet changes in synchronization that were positively correlated with stimulus drive. These results suggest that repetition leads to a sharpening of the stimulus representation.
However, the study repeated a small fixed set of gratings many times, and it therefore remained unclear whether changes were specific to the repeated stimuli, or whether a new set of stimuli

would be affected by those changes. Moreover, the exclusive use of grating stimuli precluded generalization to more naturalistic stimuli. Here, we investigate the stimulus specificity of repetition effects by presenting pseudo-randomly repeating natural images. Monkeys were engaged in a change detection task on the presented objects (20 repetitions per image, ~2s presentation duration, with maximally 4 intervening stimuli between repetitions). Neural data were recorded from V1 and V4 in several rhesus monkeys. Visually induced MUA in both V1 and V4 showed repetition-related decreases. All natural stimuli induced gamma-band activity, ranging from few ten to few thousand percent power increases. Repetition could induce either decreases, increases or an initial decrease followed by an increase, depending on the particular stimulus, yet reliable across recording sessions. The interleaved and independent dynamics per stimulus demonstrate that repetition-related changes of gamma-band synchronization are specific to the respective stimulus. We provide preliminary evidence that repetition-related changes in V1 gamma are stimulus-location specific and therefore likely a local phenomenon. Ongoing recordings investigate the effects of massed repetitions. Collectively, these analyses demonstrate changes in stimulus representation on the timescale of seconds.

Disclosures: A. Peter: None. J. Dowdall: None. L. Klein: None. J. Klon-Lipok: None. K. Kouroupaki: None. M. Schoelvinck: None. J. Schmiedt: None. K. Shapcott: None. M. Schmid: None. W. Singer: None. P. Fries: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.06/NN7

Topic: D.06. Vision

Support: NSF

NIH

IARPA

Title: Effect of training on stimulus representation in V1 and V2 neuronal ensemble.

Authors: ***T. LEE**, G. HUANG, Y. ZHANG;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: We studied how familiarity due to repeated exposure to a class of patterns or object images could modify neural representation of V1 and V2 neurons at the population level. In this study, we exposed awake behaving monkeys to specific classes of patterns or specific classes of

objects seen in different views, and monitored the evolution of the representation of V1 and V2 neuronal population responses during this process. To monitor neuronal activities, we used semi-chronically implanted SC32 multi-electrode arrays with independently movable electrodes from Gray Matter Research which can track the same neurons over weeks. We found that (1), intense exposure to specific stimuli or specific class stimuli over days leads to a sharpening of the tuning curves of individual neurons, as well as an increase in the sparsity of the population responses to the trained stimuli; and (2) exposure training leads to a change the stimulus representation of the neuronal population for this class of stimuli, resulting in better stimulus discrimination. We compared these results with simulation results of a variety of neural models. Preliminary results suggest hierarchical models with predictive coding and sparse coding constraints can account for these observations.

Disclosures: T. Lee: None. G. Huang: None. Y. Zhang: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.07/NN8

Topic: D.06. Vision

Support: NIH

NSF

IARPA

Title: Contextual modulation is responsible for image familiarity effect in V1 and V2 neurons

Authors: ***G. HUANG**, S. RAMACHANDRAN, J. SAMONDS, T. LEE, C. R. OLSON;
Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Neurons in monkey inferotemporal cortex (IT) show truncated responses to images of complex objects that the monkey is highly familiar with ('familiar images'), compared to images the monkey encounters for the first time ('novel images') (Mruczek and Sheinberg (2007)). Ramachandran, Lee and Olson (2014) have demonstrated that similar effects can be observed in neurons with much smaller receptive fields in earlier visual areas (V2 and V4) in the ventral stream. Do these neurons become familiar with the global images or the local receptive field features? This familiarity truncation effect could arise from feedback from the object recognition area (IT), or from recurrent horizontal connections within each early visual area, or arise from the modification of bottom-up feed-forward connections responsible for receptive field tuning

properties. To dissociate these causal factors, we familiarized the monkeys with a set of 25 images for up to 21 days, using a semi-chronic SC32 multi-electrode array (Gray Matter Research) to monitor V1 and V2 neurons' responses in awake behaving monkeys during this familiarization process. First, we found that the familiarity truncation of neural responses observed in V2 and V4 can also be observed in V1. More importantly, we found that the familiarity truncation effects observed in V2 neurons were greatly attenuated or eliminated when the familiar images were presented in a 3° aperture, which still covered all the receptive fields but eliminated much of the global context of the image. This second observation suggests that the familiarity truncation effects observed in V2 were likely not due to modification in the feed-forward circuits for processing receptive field stimuli, but due to changes in recurrent circuits for mediating contextual modulation. Given the latency of the truncation was earlier in V2 and V4 neurons compared to the latency of the effect in IT neurons, our observations suggest that the familiarity truncation effect might be mediated by the horizontal connections within each visual area.

Disclosures: G. Huang: None. S. Ramachandran: None. J. Samonds: None. T. Lee: None. C.R. Olson: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.08/NN9

Topic: D.06. Vision

Support: NIH 2R01EY020851

Title: Single trial familiarity judgments are reflected in the IT population response

Authors: *T. MEYER, N. C. RUST;
Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Extensive exposure of images is known to markedly alter responses in macaque inferotemporal (IT) cortex. Typically, these changes are manifest as firing rate response reductions for familiar as compared to novel images. Given that familiarity memories are stored after single exposures, we wondered whether IT neurons reflect visual familiarity memories after just a single image viewing. Previous investigations were limited in two ways: (1) traditional (largely single-neuron) analyses relied on averaging responses across many trials, however a "novel" image cannot be repeated multiple times; and (2) prior experimental designs utilized passive viewing and thus could not explore the relationship between putative familiarity signals

and behavior. To address these issues, we applied single-trial population-based approaches to analyze neural responses in IT collected from monkeys performing a visual familiarity task. Monkeys were able to report whether individual images were “novel” (never seen before) or “familiar” (seen only once). For each recording session, we computed the cross-validated performance of a linear population read-out optimized to classify images presented as “novel” versus “familiar”. We found that on average, novel and familiar conditions were correctly classified on trials in which the monkey reported the correct answer, whereas novel and familiar conditions were significantly misclassified on trials when the monkey made errors. These results establish that behaviorally-relevant familiarity signals are reflected in IT following single image exposures.

Disclosures: T. Meyer: None. N.C. Rust: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.09/NN10

Topic: D.06. Vision

Title: A neural mechanism of retaining category information in prefrontal cortex

Authors: *Y. KASHIMORI;
Univ. Electro-Communications, Tokyo, Japan

Abstract: The ability to categorize objects is a fundamental function of visual recognition. In daily life, we can rapidly and effortlessly categorize huge objects. Much effort has been devoted to the studies of how category information is processed in the brain circuits. Freedman et al. trained monkeys to categorize the computer-generated images of dogs and cats and demonstrated the involvement of the interaction between inferior temporal (IT) cortex and prefrontal cortex (PFC) in the categorization task. Moreover, they showed that neurons in the IT were more sensitive to object features, whereas neurons in the PFC exhibited a larger sensitivity to object categories. However, it still remains unclear how working memory of category information is formed in the PFC and what a role the top-down from the PFC to IT plays in the categorization task. To address these issues, we develop a model of visual system which consists of a PFC and an IT network. Each network has two-dimensional array of neurons, and single neuron was modeled with the leaky-integrator model. According to the categorization task conducted by Freedman et al., three dog images and three cat ones were memorized in the IT network, and the category information of dog and cat was represented by the PFC network. Each object or category information was represented as a dynamical attractor in the respective networks. Using

the model, we show that PFC neurons with lower firing thresholds are dominantly responsible for the formation and retention of working memory whereas those with higher firing thresholds have high sensitivity to category information of dogs/cats images. The lower-threshold neurons stabilize the attractors of category information and are capable of retaining recent history of neuronal activity even after stimulus is turned off. We also show that top-down from PFC to IT enables PFC neurons to improve the categorization ability of the dogs/cats images that are hard to discriminate between dog and cat categories. The improvement arises from the mechanism by which top-down signal elevates membrane potential of IT neuron in subthreshold region and allows IT neurons to be sensitive to forthcoming stimulus. These results suggest that the difference in sensitivity of PFC neurons and the top-down from PFC to IT may be involved in efficient performance of categorization task.

Disclosures: Y. Kashimori: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.10/NN11

Topic: D.06. Vision

Support: NIH R01 EY022930 (MRC)

NIH P30 EY008098 (MRC)

Whitehall Foundation Grant (MRC)

Klingenstein-Simons Fellowship (MRC)

Simons Foundation (MRC)

Sloan Research Fellowship (MRC)

McKnight Scholar Award (MRC)

Title: Neuronal population changes underlying visual perceptual learning and attention

Authors: *A. M. NI, D. A. RUFF, J. J. ALBERTS, J. SYMMONDS, M. R. COHEN; Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Both visual attention and perceptual learning improve performance on visually guided tasks: attention allows observers to focus on particular parts of a crowded scene, and perceptual

learning improves perception of specific stimuli. But while these two processes can both improve perception, their effects differ in timescale: attention can fluctuate on the scale of hundreds of milliseconds, while perceptual learning improves performance over weeks to months of repeated practice. Attention is known to affect the extent to which trial-to-trial variability is shared between pairs of neurons in visual cortex (noise correlations), which may affect the amount of visual information that can be decoded from the population of neurons. However, the effects of learning on neuronal populations have not been as well studied, and the neuronal underpinnings of attention and learning have never been directly compared. To test the hypothesis that all processes that improve perception share a common neural mechanism, we simultaneously measured effects of attention and learning on behavior and populations of neurons in visual cortex. We implanted two rhesus monkeys with multi-electrode arrays in area V4. We then recorded neuronal activity while the monkeys learned to perform a change detection task that required that they switch spatial attention between two visual stimuli. We found that both attention and learning improved behavioral sensitivity and decreased noise correlations. We observed attention-related changes in performance and noise correlations within individual experimental sessions. The animals' behavioral sensitivity for both the attended and unattended stimulus increased slowly over many training sessions, and noise correlations in responses to both attended and unattended stimuli decreased at the same rate. These changes were location-specific: sensitivity worsened and correlations increased when we switched stimulus locations and then gradually changed during learning. The changes in noise correlation were also task-specific: noise correlations remained stable during a passive fixation task across the course of training. Additionally, these multi-neuron data sets allow us to differentiate whether these attention- and learning-related perceptual improvements reflect improved information encoding by the neuronal population or the animals' improved ability to optimally decode the available information. Similarities between the neural signatures of attention and learning support the hypothesis that all processes that improve perception have similar neural mechanisms.

Disclosures: A.M. Ni: None. D.A. Ruff: None. J.J. Alberts: None. J. Symmonds: None. M.R. Cohen: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.11/NN12

Topic: D.06. Vision

Support: NSF Grant IIS-1219252

NSF Grant SMA 1041755

Title: Central versus peripheral vision in scene recognition: a modeling exploration

Authors: *P. WANG[1], G. COTTRELL, 92093[2];
[1]Electrical and Computer Engin., UCSD, LA Jolla, CA; [2]Computer Sci. and Engin., UCSD, La Jolla, CA

Abstract: What are the roles of central vision and peripheral vision in human scene recognition? While brain imaging studies show that the scene recognition region (Parahippocampal Place Area; PPA) is associated with peripheral vision, a careful examination of the relative contribution for central versus peripheral vision is needed. In a behavioral study done by Larson and Loschky (2009), scenes were presented using the "Window" and "Scotoma" paradigm, in which only central or peripheral information is shown inside or outside a circular region. They showed that peripheral vision contributes more than central vision in obtaining the maximum scene recognition accuracy. However, central vision is more efficient for scene recognition than peripheral based on the amount of visual area needed for accurate recognition. In our study, we modeled and explained the results of Larson and Loschky (2009) by using a neurocomputational modeling approach.
We have four main findings. First, we showed for the first time that the advantage of peripheral vision in scene recognition can be replicated nicely using state-of-the-art deep neural network models. We trained different models (namely AlexNet, VGG, and GoogLeNet) on the ten scene categories used in the behavioral study, and all models demonstrated the peripheral advantage in scene recognition. All networks also modeled the efficiency of central vision. Second, we proposed and provided support for the hypothesis that the peripheral advantage comes from the inherent usefulness of peripheral information, which is consistent with the result of Thibaut et al. (2014), who showed that patients with central vision loss can still categorize natural scenes efficiently. Third, by using a deep mixture-of-experts model ("The Deep Model", or TDM) that receives central and peripheral visual information on separate channels simultaneously, we showed that the peripheral advantage emerges naturally in the learning process: The model “attends” to the peripheral pathway more than the central pathway. As we have seen in our previous modeling work, learning creates a transform that spreads different scene categories into different regions in representation space. Finally, we visualized the features for the two pathways, and found that the central and peripheral pathways complement each other rather than competing: different preferences for scene categories emerge for the two pathways during the training process.

Disclosures: P. Wang: None. G. Cottrell: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.12/NN13

Topic: D.06. Vision

Support: The Intramural Research Program at the National Institutes of Health, National Eye Institute

Title: Long-term high-capacity memories of object values in the primate brain - fMRI study on macaque monkeys

Authors: *A. GHAZIZADEH[1], W. GRIGGS[1], D. A. LEOPOLD[2], O. HIKOSAKA[1,3];
[1]LSR NIH, Bethesda, MD; [2]NIMH NIH, Bethesda, MD; [3]NIDA NIH, Baltimore, MD

Abstract: During our lifetime we are bombarded by a large number of visual stimuli that need to be memorized and discriminated based on their ecological relevance such as their reward value. The basal ganglia, especially their posterior part, play an important role in such visual discrimination (Hikosaka et al 2014). Beyond this basal ganglia circuitry, the neural mechanism involved in such discrimination is not well-known. We addressed this issue by doing whole brain functional magnetic resonance imaging (fMRI) in two rhesus monkeys. Before the scans, the monkeys repeatedly viewed many computer-generated fractals (n>80) which were consistently associated with a large reward (Good objects) or a small reward (Bad objects) for >10 days. During block design scans (>1 day after training), these objects were passively viewed in the periphery (left or right, 6deg) while the monkey kept central fixation with no contingent reward. Thus the differential coding of objects in passive viewing would represent acquired stable object values rather than immediate reward expectations. Scan results showed widespread object Goodness coding (Good > Bad) along the ventral bank of superior temporal sulcus (STS) particularly in areas V4, TEO, TEa as well as in orbitofrontal cortex (OFC) and lateral prefrontal cortex (LPFC). Weaker effects were also observed in early visual areas (V1-3) and lateral intraparietal area (LIP). Various subcortical areas such as basolateral amygdala, ventral claustrum also showed significant Goodness coding (cluster corrected, alpha<0.01). Concomitant with neuronal discrimination, we found strong behavioral discrimination outside the scanner measured as strong gaze bias toward Good objects during free viewing sessions. To test whether neural and behavioral discrimination is maintained in long-term memory, we retested the same objects after 8-12 months with no visual exposure (memory period). Importantly many of the same visual areas along the ventral bank of STS fully retained their Good object discrimination despite long memory interval. There was significant discrimination in areas such as LPFC but with reduced magnitude. Notably free viewing of objects following the memory period revealed persistent gaze bias toward Good objects despite absence of any encounter for many months. Our

results reveal a robust and large capacity neural mechanism for discriminating and orienting toward valuable objects. It is not known how such diverse brain areas acquire and maintain Goodness coding. One possibility is that Goodness coding arises in basal ganglia first which in turn train cortical areas. This hypothesis remains to be tested.

Disclosures: A. Ghazizadeh: None. W. Griggs: None. D.A. Leopold: None. O. Hikosaka: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.13/NN14

Topic: D.06. Vision

Support: ISF Israel Science Foundation

Title: Representing features and summary statistics

Authors: *S. HOCHSTEIN;
Hebrew Univ. Jerusalem, Jerusalem, Israel

Abstract: Intensive research has uncovered diverse dimensions of summary statistic perception, including simple and complex dimensions (from circle size and Gabor orientation to face emotion and attractiveness), the type of statistics acquired (mean, variance, range), and our ability to summarize elements presented simultaneously or sequentially and to divide displays into separate groups, detecting statistics of each. But how does the brain compute scene summary statistics without first attaining knowledge of each scene element? One possible solution is that the brain uses implicit individual element information to compute summary statistics, which become consciously accessible first. This added step is superfluous. On the contrary, direct acquisition of summary statistics is not surprising and no novel computational principle is required for summary perception. A simple population code representation, as found for single element parameters, may be scaled up to compute mean values for element groups. The range of active neurons is broader, but the computation is the same for sets of elements as for a single element. Using a population code adds tremendous power, as it allows direct determination of which elements to include in the set, which elements are outliers - to be excluded and trigger pop out attention - and how to divide between simultaneously presented sets. Population coding provides a direct and efficient representation of set summary statistics. As suggested by Reverse Hierarchy Theory, conscious perception may begin with summary statistics, seeing many similar elements as a group, and only later focus attention to individual elements. Interestingly, a similar

population code representation may underlie categorization, including both category prototype and its boundaries.

Disclosures: S. Hochstein: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.14/OO1

Topic: D.06. Vision

Support: NSFC Grant 81371631

NSFC Grant 81422024

NSFC 31500923

Title: Cooperative encoding of visuo-spatial categories in human posterior parietal cortex and primary visual cortex

Authors: *L. WANG[1], Y. LI[1], X. HU[2], K. ZHAO[1], Y. YU[2];
[1]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [2]First Affiliated Hosp. of Anhui Med. Univ., Hefei, China

Abstract: Categorization is a fundamental cognitive process by which the brain assigns meaning to incoming sensory stimuli in our environment. Human and/or non-human primate studies have found categorical representations of stimuli in higher-order lateral prefrontal, posterior parietal and lateral occipital cortex. Because higher-order areas lack visual specificity of early sensory areas, it has remained unclear how human can process fine-scale features that aid in refining categorization. One proposal is that coarse categories processing occurs first in a higher-order cortical area, whereas fine categories processing occurs later in early visual areas. Here, using multi-voxel pattern analysis (MVPA), we show that higher-order posterior parietal cortex (PPC) reflected coarse categories information; whereas primary visual cortex (V1) was involved in processing categories information at a finer resolution when humans perform visuo-spatial categories task. By analyzing time series in the two areas, we obtained evidence that categorical signal was selectively in a top-down direction from PPC to V1, and stronger influence from PPC to V1 was associated with better categorical performance. Moreover, the selectivity of PPC and V1 responses shifted markedly with retraining to group the same stimuli into two new categories. These findings suggest that both PPC and sensory cortex, which processes diagnostic features for each category, abstract experience-dependent categorical representations cooperatively.

Disclosures: **L. Wang:** None. **Y. Li:** None. **X. Hu:** None. **K. Zhao:** None. **Y. Yu:** None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.15/OO2

Topic: I.07. Data Analysis and Statistics

Support: DFG GA 730/3-1

Title: MVPA investigation of working memory maintenance period: Decoding memory content and retrieval success from EEG

Authors: ***S. ALIZADEH**[1,2], M. SCHÖNAUER[1], H. JAMALABADI[1,2], S. GAIS[1];
[1]Med. Psychology and Behavioral Neurobio., Univ. of Tuebingen, Tuebingen, Germany;
[2]IMPRS for Cognitive and Systems Neurosci., Tübingen, Germany

Abstract: Recent advances in multivariate pattern analysis (MVPA) methods have further improved our understanding of memory processes by decoding what kind of information is stored in memory representations. In particular, combining these methods with measures of brain oscillations that feature high temporal resolution (e.g. electroencephalogram (EEG) or magnetoencephalography (MEG)) can provide new insights about the underlying mechanisms of human memory function. Here, we employed an MVPA based decoding approach see if electrical brain activity during working memory maintenance period contains information about the content and subsequent performance of the working memory. To do this, 19 healthy subjects underwent high-density EEG recording in two sessions. Each subject performed two Sternberg tasks in each session, once pictures of faces and houses and once picture of digits and letters were presented. During each trial of the first task, a random sequence of 8 pictures of either faces or houses was presented to the subjects with the instruction to remember all pictures of the sequence. After a 4-s retention phase, one picture of either a face or house was presented and the subjects had to decide if the probe item was part of the previously viewed sequence. The same procedure was repeated in the second task with digits and letters stimuli.
We then used MVPA to test whether we can predict solely based on EEG during maintenance period (1) if a trial is subsequently remembered or not, and (2) what kind of stimulus is maintained during the delay period. We used a two-step channel-specific frequency-domain classifier based on an ensemble of linear support vector machines. This procedure aims at reducing dimensionality of data while simultaneously increasing signal-to-noise ratio which improves the classification performance. Our results show that the subsequent working memory performance can be reliably predicted across subjects for both kinds of weak (digits/letters) and

strong (faces/houses) stimuli. In addition, we can successfully decode the working memory content for the face/house stimuli. Classification accuracy was positively correlated with the subsequent memory performance of subjects, i.e. the more confident the classifier was in decoding the memory content, the higher the retrieval success rate of subjects. Finally, we adopted a searchlight approach to localize the spatiotemporal sites of information. Our data suggest that activity in frontal and parietal areas within the gamma, beta, and alpha frequency bands predicts the subsequent retrieval success while temporal alpha and beta contain information about the content of working memory.

Disclosures: S. Alizadeh: None. M. Schönauer: None. H. Jamalabadi: None. S. Gais: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.16/OO3

Topic: D.06. Vision

Title: Behavioral and neuronal outcome of generalization following visual aversive learning

Authors: L. SHALEV[1], R. PAZ[2], *G. AVIDAN[1];
[1]Dept. of Psychology, Ben-Gurion Univ. of the Negev, Beer Sheva, Israel; [2]Dept. of Neurobiology, Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The ability to generalize across similar stimuli and respond appropriately is a basic survival skill. Studies have shown that aversive learning (pairing of a neutral conditioned stimulus to an aversive stimulus) increases discrimination thresholds of the neutral stimuli (generalization). This finding was demonstrated under a number of aversive conditions such as pain, negative odors or sounds and monetary loss. A number of brain regions, including the amygdala, the insula, the anterior cingulate cortex (ACC), and the relevant sensory cortices, were suggested to underlie this effect. Our work is focused on extending this line of studies to the visual modality. We first tested participants in a behavioral setting, while they viewed images from the International Affective Picture System (IAPS) database with either aversive or neutral valance and were asked to learn to associate auditory or visual neutral stimuli with the appearance or absence of those images. Generalization for the conditioned stimuli was tested immediately prior to, and following learning. We found wider generalization curves for the neutral stimuli that predicted aversive visual outcomes. This effect was demonstrated with auditory (tone discrimination) as well as visual (contrast discrimination and discrimination of oriented stripes) neutral stimuli. Next, we set out to examine the neural basis underlying these behavioral changes. We conducted similar experiments to those described above using fMRI

during learning and found that activity in the amygdala, insula, the ACC, and the primary visual cortex (V1) was associated with aversive learning. These findings further strengthen the notion that generalization resulting from aversive learning is not modality specific, and suggest a global neuronal mechanism, which is utilized during the exposure to dangerous and emotionally arousing situations.

Disclosures: L. Shalev: None. R. Paz: None. G. Avidan: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.17/OO4

Topic: D.06. Vision

Support: China NSF

Title: Perceptual learning with minimized early cortical plasticity and neuron-specific response reweighting

Authors: ***C. YU**, X.-Y. XIE;
Peking Univ., Beijing, China

Abstract: Two types of mainstream theories explain visual perceptual learning (VPL): Early cortical neural plasticity (e.g., Karni & Sagi, 1991; Stoups et al., 2001) and neuron-specific response reweighting (e.g., Dosher & Lu, 1987; Law & Gold, 2008), as motivated by observed learning specificities. However, our double training studies (e.g., Xiao et al., 2008; Zhang et al., 2010; Wang et al., 2016) demonstrate that VPL often shows complete transfer to untrained locations, orientations/directions, and physical properties, indicating that VPL is primarily a rule-based high-level process operating at a conceptual level. Here we test a hypothesis that VPL requires neither early cortical neural plasticity nor neuron-specific response reweighting.
In a peripheral orientation-discrimination learning task, training took place with the Gabor stimulus either rotated in 12 locations (anti-clockwise) and 4 orientations 90 deg apart (clockwise), or in a random order. Each location/orientation combination received 2 trials per block of trials, 12 trials per 2-hr session, for a total of 60 trials over 5 daily sessions, which minimizes the possibility of training-induced early neural plasticity and neuron-specific response reweighting. A single staircase controlled the base vs. target orientation difference for all location/orientation conditions. The pre/post condition was never practiced. Training with rotating and random stimuli both produced significant orientation learning, indicating that the observers can learn orientation discrimination with multiple stimulus conditions that minimize

early neural plasticity and neuron-specific response reweighting. Learning transferred to the untrained pre/post condition, so that the improvement was comparable to that in a control group that only practiced the pre/post condition for an equal number of trials. Therefore, orientation learning with multiple stimuli is genuine and unspecific to the more difficult multiple stimulus task. Similar results were obtained in a motion-direction learning task with 6 rotating locations and 8 rotating directions 45-deg apart, which also showed no direction and location specificity. These results indicate that early cortical plasticity and neuron-specific input reweighting are unnecessary for VPL. Instead sensory inputs can be reweighted at a later stage in a neuron unspecific manner.

Disclosures: **C. Yu:** None. **X. Xie:** None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.18/OO5

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

Support: CIHR Grant to Robert Hess

Title: Investigating neural plasticity in the amblyopic brain with perceptual learning in a global motion discrimination task.

Authors: *Y. GAO, A. BALDWIN, R. HESS;
Ophthalmology, McGill Univ., Montreal, QC, Canada

Abstract: Neural plasticity after the critical period holds implications for the treatment of brain damages, amblyopia and learning difficulties. It is also involved in improving cognitive functions through physical exercises. Amblyopia is a neural developmental disorder caused by deprived visual input from one eye during the critical period of the visual system's development. It was widely believed that the effects of this deprivation are permanent unless corrected during the critical period. Recent work however has demonstrated plasticity in the brains of adult amblyopes, and raised the possibility of repairing visual functions.
Perceptual learning of simple tasks such as orientation and motion direction discrimination is a common method to study neural plasticity. Former studies found that perceptual learning can induce significant improvements in the impaired visual acuity, contrast sensitivity, stereo-acuity and binocular combination in amblyopic observers. Amblyopes show a deficit for the processing of global motion which, unlike those mentioned previously, also affects monocular perception in the eye that was not deprived during development (the "fellow" eye). Although learning for

global motion has been demonstrated for normals it has not been shown in human amblyopes. The current study investigates i) whether perceptual learning can improve global motion processing in amblyopic observers, and ii) whether the learning effects are specific to the trained eye.
Amblyopic observers and normal controls were trained on a motion direction discrimination two-interval forced choice task for 5 days. The stimuli were a field of isotropic log-Gabors (spatially band-pass "dots") with peak spatial frequencies of 3.8 c/deg and contrasts of 10 times or higher of the detection thresholds of each observer. The difficulty of the task was varied by modifying the offset angle. Amblyopes were split into two groups and trained either with their amblyopic or fellow eye.
Even though there was considerable individual variability, we found a learning effect in the trained eye and no transfer to the untrained eye in both amblyopic and normal observers. Specifically, we found that for the amblyopic observer who was trained with the fellow eye and for all the normal observers, training improves the performance of the trained eye in global motion processing while the performance in the untrained eye hardly changed. Yet for the amblyopic observers who were trained with the amblyopic eye, training induces much smaller improvement in the trained amblyopic eye and the untrained fellow eye was even worse-off. We conclude that amblyopes show less perceptual learning on this task.

Disclosures: **Y. Gao:** A. Employment/Salary (full or part-time): McGill University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; China-Canada Joint Health Research Initiative. **A. Baldwin:** None. **R. Hess:** None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.19/OO6

Topic: D.06. Vision

Support: NIH R01 EY023384

Title: The feature-weighted receptive field: an interpretable encoding model for complex feature spaces

Authors: *T. NASELARIS[1], G. ST-YVES[2], N. DESISTO[3];
[1]Neurosciences, [2]Med. Univ. of South Carolina, Charleston, SC; [3]Physics, Col. of Charleston, Charleston, SC

Abstract: Voxel-wise encoding models used in studies of the visual system can vary widely with respect to their interpretability and expressiveness. For example, the population receptive field (pRF) is a highly interpretable model because it has a small number of parameters that explicitly reveal the size and spatial location voxel-wise receptive fields. The pRF is a very effective tool for delineating area- and state-dependent variations in receptive field size; however, it's expressiveness is limited because it does not characterize variation in feature tuning. More expressive models include the Gabor wavelet model and encoding models based on deep convolutional neural networks. Such models are expressive because they can reveal variations in feature tuning even when the feature spaces are extremely complex; however, they typically contain thousands of parameters and so can be difficult to interpret.
We introduce the feature-weighted receptive field (fwRF), an encoding model designed to balance interpretability and expressiveness. The fwRF is organized around the notion of a feature map—an arbitrary transformation of visual stimuli into visual features that preserves the topology of visual space (but not necessarily the native resolution of the stimulus). According to the model, activity in each voxel encodes stimulus variation in a spatially localized region across multiple feature maps. This region is fixed for all feature maps; however, the contribution of each feature map to voxel activity is weighted. Thus, the model has two separable sets of parameters: "where" parameters that characterize the size and position of the receptive field, and "what" parameters that characterize tuning to visual features. This space/feature separability makes it possible to estimate explicit receptive field parameters without compromising model expressiveness. It also drastically reduces the number of adjustable parameters relative to models with non-separable space-feature terms (e.g., the Gabor wavelet model).
We describe an optimization algorithm for fitting the fwRF parameters, as well as a fast GPU-based implementation in Theano. We then demo the model in three use cases: population receptive field mapping; Gabor wavelet modeling; and modeling with a feature space supplied by a deep convolutional neural network. We show that the fwRF models recover consistent receptive field size and locations, as well as complex feature tuning. Our results suggest that the feature-weighted receptive field model will be particularly useful as a method for regressing the activities of deep neural networks onto measured brain activity.

Disclosures: T. Naselaris: None. G. St-Yves: None. N. DeSisto: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.20/OO7

Topic: D.06. Vision

Support: RO1 EY023384

Title: Imagery receptive fields

Authors: ***J. BREEDLOVE**[1], G. ST-YVES[1], T. NASELARIS[1], C. OLMAN[2];
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Abstract: Mental imagery is difficult to study due to the inevitable loss of signal that results from the absence of a visual stimulus. Most previous fMRI studies of mental imagery have taken a bootstrapping approach to the problem by applying a model derived from a perceptual experiment to activity measured during imagery. In the current study, we modelled imagery directly. We measured whole-brain BOLD activity as participants viewed and then imagined previously memorized objects at 8 distinct locations in the visual field. We then estimated for each voxel a perceptual receptive field model (pRF) and an imagery receptive field model (iRF) using the activity generated during perception and mental imagery, respectively. The fitting procedure assigned a receptive field size and center to each voxel, as well as a spatial frequency and orientation tuning profile.

We found that it is possible to accurately predict imagery activity using the iRF; it is also possible to decode the location and extent of imagined objects. These findings demonstrate the feasibility and potential power of the iRF for quantitatively characterizing mental imagery. Furthermore, our analysis revealed that the predictive accuracy of the iRF increased with ascent of the visual hierarchy: predictions were worst in early visual cortex, but by V3A on the dorsal side and LOC on the ventral side prediction accuracy of the iRF was on par with accuracy of the pRF.

Consistent with previous findings, pRFs cross-predicted activity during mental imagery, strengthening support for a conservation of spatial and feature tuning between mental imagery and perception. However, differences were also apparent. Because of the weak signal during imagery, the centers, sizes, and locations of iRF's were scattered relative to the pRFs. More interestingly, underneath this scatter we uncovered what appear to be some systematic differences in tuning: in many visual areas, iRFs appear to be larger in size, and show a shift toward smaller preferred spatial frequencies than the pRFs.

Our results suggest that during mental imagery, activity is propagated toward early visual areas from higher-level areas that have relatively large receptive fields and low spatial resolution, and in which patterns of activity during imagery are largely equivalent to patterns of activity during perception.

Disclosures: J. Breedlove: None. G. St-Yves: None. T. Naselaris: None. C. Olman: None.

Poster

242. Visual Learning, Memory, and Categorization

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 242.21/OO8

Topic: D.06. Vision

Support: Grant R01 EY023384 to T.N.

Title: A deep predictive coding model of mental imagery

Authors: *G. ST-YVES[1], T. NASELARIS[2];
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Abstract: There is solid empirical evidence that mental imagery (i.e. the conjuring of a visual percept in absence of external visual stimuli) uses the same cortical machinery as visual perception. The process of generating mental images is thus apparently very closely related to the perception of retinal ones. We present a predictive coding account of the relationship between mental imagery and perception in the form of a deep predictive coding network. Mental images are equated with predictive signals in the network that reconcile the multiple representations of a retinal image that arise at distinct stages of visual processing. Mental imagery is experienced most strongly when the retinal image is removed (e.g., when eyes are closed) and the strength of the predictive signal is increased relative to its strength during perception. A mental image of a specific visual scene is realized when activity at one stage of visual processing is clamped to the pattern induced during retinal perception of the same scene. We show that in our network, activity induced during mental imagery is invariably weaker than its corresponding activity during perception. Mental images are also inherently imprecise and unstable. The precision of mental imagery is constrained by the pooling structure of the network, while the stability is limited by the competition of the predictive feedback and the feed-forward propagation of information. These aspects of the model are consistent with known empirical results, and vindicate the commonly shared experience of imagery as a weak form of perception. However, we show that the strength of activity at any stage of in the network depends strongly upon the stage that is clamped during imagery, and that this dependence is largely asymmetric with respect to the clamped stage. Importantly, we show that a significant increase in the strength of predictive signaling during mental imagery can alter the receptive field properties of the network in an intuitive way that reflects the pooling structure of the network and the presence of feedback. Our model provides a normative theory of mental imagery by showing how it might arise as a natural consequence of predictive coding, and accounts for known empirical findings and intuitions about mental imagery while making new and directly testable predictions.

Disclosures: **G. St-Yves:** A. Employment/Salary (full or part-time): medical university of south carolina. **T. Naselaris:** A. Employment/Salary (full or part-time): medical university of south carolina.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.01/OO9

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC

FRQ-S

Title: Oscillatory activity in motor cortical regions is modulated by expected visual reafferent feedback

Authors: ***P.-M. BERNIER**[1], B. DUFOUR[2], F. THÉNAULT[2], K. WHITTINGSTALL[2]; [1]Dept. de Kinanthropologie, Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: The planning of voluntary movement involves predicting its associated sensory consequences. While the primary motor cortex plays a critical role in the specification of motor commands, recent fMRI work suggests it is also modulated by the expected visual consequences of an action (Eisenberg et al., 2011). Here electroencephalography was used to investigate oscillatory activity over motor and parietal regions during a visual-motor dissociation task. Participants planned and executed reaching movements toward a single visual target positioned straight-ahead of midline, with visual feedback of the hand provided via a cursor. Before every trial, they were precued as to the direction of the visual feedback, which could be either spatially congruent with the actual hand position, rotated leftward or rightward by 20º, or not provided at all. Results revealed that subjects produced similar movement kinematics across conditions, suggesting similar motor commands. EEG activity during planning revealed strong alpha-band (8-15 Hz) desynchronization at parietal electrodes contralateral to the expected visual feedback. In contrast, neither alpha- nor beta-band (15-30 Hz) activity at motor electrodes was modulated by the direction of the expected visual feedback. Interestingly, theta-band (3-7 Hz) activity at motor electrodes showed greater desynchronization in anticipation of visual feedback, irrespective of its direction, as compared to no visual feedback. These results demonstrate that while oscillatory activity in motor regions is sensitive to the upcoming presence or absence of visual feedback (i.e. theta-band), it is not modulated by its direction.

Disclosures: P. Bernier: None. B. Dufour: None. F. Thénault: None. K. Whittingstall: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.02/OO10

Topic: D.08. Visual Sensory-motor Processing

Support: DARPA Contract No.N66001-15-C-4015

NSERC Alexander Graham Bell Canada Graduate Scholarship - Master's

Title: Establishing normative eye movement patterns during upper-limb functional tasks

Authors: *E. B. LAVOIE[1], E. A. CROCKETT[1], O. KOVIC[1], A. M. VALEVICIUS[2], Q. A. BOSER[2], P. M. PILARSKI[3], A. H. VETTE[2], J. S. HEBERT[3], C. S. CHAPMAN[1];
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Abstract: Recent advances in eye-tracking technology have allowed researchers to move from testing stationary and seated individuals to testing fully mobile participants engaging with real objects. However, even with these advances, the majority of studies still use restrictive lab-based tasks that are not representative of the demands on the eye-movement system in the real world. Akin to the Cognitive Ethology research approach (Kingstone et al., Brit J Psychol, 2008), it is necessary to connect lab-based tasks to real-world tasks in order to gain an understanding of natural human behaviour. Thus, we recorded eye movements in two tasks mimicking real-world demands, establishing a normative model for functional eye gaze.
The first task emulates moving a box of pasta from a counter-top into a cupboard (PB) and the second task emulates moving cups across a table (C). The tasks were designed to mimic everyday activities and each had unique requirements: the PB task required the individual to turn their head and body and interact with different heights while the C task has smaller objects filled with beads requiring more precision and putting a consequence (spilling) on errors. We used a Dikablis Professional 2.0 eye-tracker to track the eye movements of 20 healthy participants while they performed 20 trials of each of the PB and C tasks.
Data analysis identified specific areas of interest (AOIs): the participant's hand, the targets (pasta box & cups) and the placement locations. Measuring the timing of eye fixation on each AOI allowed derivation of measures such as fixation duration and the offset between when the eyes reach a target and when the hand reaches the same target. In line with previous work (Mennie et al., Exp Brain Res, 2007), we found that the eyes lead the hand, and that these "anticipatory

saccades" are modulated by task demands such that tasks requiring greater precision and having error consequences (e.g. the C task) show reduced anticipatory looking. As well, there is very little, if any, eye fixation on a participant's own hand. Likely other sensory systems, predominantly proprioception, mediate the lack of requirement for continual eye fixation on the hand and object of interest.
An important extension of this work will be to test individuals who have compromised proprioception – most notably, upper limb prosthetic users. The strong prediction is that these participants will need to fixate on their moving prosthetic limb as it interacts with objects because they do not have proprioceptive feedback from the device. This project has made these comparisons possible by establishing a normative data set for eye movement patterns during functional tasks.

Disclosures: E.B. Lavoie: None. E.A. Crockett: None. O. Kovic: None. A.M. Valevicius: None. Q.A. Boser: None. P.M. Pilarski: None. A.H. Vette: None. J.S. Hebert: None. C.S. Chapman: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.03/OO11

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC

Title: Reach-related sensory prediction errors produce event-related potentials similar to feedback-related negativity during visuomotor rotation.

Authors: *F.-A. SAVOIE[1], K. WHITTINGSTALL[2], P.-M. BERNIER[3];
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Abstract: INTRODUCTION: Feedback-related negativity (FRN), a negative event-related potential (ERP) with a mid-frontal topography, has typically been involved in the monitoring of reward prediction errors (i.e. a mismatch between the actual and expected behavioral outcomes) (Walsh & Anderson, 2012). However, it is unclear whether the FRN is sensitive to other types of mispredictions, such as sensory prediction errors (i.e. a mismatch between the actual and expected sensory consequences of motor commands), which results in sensorimotor learning. In this light, the purpose of this study was to investigate whether sensory prediction errors produce an FRN-like ERP in absence of reward prediction errors. METHODS: Electroencephalography

(EEG) was recorded while participants (n =17) completed a center-out reaching task to one of two visual targets positioned 22.5° left and right of a central fixation point. Visual feedback of a cursor corresponding to the position of the hand was provided during movement. In a strategy condition (STR, 96 trials), reaches were made under 45° visuomotor rotation, though participants were explicitly given the strategy to counter the rotation. To prevent visuomotor remapping from occurring in STR trials, these were interspersed with ≥ 2 non-rotated trials. Once all STR trials were completed, participants were exposed to a constant 45° rotation (196 trials) to induce visuomotor remapping. The last 96 of these served as control (CTRL) trials. Because CTRL and STR were matched for end-point errors, sensory inputs and motor outputs, the sole difference between these conditions was that visuomotor remapping had occurred in CTRL. Hence, EEG signal differences obtained by contrasting CTRL and STR after movement onset would reflect neural activity solely attributable to the processing of sensory prediction errors. RESULTS: To prevent potential reward prediction errors from confounding results, trials in which participants failed to displace the cursor through the correct target were removed. No significant differences were detected between STR and CTRL for reaction times (STR: 350 ± 36 ms, CTRL: 359 ± 49 ms, $p = 0.11$) or movement times (STR: 300 ± 35 ms, CTRL: 289 ± 40 ms, $p = 0.19$). However, a mid-frontal ERP, akin to the FRN, was observed after movement onset in STR but not CTRL. Specifically, this ERP consisted of a negative deflection, which peaked at electrode C2 (~ 225 ms), followed by a positive deflection, which peaked at electrode Cz (~ 285 ms). CONCLUSION: These findings suggest that the neural processing of sensory prediction errors, which lead to the updating of internal models, may share neural substrates with reward prediction error processing.

Disclosures: F. Savoie: None. K. Whittingstall: None. P. Bernier: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.04/OO12

Topic: D.08. Visual Sensory-motor Processing

Support: Radboud Excellence Fellowship

European Research Council

Title: Decoding perceptual consequences of swiping movements

Authors: ***S. FABBRI**, K. SUTTER, I. TONI, P. MEDENDORP;
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Abstract: Interacting with objects is a fundamental characteristic of human behavior. With the advent of touchscreen technologies, the interaction with 3D virtual objects on touch screens evokes new computational challenges compared to manipulation of real objects. Swiping movements on touch screen, even though the swipes themselves are constrained to a 2D plane. Due to noncommutativity of rotations ($a \times b \neq b \times a$), a horizontal swipe followed by a vertical swipe evokes a different final orientation of the virtual object than a vertical swipe followed by a horizontal swipe, even though the swiping movements themselves commute (i.e. order makes no difference for the final position of the finger on the touch screen). In this experiment, we measured brain activity while participants performed swiping movements to rotate a 3D virtual object on an MR-compatible touch screen. By manipulating the order of the swipes and the initial orientation of the object, we could disentangle between commutative processes (when different orders of swipes led to the same position of the finger on the screen) and noncommutative processes (when different orders of swipes led to different orientations of the object). The univariate contrasts on functional magnetic resonance imaging (fMRI) data identified distinct networks of regions involved during non-commutative compared to commutative processes. We currently use Representational Similarity Analysis (RSA) to further characterize the neural representation of these processes. We conclude that the brain makes use of commutative and noncommutative computations to implement the mapping between 2D motor actions and the 3D perceptual consequences.

Disclosures: S. Fabbri: None. K. Sutter: None. I. Toni: None. P. Medendorp: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.05/OO13

Topic: D.08. Visual Sensory-motor Processing

Support: NSF STC award CCF-1231216

Title: Subtle preparatory movements reveal future actions

Authors: *M. VAZIRI PASHKAM[1], R. GONZALEZ[2], S. CORMIEA[1], K. NAKAYAMA[1];
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[2]Massacheusetts Inst. of technology, Cambrdige, MA

Abstract: To act quickly and appropriately in the social environment, we need to predict others' goals and infer their intentions. To study this predictive ability in the lab, we designed a two-person competitive reaching task in a naturalistic setting. Two otherwise naïve subjects faced

each other separated by a plexiglass screen. One (Attacker) was instructed to tap one of two targets on the screen and the other (Blocker) was told to tap the same target as quickly as possible. Blocker reaction times were surprisingly fast, almost 100 milliseconds faster than reaction times to a dot projected on the screen moving in the same manner. Using video recordings of an Attacker and through systematic manipulation of the videos, we showed that Blockers use subtle preparatory movements of Attackers to predict their goal. We occluded various body parts of the Attacker and showed that reaction times slowed down only when most of the Attacker's body was occluded. This indicates that preparatory cues are widely distributed over the body of the Attacker. There was no learning during the session indicating that the blockers already have this built in capacity prior to the experiment. To determine the exact spatiotemporal profile of the preparatory cues, we used a machine vision approach to predict Attacker's movements from the videos. Attacker videos were analyzed using an optic flow algorithm and the optic flow frames were used to measure the performance of a linear support vector machine (SVM) classifier in discriminating leftward from rightward movements at various points in time and space. The accuracy of the classifier's started at chance level in the earlier frames of the video and gradually increased to reach 100% accuracy in the later frames. The shape of the classifier's psychometric function closely matched that of the human performance in predicting the movement goal. We then used the weights of the SVM to determine the spatiotemporal profile of the informative cues. In the beginning high weights were located on the head and neck region. They later moved to the torso, shoulder and arms and finally concentrated on the hand and arm region. These results demonstrate that humans possess a remarkable action prediction ability and are able to use any and all predictive cues from the body for accurate and speeded responses.

Disclosures: M. Vaziri Pashkam: None. R. Gonzalez: None. S. Cormiea: None. K. Nakayama: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.06/OO14

Topic: D.08. Visual Sensory-motor Processing

Title: The effects of varying cognitive and motor demand on an attention-mediated reaching task

Authors: *C. FUEGER, L. PETROVSKA, W. E. HUDDLESTON;
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Abstract: Every day, vision guides one's actions to facilitate successful navigation through a complex environment. Yet, one's available neural resources needed to successfully perform visually-guided movements do have limits. The purpose of this study is to examine the effects of both cognitive and motor demand on an attention-mediated reaching task. Ten females (20-29 y.o.) quickly and accurately performed a delayed reach to a previously cued location. Participants sat in front of a vertical screen with 8 peripheral targets, while visually focusing on the center of the screen. The trial sequence was as follows: a brief location cue in 1 of the 8 peripheral targets, a delay, and a central go cue. Participants performed each of two trial types. The first was a simple reach (SIMPLE) to the previously cued location. The second involved touching a target 3 locations clockwise from the initial cue (SPatial REmapping; SPRE). Participants performed the 2 types of delayed reaches under 4 task conditions. The two BASELINE conditions included either only SIMPLE trials or SPRE trials. The choice condition (CHOICE) included both trial types. The final condition was the same as CHOICE; however, during the delay participants touched 4 cued targets (i.e., a motor mask; MOTOR). Dependent measures included reaction time and variability, reach endpoint accuracy and variability, and normalized path length and variability. In the MOTOR condition, the SPRE trials compared to the SIMPLE trials had increased reaction times ($p < .001$), longer normalized path lengths ($p = .007$) and greater path length variability ($p = .008$), but no difference in reaction time variability ($p = .082$). Participants had increased reaction times ($p = .008$) on the SPRE MOTOR trials versus SPRE CHOICE trials, with no differences in reaction time variability ($p = .192$), normalized path length ($p = .080$) and variability ($p = .041$). Therefore, the addition of a motor task during the delay lengthened participants' reaction times when the task required spatial remapping. Alternately, path length increased only with a combination of an intervening motor task and spatial remapping, but not with spatial remapping only. Spatial remapping (a presumably cognitive task component) was either interrupted or slowed during the motor mask-related reaching. Thus, the completion of the motor and cognitive components of the reaching task were limited, possibly due to the task exceeding the limits of available neural resources. The next step is to replicate this experiment with a patient population, such as individuals whom have sustained a mTBI, to explore the effects of this injury on attentional resource limits.

Disclosures: C. Fueger: None. L. Petrovska: None. W.E. Huddleston: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.07/PP1

Topic: D.08. Visual Sensory-motor Processing

Support: NSERC (Canada)

CFI (Canada)

Botterell Fund (Queen's University)

ORF (Canada)

Title: Hd-tDCS modulated EEG correlations during reaching

Authors: *J. JEYACHANDRA[1,2,3], S. XU[1,2,3], J. P. GALLIVAN[1], G. BLOHM[1,2,3];
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Abstract: EEG signatures can indicate upcoming reaching movements in the dorsal premotor cortex (PMd) and medial intraparietal sulcus (mIPS) (Hoshi and Tanji, 2004; Grefkes and Fink, 2005). However, the exact contributions of PMd and mIPS to reaching behavior is unclear. To address this, we used EEG to investigate if PMd and mIPS activity was related to reaching metrics. Furthermore we modulated PMd and mIPS activity with HD-tDCS and investigated whether changes in EEG were predictive of changes in behavioral performance.
Both left mIPS and PMd were localized for each participant using a combination of functional magnetic resonance imaging and neuronavigation software. Participants performed memory-guided reaches from one of two initial hand positions (IHP) to one of four randomly chosen targets. HD-tDCS electrodes were placed in a 4x1 placement and either cathodal or anodal stimulation was administered (20min duration, 2mA). EEG recordings were taken prior to stimulation and after stimulation using the same electrode placement. Each participant completed 250 trials for pre-stimulation, stimulation and post-stimulation conditions. Time-frequency response (TFR) analysis was performed on the EEG data using Morlet wavelets and baseline was selected as the period stretching 500ms prior to target onset in order to compute z-scores during the time after target onset.
We examined whether the TFR derived from EEG was correlated to behavioural performance across all possible movement directions. Prior to stimulation, vertical endpoint error was strongly correlated with the beta band frequencies for both mIPS and PMd. This suggests a role of both mIPS and PMd in determining movement amplitude. Next we examined how relative changes in performance from baseline between anodal and cathodal stimulation polarities corresponded to changes in the TFR. Vertical endpoint errors were strongly correlated with changes in the beta band for both areas (mIPS $r = -0.680$, $p < 0.05$; PMd $r = -0.717$, $p < 0.05$) suggesting that behavioural differences due to HD-tDCS are reflected in changes of EEG activity. Finally we examined whether the difference in anodal and cathodal post-stimulation EEG was related to baseline activity. We found a strong correlation between pre-stimulation and post-stimulation EEG signals between stimulation polarities for both areas (mIPS $r = 0.906$, $p < 0.01$; PMd $r = -0.719$, $p < 0.05$). This suggests that HD-tDCS effects may be predicted by EEG activity prior to stimulation.
We conclude that measuring EEG activity before and after applying hd-tDCS might provide

useful complementary information regarding the actual effect of tDCS on the brain (as compared to the intended effect).

Disclosures: J. Jeyachandra: None. S. Xu: None. J.P. Gallivan: None. G. Blohm: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.08/PP2

Topic: D.08. Visual Sensory-motor Processing

Support: Penn State SSRI, Level 1 Award

Title: Reaching across midline is increased by cognitive load

Authors: *J. LIANG[1,2], K. M. WILKINSON[2], C. REGIEC[4], R. L. SAINBURG[3];
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Abstract: Previous studies have suggested that hand selection for reaching is modulated by task difficulty (Mamolo, Roy, Bryden, & Rohr, 2004, 2005), although exactly which features of a task contribute to this 'difficulty' have not been systematically determined. We have previously shown that biomechanical and kinematic factors such as smoothness and energy can predict patterns of selection across the workspace, emphasizing the role of predictive cost-analysis in selection. We hypothesize that the decision of which hand to use when reaching to various locations in space requires implicit cognitive processes, and thus predict that increasing the cognitive load of the task will alter the hand selection patterns. We designed a memory and search task with 3 levels of difficulty: In the control condition, a stimulus was presented in the middle of the workspace for 2 seconds, then replaced by the same stimulus presented as a target in one of 16 positions across the workspace. Participants were given a go signal and asked to reach to it with either hand. In our cognitive load conditions, the stimulus was replaced by an array of 16 pictures with one matching the cued stimulus. They were required to find and reach to the previously cued stimulus. In our lower-load condition, the 16 stimuli were clustered into one of 4 color groups to assist in the search process. In the higher-load condition the stimuli were distributed throughout the workspace, regardless of color.
Our findings indicate a strong influence of cognitive load on arm selection, such that the dominant arm was selected progressively more often under higher load conditions. Surprisingly,

however, the increased load led to an increase in cross-midline reaches by both arms. Because crossing midline is more costly in terms of kinematic and kinetic factors, our findings suggest that cognitive processes are required to avoid crossing midline. We suggest that kinetic and kinematic costs are inadequately assessed under higher cognitive load conditions and hand selection becomes biased by which hand was used on the previous trial, regardless of efficiency. We conclude that the choice to NOT cross midline requires cognitive resources.

Disclosures: J. Liang: None. K.M. Wilkinson: None. C. Regiec: None. R.L. Sainburg: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.09/PP3

Topic: D.08. Visual Sensory-motor Processing

Support: FIRB 2013 To Luca Turella

Title: Human neuroimaging suggests overlapping but distinct representations for planning vs. imagining hand actions

Authors: *S. MONACO[1], G. MALFATTI[1], J. C. CULHAM[2,1], L. TURELLA[1], L. CATTANEO[3,1];
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Abstract: The role of motor imagery is relevant in patients with motor deficits as well as in neurologically intact individuals. In patients with motor impairments, the successful use of brain-computer interfaces follows extensive training that consists of having the patients imagining what they want the effector to do (Green & Kalaska 2001; Alonso-Valerdi et al. 2015). In neurologically intact individuals, motor imagery improves the performance of acquired skills and acquisition of new ones (Annett 1995). Previous studies have compared the neural activity elicited during imagery tasks and action performance (Oosterhof et al. 2012). However, real actions require more efforts than imagined ones and involve somato-motor components that are absent in imagery tasks. The behavioral impact of motor imagery on real actions might be related to the fact that action representations generalize between planning real actions and imagining them. We explored: i) whether areas traditionally implicated in hand actions, such as the anterior intraparietal sulcus (aIPS), have a shared representation for planning and imagining specific hand movements, and ii) the role of early visual cortex (EVC) in planned and imagined actions.

We used a slow event-related functional magnetic resonance imaging (fMRI) paradigm in which participants (N=16) performed or imagined performing actions with the right dominant hand towards a centrally located object composed of a small shape attached on a large shape. The actions consisted of grasping the large shape, grasping the small shape, or reaching to touch the center of the object while fixating a point above the object.
At the beginning of each trial an auditory cue instructed participants about the task (Imagery, Movement) and the action (Grasp large, Grasp small, Reach-to-touch) to be performed at the end of the trial. A delay of ten seconds was followed by a go cue to perform or imagine performing the action (go phase). Importantly, for both Imagery and Movement only the object, but not the hand, was visible to the participants.
Using multi-voxel pattern analysis, we decoded action type in the planning phase of Movement tasks as well as in the go phase of Imagery tasks in bilateral anterior intraparietal sulcus (aIPS) and in early visual cortex (EVC), but only in the right hemisphere. Moreover, we found cross-decoding between planning and imagery in aIPS, but not in EVC. Our results suggest a shared representation for planning and imagining specific hand movements in aIPS but not in low-level visual areas, such as the EVC. Taken together, these results suggest that planning and imagining actions have overlapping but not identical neural substrates.

Disclosures: S. Monaco: None. G. Malfatti: None. J.C. Culham: None. L. Turella: None. L. Cattaneo: None.

Poster

243. Visually Guided Reaching

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 243.10/PP4

Topic: D.08. Visual Sensory-motor Processing

Support: CFI (Canada)

NSERC (Canada)

ORF (Canada)

Title: Differential effects of hd-tDCS on mIPS and PMd in reach planning

Authors: *S. XU[1,2,3], J. JEYACHANDRA[1,2,3], J. GALLIVAN[1], G. BLOHM[1,2,3];
[1]Queen's Univ., Kingston, ON, Canada; [2]Canadian Action and Perception Network (CAPnet), Toronto, ON, Canada; [3]Assn. for Canadian Neuroinformatics and Computat. Neurosci. (CNCN), Kingston, ON, Canada

Abstract: Making a reaching movement to a target requires a movement plan, i.e., a difference vector of the target position with respect to the starting hand position. While it is known that activity in the PMd and mIPS reflects aspects of a kinematic plan for a reaching movement, it is unclear how the two regions may differ.

We investigated the functional roles of the left mIPS and PMd in the planning of reaching movements using high definition transcranial direct current stimulation (hd-tDCS) and examined changes in reaching performance when participants were subjected to anodal and cathodal stimulation at each site. The left mIPS and PMd were functionally localized with fMRI using an interleaved center-out pointing and saccade task and mapped onto the scalp using Brainsight. We adopted a randomized, single-blind design and applied anodal and cathodal stimulation (2mA for 20 min; 3cm radius 4x1 electrode placement) during separate visits scheduled at least a week apart. Each participant performed 250 baseline, stimulation, and post-stimulation memory-guided reaches starting from one of two initial hand positions (IHPs) to one of 4 briefly flashed targets (20 cm distant, 5 cm apart horizontally) while fixating on a straight-ahead cross located at the target line.

In separate 2-way ANOVAs of horizontal endpoint error difference, we found a significant IHP by target interaction effect when cathodal stimulation was applied at the mIPS, but only significant IHP and target main effects when applied at the PMd. Cathodal stimulation at the mIPS produced a IHP-dependent contraction, such that reaches from the left IHP undershot and reaches from the right IHP overshot targets. Contrastingly, the movement vector was modulated independently of IHP when cathodal stimulation was applied at the PMd. These results suggest that the movement vector is not yet formed at the input level of mIPS, but is encoded in the input of PMd.

Furthermore, there were differences in the extent to which anodal and cathodal stimulation had on behavior across sites of stimulation. We quantified the effect of stimulation on movement amplitude using the difference of amplitude error during and post-stimulation from baseline performance. At the mIPS, cathodal stimulation reduced amplitude error compared to anodal stimulation. Contrastingly, the effects are reversed at the PMd, such that anodal stimulation reduces amplitude error compared to cathodal stimulation. This difference might be due to competitive vs non-competitive dynamics in PMd and mIPS respectively.

In summary, we conclude that tDCS is a viable, useful method in investigating movement planning through temporary perturbations of the system.

Disclosures: S. Xu: None. J. Jeyachandra: None. J. Gallivan: None. G. Blohm: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.01/PP5

Topic: D.09. Multisensory Integration

Title: Cross-modal sensory information integration in modulation of vertebrate visual system functions

Authors: *S. BANERJEE[1], W. J. SCHEIRER[1], L. LI[2];
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Abstract: Cross-modal signaling interactions between different sensory systems, specifically the centrifugal signals in modulation of the visual system, in vertebrate animals have been reported. While much progress has been achieved in understanding the anatomy, our knowledge of the underlying regulatory mechanism and physiological roles of centrifugal input to the retina is still in its nascent stage. Understanding the impact of centrifugal signals for retinal sensitivity will have far reaching consequences not only in our quest for understanding brain functions across species, but also in the development of computer algorithms that can integrate information from heterogeneous sources.

We use zebrafish as model organisms for *in vivo* and *in vitro* studies of brain centrifugal feedback in visual system functions. Zebrafish possesses a prominent olfacto-retinal centrifugal (ORC) pathway, which originates from the terminalis neurons (TNs) in the olfactory bulb and terminates in the retina. The TNs synthesize GnRH as a major neurotransmitter. In the retina, the TN fibers synapse with dopaminergic interplexiform cells (DA-IPCs) and retinal ganglion cells (RGCs), and possibly other retinal cell types. The function of the ORC pathway is regulated by the olfactory input. It may also be affected by the presence of endocrine hormones, such as melatonin.

Based on the results from wet-bench research examining the above circuit-level phenomena, we created computational neural models that leverage the principles of the statistical extreme value theory (EVT) to simulate and predict the consequence of sensory integration in retinal function. The overall objective of this work is to uncover facets of the mathematical and algorithmic underpinnings of sensory integration in the brain, with the dual goal of advancing understanding in biology and building more robust and powerful artificial information processing systems.

Disclosures: S. Banerjee: None. W.J. Scheirer: None. L. Li: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.02/PP6

Topic: D.09. Multisensory Integration

Support: MOE AcRF Tier 2

Title: Claustrum neurons projecting to anterior cingulate cortex are topologically organized and exhibit sexual dimorphism

Authors: *Z. CHIA[1,2], G. J. AUGUSTINE[1,3];
[1]Lee Kong Chian Sch. of Med., [2]Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore; [3]Inst. of Mol. and Cell Biol., A*STAR, Singapore, Singapore

Abstract: Among the reciprocal anatomical connections the claustrum shares with cortical regions, the connection with the Anterior Cingulate Cortex (ACC) appears the densest and most widespread. However, the function of this connection is unknown. To address this question, we have characterized the neurons projecting from the claustrum to the ACC. Claustrum cells that project to the ACC were identified by injecting into the ACC fluorescent beads that were retrogradely transported to the sites of ACC input. To identify the claustrum in live slices, we used a transgenic mouse line that expresses YFP-tagged *Volvox* channelrhodopsin-1 at high levels within the claustrum (Front. Neural Circ. 7:160). Many bead-labelled neurons were found in the claustrum, while few were found in the insular cortex; this indicates a preferential and monosynaptic connection between claustrum and ACC. The majority of ACC-projecting claustrum neurons were found ipsilateral to the bead injection site, with a greater density of labelled cells observed in the claustrum lateral to the preoptic area. Whole-cell patch clamp recordings from bead-labelled claustrum neurons were used to characterize the intrinsic properties of these neurons. These properties varied in different regions of the claustrum: labelled projection cells were predominantly (44%) type 4 Strongly Adapting cells (SA4) in the most anterior part of the claustrum, while type 2 Mildly Adapting cells (MA2) were predominant (77%) in claustrum lateral to the preoptic area, and SA4 cells were the majority (63%) of neurons found in the posterior part of the claustrum. When sorted by sex, the male claustrum showed a significant bias toward SA3 type cells (53%) within the anterior claustrum and no SA4 type cells were found, while in female mice there was a bias for SA4 cells in the anterior claustrum (76%) and no SA3 cells. Our results show that there is ipsilateral dominance for ACC-projecting claustrum neurons, with topological selectivity varying along the anterior-posterior axis. We have also found, for the first time, sexual dimorphism in ACC-projecting claustrum cells. These results demonstrate a direct pathway from claustrum to ACC, consistent with the

hypothesis that the claustrum serves as a link for information flow between the insular cortex and the ACC, a circuit that may play an important role in the salience network.

Disclosures: Z. Chia: None. G.J. Augustine: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.03/PP7

Topic: D.09. Multisensory Integration

Support: DFG Grant WE4880/2-1

Title: Expression patterns of NF200, $Na_v1.6$, Ankyrin G and related proteins in a multimodal cell type of the avian optic tectum

Authors: K. LISCHKA[1], S. LADEL[1], H. LUKSCH[1], *S. WEIGEL[2];
[1]Chair of Zoology, Tech. Univ. Munich, Freising, Germany; [2]Technische Univ. München, Freising-Weihenstephan, Germany

Abstract: The vertebrate midbrain is an important subcortical area involved in different functions such as integrating different sensory modalities, movement initiation and bottom-up attention. Our group is particularly interested in cellular computation of multisensory integration. We focus on the visual part of the avian midbrain, the optic tectum (TeO, homologous to the mammalian superior colliculus). In non-mammalian vertebrates, this area has a complex layered structure with the great advantage of distinct in- and output regions. In chicken, the TeO is organized in 15 layers where visual input targets the superficial layer while auditory input terminates in deeper layers.
One specific cell type, called Shepherd's crook neuron (SCN), has dendrites in both input regions. The characteristic feature of these neurons is the axon that originates up to 120 µm from the soma at the apical dendrite. The molecular identity of this characteristic area and thus, the site of action potential generation are of particular importance to understand signal flow and cellular computation. Based on the morphology of the SCN, action potentials at the axon could be evoked either by summation of auditory input at the basal and visual input at the apical dendrite, or maybe just by a strong visual input to the apical dendrites that bypasses the soma. This axopetal information flow was already proposed by Ramon y Cajal. However, despite the involvement of SCN in bottom-up attention little is known about the detailed neuroanatomy and cellular computation.
Here we present immunohistochemical data of the structural proteins NF200 and Ankyrin G, ion

channels $Na_v1.6$ and $K_v1.2$ and myelin. The structural protein NF200 is strongly expressed in the entire axon from its origin on. In contrast, the distribution of $Na_v1.6$ channels on the axon is primarily located at a specific region on the axon. The voltage-gated potassium channel subtype 1.2 is restricted to the cell soma. The structural protein Ankyrin G is mainly expressed from the axon origin and ends at the position where $Na_v1.6$ expression begins. Combining these expression patterns we can locate the initial segment of the axon. Interestingly, we find variations in the expression patterns which suggest diverse SCN subtypes. The molecular identification of the axon and ion channel distribution in SCN allows delineating the information flow and particularly the integration of sensory modalities in the TeO of the avian midbrain.

Disclosures: K. Lischka: None. S. Ladel: None. H. Luksch: None. S. Weigel: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.04/PP8

Topic: D.09. Multisensory Integration

Support: HFSP

Title: *In vivo* investigation of the morphogenesis of sensory spinal neurons in zebrafish

Authors: *L. DESBAN[1], A. E. PRENDERGAST[2], C. WYART[2], P.-L. BARDET[2];
[1]Wyartlab, Inst. For Brain and Spinal Cord, Paris, France; [2]ICM, Paris, France

Abstract: During development, neurons undergo various differentiation processes to acquire a highly specialized morphology intimately connected with their function. Little is known about the actual regulation of the morphological changes during their differentiation. This project aims to understand the cellular mechanisms underlying neuromorphogenesis. We take advantage of the transparency of the zebrafish embryo to investigate *in vivo* the differentiation of sensory spinal neurons, the cerebrospinal fluid-contacting neurons (CSF-cNs). Spinal Spinal CSF-cNs display a very characteristicdistinctivecharacteristic morphology with the projection into the central canal of an unusual apical extension, reminiscent of sensory organelles, in contact with the CSF.
Using LifeAct, a marker of F-actin, we undertook a descriptive approach of time-lapse imaging to characterize the main steps of CSF-cN morphogenesis. This allowed the identification of the early stages leading to the elaboration of the apical extension. First, F-actin seems to beis recruited at the level of a transient ring of actin. From this structure starts the initiation, and followed by thethen the elongation, of microvilli, structures usually supported by bundles of

actin filaments. Our timelapse approach also enabled us to characterize various shapesforms of the apical extension developed bygenerateddeveloped by distinct subpopulations of spinal CSF-cNs.
We To understand the molecular mechanisms underlying the formation of the apical extension, we investigated an actin-bundling factor of interest named Espin. W we showeded that Espin is highly expressedpresentexpressed in CSF-cN apical extension from the beginning of its formation. We generated *espin* mutants using the CRISPR/Cas9 system to test the contribution of this factor in the formation/maintenance of the apical extension. Characterization of these mutants is underway. Altogether, this work enables us to dissect *in vivo* mechanisms underlying the acquisition of an apical extension by sensory neurons of their functional mature morphology.

Disclosures: **L. Desban:** A. Employment/Salary (full or part-time): Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, ICM, F-75013. **A.E. Prendergast:** None. **C. Wyart:** None. **P. Bardet:** None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.05/PP9

Topic: D.09. Multisensory Integration

Support: NSERC

Title: An implicit approximate normalization model for multisensory integration across reference frames

Authors: ***P. ABEDI KHOOZANI**[1], D. STANDAGE[1], G. BLOHM[1,2,3];
[1]Queen's Univ., Kingston, ON, Canada; [2]Canadaian Action and Perception Network, Toronto, ON, Canada; [3]Assn. for Canadian Neuroinformatics and Computat. Neurosci., Toronto, ON, Canada

Abstract: Many brain processes - such as primary sensory processing, attentional modulation, multisensory integration, reference frame transformations, decision making, etc. - can be performed by probabilistic inference. It has been suggested that inference can be implemented in the brain by marginalization across variables through explicit divisive normalization, requiring intractable sums/integrals. Here, we propose an alternative, more physiologically feasible mechanism to perform marginalization. This alternative mechanism (implicit approximate normalization: IAN) is based on well-established parallel computing and machine learning principles and is functionally equivalent to divisive normalization without requiring divisive

operations. Specifically, we implemented a multi-layer feed-forward neural network using different neural coding schemes within the same network (i.e. probabilistic spatial codes and probabilistic joint codes) and trained it to perform multisensory integration across different reference frames in one step using a standard pseudo-Newton method with preconditioned conjugate gradient descent. The performance of this network was comparable to a probabilistic population code network, but without requiring non-linear or divisive operations. IAN produces a wide range of behaviors similar to recorded activity in the brain. These behaviors include inverse effectiveness, the spatial correspondence principle, gain-like modulations, super-additivity, and multi-sensory suppression. In addition to key empirical principles of multisensory integration, IAN accounts for quantitative features of cue combination: we observed modulation of neural activity in our network by varying the cue reliability, similar to area MSTd. The strength of IAN is that it performs well with a fraction of the neurons required by explicit methods (i.e. in a network with two cues to be combined in 3-D and 100 units in each dimension, divisive normalization requires 10^{12} while IAN requires 10^3 units). Furthermore, IAN doesn't require a neat preconfigured connectivity structure between neurons or explicitly matching population codes for individual neurons, in contrast to explicit divisive normalization. In conclusion, the results of this study demonstrate that marginalizing operations can be done in simple feed-forward networks of purely additive units without the requirement of explicit divisive normalization.

Disclosures: P. Abedi Khoozani: None. D. Standage: None. G. Blohm: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.06/PP10

Topic: D.09. Multisensory Integration

Title: Neural circuit basis for decision-making in *C. elegans* chemotaxis

Authors: T. MURAYAMA[1], *I. MARUYAMA[2];
[2]Senior Investigator, [1]Okinawa Inst. of Sci. & Technol. Grad. Univ., Okinawa, Japan

Abstract: Monitoring of environmental and tissue pH is crucial for the survival of animals. The nematode *C. elegans* is an excellent model organism for the analysis of neural circuits that regulate behavioral responses to environmental changes, because of its simple nervous system. We are interested in neural circuits responsible for *C. elegans* chemotactic behavior to environmental alkalinity. The animal is attracted to mildly alkaline pH, and avoids strongly alkaline pH higher than pH 10. Previous genetic dissection and Ca^{2+} imaging demonstrated that

ASEL and ASH are major sensory neurons responsible for attraction to mildly alkaline pH and repulsion from strongly alkaline pH, respectively. ASEL was activated by alkaline pH ranging from pH8 to pH 11. ASH was activated by alkaline pH higher than 10. Moreover, ASEL was transiently activated by pH up-step, whereas ASH showed long-lasting activation during stimulation. These results suggest that ASH and ASEL activities compete each other and ASH activity overrides that of ASEL upon stimulation with strongly alkaline pH. The long-lasting ASH activity may override the transient activation of ASEL. Since there is no chemical or electric connection between ASEL and ASH, signals from these 2 alkaline pH sensors must be integrated by downstream circuits. We have now analyzed interneurons responsible for the behavioral switch, and will discuss how the decision-making occurs.

Disclosures: T. Murayama: None. I. Maruyama: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.07/PP11

Topic: D.09. Multisensory Integration

Support: NIH Grant NIDCD 005640

Title: Adaptive shifts in owls reared with single pane prisms

Authors: *W. M. DEBELLO, D. SANCULI, D. TOTTEN;
UC Davis, Davis, CA

Abstract: Prism adaptation in barn owls is a valuable model for understanding the neural mechanisms of plasticity and learning. Previous work has employed dual pane (DP) prism lenses, which can be knocked out of alignment during aviary life. We investigated the utility of a single pane (SP) prism lens that ensures binocular registration. Auditory and visual receptive fields were measured in normal owls (n=13), owls adapted to dual pane prisms (n=6) and owls adapted to single pane prisms (n=7). Visual spatial receptive fields of multi-units within the optic tectum (OT), measured using static or moving dots displayed via a digital light projector, were displaced horizontally as expected but otherwise similar with slight changes reflecting optical distortion artifacts. Auditory spatial receptive fields measured using a free-field speaker array were indistinguishable before and immediately after mounting a prototype design, indicating that SP prisms do not alter the binaural cues on which localization is based. The chronic effects were studied by rearing juvenile barn owls with surgically attached prisms (19^{o} optical displacement) for up to two years. Adaptive shifts in tuning for interaural time difference (ITD) measured using

dichotic stimuli were robust and significantly larger in the SP group (mean = 42 μs) than in the DP group (mean = 33 μs). Site-to-site variability in auditory-visual alignment was reduced with SP prisms, and auditory responses in the deep layers of optic tectum were slightly faster. Yet, site-to-site variability with SP prisms was still larger than in normal owls. Across all groups no significant changes were observed in visual receptive field structure or latency of visual responses. In total, these results demonstrate that SP prisms promote modest improvement in the degree and reliability of adaptive shifts. The fact that prism-adapted owls come to close but not quite equal to normal owls in neuronal performance metrics indicates that the prism-adapted circuit is altered in ways that could impact population code read-out.

Disclosures: W.M. DeBello: None. D. Sanculi: None. D. Totten: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.08/PP12

Topic: D.09. Multisensory Integration

Support: NIH Grant DC014765

Title: Sensory cross-modulation in thalamo-reticular interactions

Authors: *K. PAUL, D. A. LLANO;
Beckman Inst., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The thalamic reticular nucleus (TRN) consists of GABAergic neurons that have been hypothesized to modulate the flow of sensory or motor information through first- and higher-order thalamic nuclei. The TRN has closed loop reciprocal connectivity with the thalamus which are well established, however, there is a growing evidence of non-reciprocal or open loop connectivity between them. While the majority of information transmission to and from the cortex occur via separate thalamic nuclei corresponding to the sensory modalities, there has also been evidence of crosstalk in the thalamus between the modalities which occur through an inhibitory disynaptic pathway involving the TRN. Previous literature (Crabtree et. al., 1998; 2000) used pressure ejection of glutamate to show disynaptic connectivity between first order and higher order thalamic nuclei. In our investigations, we have used laser scanning photostimulation with glutamate uncaging to systematically identify the pattern of functional intrathalamic connectivity via TRN. Whole-cell patch-clamp recordings were done in cells in discrete thalamic nuclei which were obtained in the horizontal and thalamocortical slice orientation from mice (P12-28). MNI-caged glutamate (Tocris) was added to recirculating ACSF

and stimulated using a pulsed UV laser (355 nm, DPSS). A grid of points (20 by 20 array) was created encompassing several thalamic nuclei and focal photolysis was accomplished by non-neighbor stimulation of points within the grid. Current responses were obtained in the voltage clamp configuration with the outward current amplitude representing strength of the disynaptic connection. We found strong disynaptic connectivity between neurons in the thalamus and TRN which were topographically organized. We also found disynaptic connectivity between nuclei in the thalamus which suggest the presence of open loop connections. By employing laser scanning photostimulation with glutamate uncaging we have performed a comprehensive investigation of the functional intralathalmic connectivity pattern to add to the previous work in the realm of sensory cross-modulation within the thalamus.

Disclosures: K. Paul: None. D.A. Llano: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.09/PP13

Topic: D.09. Multisensory Integration

Support: CIHR MOP-12675

CIHR FDN-143209

Title: Multiple spatial and temporal orders of cortical mesoscale spontaneous activity are present in in GCaMP6 mice

Authors: ***M. P. VANNI**, G. SILASI, Y. SEKINO, M. BALBI, A. CHAN, J. LEDUE, D. XIAO, F. BOLANOS, T. H. MURPHY;
Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: While behavior is expected to be strongly locked with neuronal activity, the link between spatiotemporal cortical dynamics and perception/action still needs to be better described experimentally and is still one of the major challenges of systems neuroscience. At the mesoscopic scale, which explores the function of large groups of neurons, cortical activity can reveal the topography of connections between areas, as well as the boundaries of systems in multiple temporal domains from slow global oscillations to fast waves. The use of transgenic mice expressing calcium indicators offers the possibility to explore mesoscale dynamics on large regions of cortex that include multiple imbricated spatial levels of organization. However, the temporal dynamics of calcium activity was never investigated in behaving mice and was

explored here for different frequency components. Transgenic mice expressing GCaMP6s or GCaMP6f (Allen Institute: Ai94 and Ai93, n=40) were implanted with a bilateral chronic window covering most the dorsal cortex. Cortical fluorescence was collected in quiet and behaving mice and temporally filtered. Brain activity as well as connectivity was analyzed using seed pixel correlation and clustering approaches such as K-means. Mouse behavior was measured by collecting videos of body movements with a webcam. Beyond 10Hz, few specific patterns of brain parcellation was observed. Around 4Hz (delta and theta rhythms), a territory of strong activity was observed in visual and retrosplenial regions in contrast to somatosensorimotor areas more associated with lower frequency components. Thus, a clear clustering was observed, delimitating two mediolateral domains that may correspond with previously defined borders of the default mode and lateral networks in mice. At lower frequency, below 1Hz, 3 functional modules were generally observed within activity regularly alternating between them. Thus, multiple cortical patterns of activity co-exist in different temporal scales and could be associated with fine balance of neuronal processing during perception, decision making, and action. Beyond the impact of monitoring cortical organization in disease models, this study could open new orientations in developing more advanced brain machine interface technologies.

Disclosures: M.P. Vanni: None. G. Silasi: None. Y. Sekino: None. M. Balbi: None. A. Chan: None. J. Ledue: None. D. Xiao: None. F. Bolanos: None. T.H. Murphy: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.10/PP14

Topic: D.09. Multisensory Integration

Support: R01 DC0005460

UC Davis BRAIN-STIM Award

Title: Toric spines in the owl inferior colliculus

Authors: *D. SANCULI[1], K. PANNONI[1], M. SUNG[1], V. POPAT[1], C. ZAHER[1], W. DEBELLO[1,2], M. ELLISMAN[2], E. BUSHONG[2];
[1]Univ. of California Davis, Davis, CA; [2]Univ. of California San Diego, San Diego, CA

Abstract: Space-specific neurons (SSNs) in the barn owl inferior colliculus compute a map of auditory space. Their function is well-characterized but their ultrastructure is not. We used serial

block electron microscopy (SBEM) and stimulated emission depletion (STED) microscopy to analyze SSNs labeled in vivo by injections of the tracer micro-ruby. Injections were targeted to the deep layers of OT at map locations representing frontal space. Retrogradely labeled cells were consistently observed in the external nucleus of the inferior colliculus (ICX) and more rarely in the lateral shell of the IC. The SBEM volume (45 x 45 x 75 μms) contained one labeled neuron whose soma and proximal dendrites were studded with unusual dendritic spines evocative of thorny excrescences (TE) found in mammalian hippocampus and amygdala. In contrast to mammalian TEs: (1) Many SSN spines had holes ("toric spines"). Some holes manifested as cytoplasmic continuities while others were formed by adhesions between filopodia. (2) They largely lacked spine head protrusions, tending towards narrow cylindrical structure, (3) They appeared more evenly distributed across the soma and dendrites, as opposed to the clustered organization of TEs, and (4) Nearly all toric spines integrated synaptic inputs arising from multiple axons. STED imaging of >40 retrogradely labeled cells revealed at least two morphological classes: one exhibiting high density of toric spines distributed widely throughout the dendritic field (a minority of cells), and one devoid of toric spines but studded with typical spines (a majority). To our knowledge, this is the first report of a toric postsynaptic structure in any species or brain region. We propose that toric spines represent an independent computational subunit of SSNs.

Disclosures: D. Sanculi: None. K. Pannoni: None. M. Sung: None. V. Popat: None. C. Zaher: None. W. DeBello: None. M. Ellisman: None. E. Bushong: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.11/PP15

Topic: D.09. Multisensory Integration

Support: Einstein

ERC

Neurocure

Mariecurie

Title: Air-Track: A real-world environment for active sensing in head-fixed mice

Authors: ***M. N. ABDELHAMID**[1,2], H. ORABY[1], S. DOMINIAK[1], R. SACHDEV[1], Y. WINTER[1], M. E. LARKUM[1];

[1]Biol. Dept., Neurocure Cluster of Excellence, Humboldt Universi, Berlin, Germany; [2]Berlin Sch. of Mind and Brain, Berlin, Germany

Abstract: Natural behavior occurs in multiple sensory and motor modalities and in particular is dependent on sensory feedback that is constantly adjusts behavior. To investigate the underlying neuronal correlates of natural behavior, it is useful to have access to state-of-the-art recording equipment (e.g. 2-photon imaging, patch recordings, etc.) that frequently requires head-fixation. This limitation has been addressed with various approaches such as virtual reality/air ball or treadmill systems. However, achieving multimodal, realistic behavior in these systems can be challenging. These systems are often also complex and expensive to implement. Here we present "Air-Track", an easy to build, head-fixed behavioral environment that requires only minimal computational processing. The Air-Track is a lightweight, physical maze floating on an air table that has all the properties of the "real, physical" world, including multiple sensory modalities tightly coupled to motor actions. To test this system, we trained mice in Go/No-Go and two-alternative forced choice tasks in a plus maze. Mice chose lanes, and discriminate apertures or textures by moving the Air-Track back and forth, and rotating it around themselves. A custom-controlled Arduino/Pixy system is used to monitor animal location, and generated an interactive environment to control stimulus presentation and reward delivery. We are currently developing the ability to use the Air-Track system to elicit and track natural behavior in concert with the ability to monitor or manipulate brain activity.

Disclosures: M.N. Abdelhamid: None. H. Oraby: None. S. Dominiak: None. R. Sachdev: None. Y. Winter: None. M.E. Larkum: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.12/PP16

Topic: D.09. Multisensory Integration

Support: BFU2012-36107

Title: Basal forebrain circuits implicated in cortical modulation

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Abstract: Previous studies indicate that there is specificity in the projection pathways linking the different basal forebrain (BF) nuclei with the cerebral cortex. The aim of present study is to

provide evidences of different circuits between BF and motor, sensory and prefrontal cortices implicated in sensory-motor integration. Sprage-Dawley rats have been used in the experiments. The Ethical Committee of the Autonomous University of Madrid approved all animal procedures. Animals received injections of the retrograde tracers: Fluoro-Gold in the Horizontal Diagonal Band (HDB) and Fast Blue in Basal Magnocelular Nucleus (B). Fluorescent and confocal microscopy was used to locate the retrograde labeled neurons. Our results show the existence of two different reciprocal circuits: 1) Projections arising the prefrontal and sensory cortices, implicated in processing and integration of sensory information, aim the HDB. 2) Projections arising the motor and sensory cortices drive forward B neurons. Since it is known that the BF cholinergic neurons degenerate at the first stage of Alzheimer disease, these circuits could be implicated in different ways in the progression of the disease.

Disclosures: I. Chaves-Coira: None. A. Nunez: None. M.L. Rodrigo-Angulo: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.13/QQ1

Topic: D.09. Multisensory Integration

Support: FAPERJ

CNPq

Title: Thalamic inputs to cortical areas involved in skilled forelimb movements and tool use

Authors: ***A. MAYER**[1], G. LEWENFUS[1], R. E. BITTENCOURT-NAVARRETE[2], J. G. FRANCA[1];
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Abstract: The posterior parietal cortex (PPC) of primates plays a crucial role in manual behaviors such as object manipulation and tool use. Corticocortical connectivity of different sectors of areas 5 and 7 in cebus and macaque monkeys suggest that segregated cortical circuits promote the somatosensory-motor and visuomotor integration necessary for implementation of such behaviors. However, the part played by thalamic nuclei projecting to those cortical circuits is not yet known. In this study we investigated the thalamic projections to areas 5v (or PEip), anterior intraparietal area (AIP), PFG, and PF in the cebus monkey.
After a brief electrophysiological mapping of the parietal cortex, five adult cebus monkeys were

injected with different retrograde tracers at different sectors of the PPC. Three injections were made in the hand/forearm representation of area 5v, five in the expected location of AIP and/or PFG, and two in area PF. After a survival of 11-14 days, animals were perfused and the diencephalon was cut coronally. Distribution of labeled thalamic neurons was represented in schematic drawings of coronal sections. Identification of thalamic nuclei was performed on cytochrome oxidase and Nissl-stained sections.
Most labeled cells were found in the lateral posterior nucleus (LP) and pulvinar complex. The main sources of thalamocortical projections to area 5v were the anterior pulvinar nucleus (PuA, 50%), the lateral posterior nucleus (LP, 18%), and the ventral posterior superior nucleus (VPs, 15%). In contrast, injections in area AIP/PFG revealed retrograde labeling mainly in LP (55%) and medial pulvinar nucleus (PuM, 37%). Despite some variability between cases, thalamocortical projection to area PF was similar to both that to area 5v and to AIP/PFG. More specifically, area PF received projections mainly from LP (57% and 20%, respectively in the two injection cases) and PuM (11% and 62%), followed by PuA (15% and 12%) and VPs (16% and 2.5%).
When projections to areas 5v and AIP/PFG were compared, different patterns of thalamocortical connectivity indicated that circuits dedicated for integration of somatosensory and visual information with motor processing were also segregated at subcortical levels. PuA seems to be the main source of higher-order inputs to somatosensory-motor circuits, whereas PuM contributes relatively more with the visuomotor processing. Additonally, thalamic inputs common to areas 5v, AIP/PFG and PF, especially from LP, suggest that the thalamus could also perform functional integration between these segregated cortical circuits involved in implementation of skilled forelimb behaviors.

Disclosures: A. Mayer: None. G. Lewenfus: None. R.E. Bittencourt-Navarrete: None. J.G. Franca: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.14/QQ2

Topic: D.09. Multisensory Integration

Support: DFG SFB TRR31

NIH RO1 DC009607

Title: Early sensory experience sculpts the development of multisensory connections of primary sensory cortices

Authors: J. U. HENSCHKE[1], A. M. OELSCHLEGEL[2], F. ANGENSTEIN[4], F. W. OHL[1], J. GOLDSCHMIDT[1], P. O. KANOLD[5], *E. BUDINGER[3];
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Abstract: Multisensory integration does not only recruit higher-level association cortex, but also primary sensory cortices like A1 (auditory), S1 (somatosensory), and V1 (visual). The underlying anatomical pathways include direct cross-modal thalamocortical and intracortical connections. Sensory loss from birth in humans results in functional recruitment of the deprived cortical territory by the spared senses. We investigated if the multisensory recruitment could be due to changes in the early development of thalamocortical and intracortical projections. Neuronal tracer injections into A1, S1, and V1 within the first postnatal month of normally developing Mongolian gerbils revealed that multisensory thalamocortical connections emerge before intracortical connections but mostly disappear during development. However, early auditory, somatosensory, or visual deprivation leads to an increase of these connections encompassing lemniscal, non-lemniscal, and multisensory pathways. The specific changes in the connectivity patterns are not due to a neurogenesis or apoptosis of projection neurons but rather to axonal remodeling. Consistent with our anatomical findings, functional single-photon emission computed tomography (SPECT) revealed altered stimulus-induced activity and higher functional connectivity specifically between primary areas in deprived animals. Together, we show that early sensory experience has a dramatic effect not only on the development of sensory thalamocortical and intracortical pathways underlying the deprived sense but also on pathways serving the non-deprived senses enabling a functional recruitment of deprived cortical areas by the spared senses.

Disclosures: J.U. Henschke: None. A.M. Oelschlegel: None. F. Angenstein: None. F.W. Ohl: None. J. Goldschmidt: None. P.O. Kanold: None. E. Budinger: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.15/QQ3

Topic: D.09. Multisensory Integration

Support: BMBF 01GY1004B, Bernstein Center Munich,

Title: Auditory input and receptive fields in the optic tectum of the chicken, an auditory generalist avian species.

Authors: *H. LUKSCH, H. A. SCHNYDER, B. NIEDERLEITNER, Q. KRABICHLER, C. GUTIERREZ-IBANEZ, U. FIRZLAFF;
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Abstract: The auditory system of birds has been intensely studied over several decades. For example, the processing of monaural and binaural cues in the brainstem has been investigated in various species, most notably the chicken and the barn owl. In contrast, the integration of auditory input to the midbrain multimodal map in the optic tectum has almost exclusively been studied in the barn owl. The latter can be considered an auditory specialist as, contrary to most other birds, it can locate auditory cues in space with great precision. As the comparison between specialist and non-specialist circuit layout allows for an assessment of essential elements, here we analyzed, for the first time, the auditory midbrain, its projection upon the optic tectum, and the auditory receptive fields of tectal neurons in the chicken, an auditory generalist. In barn owls an auditory spatial map is formed in the external nucleus of the inferior colliculus (ICX) and then conveyed to the TeO by a direct projection. In the chicken, we found this projection to be quite weak, but discovered a second projection from the ICx that reaches a lateral part of the Formatio reticularis (FRLx), which then projects broadly upon the TeO. Intracellular labeling demonstrated that individual neurons cover up to 40 degrees of visual space in the lateral aspects of the TeO. Extracellular recordings of units in IC, TeO, and FRLx stimulated with virtual acoustic stimuli revealed spatially defined auditory receptive fields that were typically broad, centered at lateral directions from the animal. Some tectal neurons had very large, conspicuous circular center-surround receptive fields (donut-shaped), which had a coarse topography in the TeO. Thus, while the chicken is a generalist without specialized auditory capabilities, spatial auditory information is integrated into the multimodal map of space in the TeO. Future comparative analysis will show whether the newly described projection via the FRLx is a plesiomorphic feature and the enlarged direct projection from the ICx to the TeO in the owl a consequence of being an auditory specialist.

Disclosures: H. Luksch: None. H.A. Schnyder: None. B. Niederleitner: None. Q. Krabichler: None. C. Gutierrez-Ibanez: None. U. Firzlaff: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.16/QQ4

Topic: D.09. Multisensory Integration

Support: NSF IGERT DGE-1069104

MD5M Lions Grant

NIH NCATS UL1 TR000114

MnDRIVE Innovation Grant

UMN Interdisciplinary Fellowship

Title: Controlling plasticity in sensory cortical regions using multisensory neuromodulation

Authors: *C. GLOECKNER, J. NOCON, H. LIM;
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Abstract: Sensory systems are vastly connected through multisensory integration. The ability to induce plasticity in cortical areas using noninvasive, controlled sensory stimulation that takes advantage of existing multimodal pathways may treat various neural sensory disorders characterized by abnormal neural firing. We investigated changes in sensory cortical activity induced by paired stimulation of two different sensory systems with specific temporal delays. We recorded sensory receptor-driven spike activity in the right olfactory piriform cortex (**OC**), gustatory cortex (**GC**), primary somatosensory cortex (**S1**), primary auditory cortex (**A1**), and primary visual cortex (**V1**) of ketamine-anesthetized guinea pigs using 32-site electrode arrays. Spontaneous and receptor-driven activity before and after paired stimulation were compared to assess plasticity effects. Subcutaneous needles electrically stimulated the skin of various body regions for somatosensory stimulation. Surface electrodes stimulated the tongue and nasal cavity for gustatory and olfactory stimulation. Broadband noise was used for auditory stimulation, and an LED light board was used for visual stimulation. Several animals also received chronic recording implants to confirm cortical plasticity effects in an awake state. All cortices were located using stereotactic coordinates and functional responses to receptor activation. Significant changes in cortical firing ($p<0.01$, ranked-order t-test, $n=13394$ total) were induced with paired sensory stimulation compared to control condition (no stimulation) that depended on sensory input and recording location. Pairing somatosensory and auditory stimulation suppressed or facilitated firing in A1 depending on inter-stimulus delay, and induced differential effects in S1 depending on body stimulation location. Paired gustatory and somatosensory stimulation suppressed or facilitated GC depending on left/right body stimulation location. OC was facilitated by paired olfactory and somatosensory stimulation, but was suppressed with paired olfactory and acoustic stimulation. V1 was facilitated by paired visual and right shoulder or acoustic stimulation, but was suppressed by paired visual and left shoulder stimulation.
We can induce differential effects in neural firing in sensory cortices through multisensory stimulation. By optimizing stimulation parameters, such as location and inter-stimulus delay, we can control the type, location, and amount of cortical plasticity, which may potentially treat neural sensory disorders (e.g. tinnitus or chronic pain) noninvasively by altering abnormal spike patterns with specificity.

Disclosures: C. Gloeckner: None. J. Nocon: None. H. Lim: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.17/QQ5

Topic: D.09. Multisensory Integration

Support: NSF Grant IOS 11471172

Professional Staff Congress City University of New York (CUNY) Research Award Program

ANPCyT grant BID-PICT 2012-1578

UBACyT

Title: Integration of auditory and visual inputs depends on differential properties of Mauthner-cell dendrites.

Authors: V. MEDAN[1], T. MÄKI-MARTTUNEN[2], J. SZTARKER[1], *T. PREUSS[3];
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Abstract: Most animals take advantage of multiple information sources to form a coherent percept of the world for adaptive behavioral decisions, however, the underlying mechanisms of such interactions are still far from clear. Here we address two essential questions (i) how do neurons process multimodal sensory inputs with different temporal characteristics, and (ii) what role do intrinsic properties of dendrites play in selective sensory filtering? For that we took advantage of the startle escape network in goldfish, which is centered on a pair of indefinable decision-making neurons, the Mauthner-cells (M-cells). These cells receive auditory and visual inputs via separate dendrites, both accessible for morphological characterization, *in vivo* recordings, and computational modeling.
Reconstructions of labeled cells (N=3) revealed that the visual dendrite (VD) is on average 30% longer and 25%, thinner than the auditory dendrite (LD), resulting in a 28 ± 8 % higher surface/volume ratio of the VD when compared to the LD (t-test; $p = 0.036$). Accordingly, our electrophysiological results showed differences in cable properties in the two dendrites. Namely, a lower transfer resistance in the VD (29.1 ± 20.2 kΩ) than in the LD (77.9 ± 33.4 kΩ; $t=3.55$, $df=13$, $p=0.004$), and a longer time constant in the VD (0.57 ± 0.23 ms) compared to the LD

(0.25 ± 0.07 ms; t=2.66, df=5, p=0.04). Similarly, the dendritic space constant of visually evoked PSPs in the VD ($\lambda_{ortho,VD}$=145 μm) is about half of that for auditory PSP in the LD ($\lambda_{ortho,LD}$=361μm).
Modeling experiments showed that these passive cable properties fully account for the observed differences in PSP decay in the two dendrites.
Interestingly, our electrophysiological experiments showed that auditory evoked PSPs originating in the LD also invade the VD, as well as the opposite, visual PSPs invading the LD. The effects of the antidromically propagating PSPs are asymmetrical between the dendrites however, with auditory PSPs being more prominent in the VD than visual PSPs in the LD. Modeling experiments imply that this asymmetry might be due to active conductances expressed in the proximal segments of the VD.
Taken together, our results suggest modality-dependent membrane specialization in the two M-cell dendrites that are suited for processing stimuli of different time domains (e.g. visual looms and auditory clicks), which might be critical for vital startle-escape decisions.

Disclosures: V. Medan: None. T. Mäki-Marttunen: None. J. Sztarker: None. T. Preuss: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.18/QQ6

Topic: D.09. Multisensory Integration

Support: NSF-GRF fellowship (BJH)

NIH Director's Innovator Award (HZ)

NIH R01 grant (NS081071; TM)

Title: Input-specific functional heterogeneity of the thalamostriatal circuitry

Authors: *B. C. JONGBLOETS, B. J. HUNNICUTT, W. T. BIRDSONG, K. GERTZ, H. ZHONG, T. MAO;
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Abstract: The basal ganglia play an essential role in movement control and learning. Their primary input station - the striatum - is responsible for sorting contextual and motor information from the thalamus into specific downstream pathways. Knowledge of the precise circuits and organizational principles between the thalamus and striatum is essential for the mechanistic dissection of how these structures orchestrate movement control and learning. We systematically

analyzed excitatory thalamic inputs to the mouse striatum based on our viral-mediated anterograde fluorescent tracing dataset (Hunnicutt et al., *Nat Neurosci*, 2014). Whole brain image datasets were obtained and analyzed using custom algorisms to generate a map of individual thalamic subregions differentially projecting to the striatum. Guided by these projection maps, optogenetic experiments were carried out to assess functional properties of thalamostriatal projections originating from specific thalamic subregions. We found that converging inputs from different thalamic subregions onto the same medium spiny neurons (MSNs) may exhibit distinct synaptic properties. The present functional data provides a framework for investigating the convergence of multimodal information onto individual MSNs. Our comprehensive thalamostriatal projectome may guide functional studies aimed at understanding the diverse functions of the striatum.

Disclosures: B.C. Jongbloets: None. B.J. Hunnicutt: None. W.T. Birdsong: None. K. Gertz: None. H. Zhong: None. T. Mao: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.19/QQ7

Topic: D.09. Multisensory Integration

Support: EMBO ALTF 549-2013

Title: Identification of multiple classes of GABAergic sensory neurons contacting the cerebrospinal fluid in the vertebrate spinal cord

Authors: *A. PRENDERGAST, L. DJENOUNE, L. DESBAN, J. GOMEZ, J. STERNBERG, P.-L. BARDET, C. WYART;
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Abstract: Multiple lines of evidence indicate that molecular and mechanical cues from the cerebrospinal fluid (CSF) can affect the development and function of the central nervous system (CNS). However, the mechanisms by which these cues are detected and relayed to the CNS remain elusive. Cerebrospinal fluid-contacting neurons (CSF-cNs), being situated at the interface between the CSF and the CNS, are in an ideal location to convey this information to local networks. These neurons extend an apical microvilliary extension into the CSF, express the transient receptor channel PKD2L1, and project an axon rostrally onto local targets. In zebrafish larva, we recently showed that CSF-cNs constitute a mechanosensory loop detecting bending of the spinal cord that tunes the frequency of locomotion. Evidence from zebrafish and mouse

indicates that CSF-cNs originate from two distinct progenitor domains. Here, we ask whether these two groups of CSF-cNs have different morphological and molecular characteristics. We first show that the morphology of the apical extension and the axonal projection differ between the two CSF-cN groups. We further identify a repertoire of peptides and neuromodulators that distinguish more subtypes within each group. Our study reveals more CSF-cNs are more heterogeneous than previously believed, suggesting that multiple neuromodulatory functions are likely carried out by distinct CSF-cNs within this sensorimotor feedback loop.

Disclosures: A. Prendergast: None. L. Djenoune: None. L. Desban: None. J. Gomez: None. J. Sternberg: None. P. Bardet: None. C. Wyart: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.20/QQ8

Topic: D.09. Multisensory Integration

Support: ERC Starting Grant 311673

Title: Pkd2l1 underlies spontaneous activity in intraspinal sensory neurons

Authors: *J. R. STERNBERG[1], L. DJENOUNE[1,2], A. PRENDERGAST[1], J. MCDEARMID[3], H. PASCAL MOUSSELLARD[1], C. WYART[1];
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Abstract: In the spinal cord, cerebrospinal fluid-contacting neurons (CSF-cNs) are sensory neurons responsible for relaying mechanical and chemical sensory information locally to motor circuits. CSF-cNs in diverse species express GABA and the transient receptor potential channel TRPP3 or PKD2L1, involved in sour taste. Here we use genetic targeting, calcium imaging, pharmacology and electrophysiology in the zebrafish to investigate the role of PKD channels in spontaneous activity in these neurons. We show that Pkd2l1 forms a complex with Pkd1l2 in CSF-cNs in zebrafish. Calcium imaging showed large long-lasting calcium transients in a subpopulation of CSF-cNs at early developmental stages that reflect high frequency firing, which were abolished in a *pkd2l1* mutant. We used whole-cell patch clamp recordings to investigate whether differences in frequency of spontaneous firing between the two subpopulations were due to differences in subcellular localization of Pkd2l1 or intrinsic properties. We found that Pkd2l1 opens spontaneously and that current from a single channel opening is sufficient to generate an action potential in all CSF-cNs. Current investigation is focused on understanding the intrinsic

and extrinsic properties that alter the probability of channel opening in these two subpopulations. These data demonstrate that a single channel opening can generate an intrinsic source of spontaneous activity in sensory CSF-cNs. Additionally, our results suggest that differences in channel activity reflect functional differences in subpopulations of CSF-cNs.

Disclosures: J.R. Sternberg: None. L. Djenoune: None. A. Prendergast: None. J. McDearmid: None. H. Pascal Moussellard: None. C. Wyart: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.21/QQ9

Topic: D.09. Multisensory Integration

Support: FIRST program

MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2012-2016

Title: Phase-locking of cortical slow waves induced by multisensory inputs at hub areas in excitatory networks

Authors: *T. YOSHIDA[1], S. KUROKI[1,3], H. TSUTSUI[2,4], M. IWAMA[1], T. MICHIKAWA[2,5], A. MIYAWAKI[2], T. OHSHIMA[3], S. ITOHARA[1];
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Abstract: Although multimodal integration is an essential brain function for sensory processing, the underlying mechanism of cortical interactions in sub-populations of different cell types remains challenging. Cortex-wide imaging is a powerful technique for visualizing intercortical activity. We established a transgenic mouse line expressing a calcium indicator, Yellow Cameleon 2.60, under the control of the Cre/loxP system and performed wide-field transcranial calcium imaging in excitatory and inhibitory networks of the cerebral hemisphere. We revealed that spontaneous slow waves represented functional connectivity between primary sensory areas and associative areas, and the wave flows formed a hub-like structure at the associative regions. Area-wide amplitude responses to combinations of somatosensory, auditory, and visual stimuli simply consisted of superposition over unimodal responses, not being cell-type specific.

Contrarily, phase responses to the combined inputs in the excitatory networks, not in the inhibitory networks, indicated phase-locked ongoing slow-oscillation for several seconds. Medial and parietal areas were locations where the phase-locking was markedly induced, and coincided with the hub areas identified from spontaneous activity. These findings suggest that association cortices serve as a hub and converged multisensory information is integrated by phase-locking.

Disclosures: T. Yoshida: None. S. Kuroki: None. H. Tsutsui: None. M. Iwama: None. T. Michikawa: None. A. Miyawaki: None. T. Ohshima: None. S. Itohara: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.22/QQ10

Topic: D.09. Multisensory Integration

Support: Grant from Singapore Ministry of Education

Title: Characterization and functional analysis of claustral VIP interneurons

Authors: *M. GRAF[1], G. J. AUGUSTINE[2];
[1]A*STAR/ Inst. of Mol. and Cell Biol., Singapore, Singapore; [2]Lee Kong Chian Sch. of Med., Singapore, Singapore

Abstract: The claustrum is a little-understood structure that is embedded within the deep layers of the insular cortex. As in all other brain regions, it is likely that interneurons play important roles in information processing within the claustrum. We have defined the morphology, intrinsic electrical properties and functional connectivity of one type of claustral interneuron, VIP expressing interneurons (VIP^+ INs). The electrical properties of these interneurons were determined via patch clamp recordings in acute brain slices. VIP^+ INs from transgenic mice expressing Channelrhodopsin-2 exclusively in VIP neurons could be identified by photostimulation when exposed to blue light. VIP^+ INs differed from other claustral INs in their somatic shape, which were either multipolar or elongated/ bipolar. Their neurites projected mainly to the shell or edge of the claustrum core, which contrasts from the enrichment of processes from PV INs within the core. VIP^+ IN had the highest input resistance, broadest AP half-width, the shallowest AHP, and lowest maximum firing rate of any claustral INs. High-speed optogenetic circuit mapping (PNAS 104: 8143) revealed that VIP^+ INs are connected to projection neurons as well as to other IN populations. Remarkably, this inhibitory input was much stronger and more efficient for INs than for projection neurons. Thus, it appears that claustral VIP^+ INs specifically target other local IN populations and might relieve projection

neurons from local inhibition imposed by PV^+ and SST^+ interneurons. In general, VIP^+ INs are strategically situated to increase signaling of claustral projection neurons via disinhibition, well-positioning these INs to regulate information flow through the claustrum during attention allocation (TINS 38: 486), consciousness (Philos Trans R Soc Lond B Biol Sci. 360: 1271), or other functions proposed for this highly interconnected brain region.

Disclosures: M. Graf: None. G.J. Augustine: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.23/DP05 (Dynamic Poster)

Topic: D.09. Multisensory Integration

Support: TLST: NIH RSFA F31 Grant (1F31NS093790-01) (NINDS)

TLST: T32 Neuroscience Advanced Predoctoral Institutional Training Grant (2T32NS062443-6A1)

JLB and CMRV: Brown University Undergraduate Teaching and Research Awards

OAC and CMRV: American Physioloical Society undergraduate fellowship

CDA: NSF IOS 1353044

Title: Cellular and behavioral correlates of the principle of inverse effectiveness in multisensory integration in the developing optic tectum

Authors: *T. L. S. TRUSZKOWSKI, O. A. CARRILLO, J. L. BLEIER, C. M. RAMIREZ-VIZCARRONDO, C. D. AIZENMAN;
Neurosci., Brown Univ., Providence, RI

Abstract: Sensory information representing different modalities is combined in the brain to provide information necessary for an organism to interact with the world. This process, termed multisensory integration, is known to occur in single cells in the optic tectum, or its mammalian homologue, the superior colliculus. However, little is known about intracellular and network level processes giving rise to multisensory integration. In this study, we use the *Xenopus laevis* tadpole optic tectum to build a multi-dimensional model of multisensory integration. The optic tectum receives sensory information from the ears, eyes and lateral line and integrates it to generate behavioral output. Using both behavioral and cellular techniques to assess multisensory

integration, we provide a robust, integrative approach to understanding multisensory integration. One important manifestation of multisensory integration, known as the principle of inverse effectiveness, states that when weak sensory stimuli are combined, the result is a supralinear enhancement of the combined response that increases its saliency, whereas combination of strong sensory stimuli that are already salient does not result in additional benefit. Behaviorally, we show that this principle is conserved across species: when tadpoles are presented with a low saliency visual stimulus, adding a subthreshold acoustic stimulus increases behavioral response likelihood, while the subthreshold acoustic stimulus provides no added benefit when paired with a high saliency visual stimulus. To assess inverse effectiveness at the cellular level, we used intracellular whole-cell current clamp recordings and found that combining large suprathreshold stimuli does not result in an increased response magnitude. However, when two subthreshold stimuli are combined, the resulting response is amplified in a supralinear manner, showing that the principle of inverse effectiveness holds at the intracellular level. We further showed that this response depends on NMDA receptor activation. To build on these results, we studied network activity in the tectum in response to visual and mechanosensory stimuli using *in vivo* Ca++ imaging. Preliminary results show that integrating cells are distributed throughout the tectum, often adjacent to cells that only respond to a single modality and have varying levels of integration. In conclusion, our data provide evidence for the conservation of multisensory processing across vertebrates and lay the groundwork for investigation of many unanswered questions.

Disclosures: T.L.S. Truszkowski: None. O.A. Carrillo: None. J.L. Bleier: None. C.M. Ramirez-Vizcarrondo: None. C.D. Aizenman: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.24/QQ11

Topic: D.09. Multisensory Integration

Support: NIH R01 DC05640

Title: Synaptic inputs to toric spines.

Authors: *K. E. PANNONI[1], D. SANCULI[1], E. BUSHONG[2], M. SUNG[1], V. POPAT[1], C. ZAHER[1], M. ELLISMAN[2], W. DEBELLO[1];
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Abstract: We recently reported the discovery of toric spines, novel dendritic structures found on a subset of space-specific neurons (SSNs) in the barn owl inferior colliculus. To determine convergence patterns we located all synaptic inputs to one SSN that had been imaged at 10nm resolution using serial block electron microscopy. Active zones made onto the 76 toric spines were identified, as well as those onto the cell body, six proximal dendrites and one thin dendrite. The total number of active zones made onto toric spines was 512, while the soma received 131 and the dendrites 386. Thus, toric spines represent a major input-output hub of SSNs. Toric spines varied in volume (>10-fold), number of holes (0-7) and number active zones received (1-49), and these features were significantly correlated. The axonal inputs to eight representative toric spines were reconstructed to the point at which they exited the local neighborhood (~20 microns). All but one toric spine received synapses originating from multiple axons with distinct incoming trajectories (range 1-11). These likely represent distinct input sources. This is in stark contrast to hippocampal thorny excrescences, which receive dominant input from a single mossy fiber termed the “detonator” synapse. The large majority of axons coursing through each local neighborhood did not synapse onto the toric spine. Thus, the filling fraction was low and on par with that observed in mammalian cortex. In addition, the large majority of local axons were unmyelinated, though hundreds of myelinated axons were embedded in the larger volume. We propose that toric spines are a subcellular locus of adaptive plasticity. Identification of the source of the diverse inputs is a current goal.

Disclosures: K.E. Pannoni: None. D. Sanculi: None. E. Bushong: None. M. Sung: None. V. Popat: None. C. Zaher: None. M. Ellisman: None. W. DeBello: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.25/QQ12

Topic: D.09. Multisensory Integration

Support: NRF-2015048055

NRF-2014R1A1A3054603

NRF-GFP-CISS-2012M3A6A6054204

KHIDI-HI14C2437

KAIST-N10140034

Title: Auditory dominance over visual perception in the mouse integrating visual-auditory conflict

Authors: *Y.-H. SONG, J.-H. KIM, H.-W. JEONG, I. CHOI, D. JEONG, K. KIM, S.-H. LEE; Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: We often experience the conflicts between the sensory modalities, and our brain resolves these conflicts through the fast and accurate integration and elicits coherent perceptual behavior. During the integration of visual-auditory conflict, one modality often dominates the other. However, we still do not understand the neural mechanism of perceptual dominance during the integration of the conflict between sensory modalities. Here, we devised the visual-auditory discrimination tasks both under the actual and artificial conditions. Mice learned to discriminate visual and auditory information, and they showed auditory-dominant perceptual behaviors during the integration of the conflict. We found that the direct inputs from V1 and A1 to the posterior parietal cortex (PTLp) are critical for inducing auditory dominance over visual perception. Inactivation of the PTLp switched the auditory dominance to visual dominance without affecting visual or auditory perception. Furthermore, only V1-selective neurons in PTLp showed significant reduction in their responses to simultaneous V1 and A1 stimulation. Our results demonstrate that the converging inputs from V1 and A1 to PTLp mediate the auditory-dominant perception during the integration of visual-auditory conflict.

Disclosures: Y. Song: None. **J. Kim:** None. **H. Jeong:** None. **I. Choi:** None. **D. Jeong:** None. **K. Kim:** None. **S. Lee:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; National Research Foundation of Korea, Korea Health Industry Development Institute.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.26/QQ13

Topic: D.09. Multisensory Integration

Support: NIH DC 014101

NSF GRFP

Klingenstein Foundation

Coleman Memorial Fund

Hearing Research Institute

Title: Visual modulation of activity in mouse auditory cortex

Authors: *R. J. MORRILL[1,2,3], A. R. HASENSTAUB[1,2,3];
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Abstract: To construct an actionable percept of the world, the brain integrates information streams from multiple sensory modalities. In the cortex, this process is traditionally held to occur in higher-order association areas; however, work over the past decade suggests that even primary sensory cortex may receive and integrate information from other modalities (Wallace et al., 2004; Ghazanfar & Schroeder, 2006). In a variety of organisms, auditory cortex responds to stimuli from other modalities, both in conjunction with and independent of acoustic stimulation (Kayser et al., 2008; Bizley et al., 2007). Despite the importance of the mouse as a systems neuroscience model for circuit dissection, it remains unknown to what degree visual stimuli modulate or drive responses in mouse auditory cortex. Here we describe the temporal and laminar organization of visual influences on neural firing in the auditory cortex of the awake mouse, using multisite probes to sample across multiple cortical layers. We demonstrate that these recording sites are located in auditory cortex based on highly characteristic neural responses to sounds such as click trains and pure tones of varied frequencies and sound levels. We then determine whether depths of the electrode penetration are within cortex through electrode track tracing using fluorescent dye. We find that spiking responses to visual stimulation are biased to deeper layers, while field potential modulation is present throughout the cortical column. We speculate as to the role of these responses in modulating auditory cortex during sound processing.

Disclosures: R.J. Morrill: None. A.R. Hasenstaub: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.27/QQ14

Topic: D.09. Multisensory Integration

Support: NIH Grant NS064013

NIH Grant NS082658

Title: Lack of intrinsic GABAergic connections in the thalamic reticular nucleus of the mouse

Authors: G. HOU, A. G. SMITH, *Z.-W. ZHANG;
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Abstract: The thalamic reticular nucleus (TRN) provides the major GABAergic input to the thalamus and is critically implicated sensory detection, selective attention, and arousal. Dysfunction of the TRN is associated with epilepsy and schizophrenia. It is generally thought that TRN neurons form GABAergic synapses with other TRN neurons and that these interconnections are important for the function of the TRN. However, the existence of such intrinsic connections is controversial. We combined two complementary approaches to examine intrinsic GABAergic connections in the TRN of the mouse. First, we expressed channelrhodopsin in nearly all TRN neurons. We found that optogenetic activation of TRN neurons and their axons evoked GABAergic postsynaptic currents (IPSCs) in TRN neurons in mice younger than 2 weeks of age but failed to do so after that age. Second, we genetically blocked synaptic release from TRN neurons through conditional deletion of vesicular GABA transporter. This had no effect on spontaneous IPSCs recorded in TRN neurons while dramatically reducing spontaneous IPSCs in thalamic relay neurons. Our results demonstrate that except for a short period after birth, the TRN of the mouse lacks intrinsic GABAergic connections.

Disclosures: G. Hou: None. A.G. Smith: None. Z. Zhang: None.

Poster

244. Multi-Sensory Integration: Circuits and Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 244.28/QQ15

Topic: D.09. Multisensory Integration

Support: Mathers Foundation award 003155-00001 (A.D.)

University of California Center for Accelerated Innovation grant U54HL119893 (I.S.)

Title: The role of ionotropic glutamate receptors in non-synaptic cross-depolarization in the mammalian dorsal root ganglia

Authors: G. CARVALHO[1], Y. MULPURI[2], A. DAMASIO[1], *I. SPIGELMAN[2];
[1]Brain and Creativity Inst., USC, Los Angeles, CA; [2]Div. of Oral Biol. & Medicine, Sch. of Dent., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Afferent pathways are thought to act largely as isolated channels relaying information from the periphery to central synapses connecting to secondary neurons. However, several

instances of orthogonal, non-synaptic communication have been described between parallel pathways that challenge this simplistic view of neuronal communication. Here we sought to investigate the physiological mechanisms of cross-depolarization (CD) in mammalian dorsal root ganglia (DRG), a phenomenon where action potentials evoked in afferent axons and propagated to the neuronal somata in DRG induce sub-threshold depolarizations in neighboring neurons. Mammalian DRG neurons are anatomically isolated from one another and are not synaptically interconnected. Yet, CD is observable in a majority of these cells, encompassing both A- and C-type fibers. CD has been proposed to be mediated by a chemical neurotransmitter(s) released from the somata of DRG neurons (Amir & Devor, J. Neurosci., 1996), however, the identity of the neurotransmitter(s) remains elusive. We used electrophysiological recording techniques in L5 DRG preparations isolated from naïve rats to begin exploring the identity of neurotransmitter(s) that may mediate CD. During intracellular recordings with 3M potassium acetate filled sharp microelectrodes, CD could be evoked by stimulation of the spinal nerve at 85% intensity necessary to elicit an action potential in the recorded neuron. CD magnitude was proportional to the frequency (10-500 Hz) of spinal nerve stimulation (n = 20 cells). Since glutamate is considered as the primary neurotransmitter at the central terminals of sensory afferents, we hypothesized that glutamate and its cognate receptors may mediate CD in DRG neurons. To test this hypothesis, we recorded CD before and after combined application of selective ionotropic glutamate receptor blockers, D(-)-2-amino-5-phosphonopentanoate (40 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM), in the recording chamber. Glutamate receptor blockade had no significant effect on the magnitude of CD (average magnitude was 85.1% ± 25.2 of the controls, n=7 cells), suggesting that ionotropic glutamate receptors do not play a central role in non-synaptic cross-depolarization in mammalian sensory ganglia.

Disclosures: G. Carvalho: None. Y. Mulpuri: None. A. Damasio: None. I. Spigelman: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.01/QQ16

Topic: E.03. Basal Ganglia

Title: Cell-type-specific plasticity at corticostriatal synapses prevents suppression of habitual control of behavior

Authors: *A. CAVACCINI[1], V. PAGET-BLANC[1], M. TRUSEL[1], A. ROCCHI[2], M. PENNUTO[3], A. CONTESTABILE[1], B. GRECO[1], R. TONINI[1];

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Abstract: The ability to adapt behavior and effectively cope with an ever-changing environment requires flexible, goal-directed control of behavior, which strictly depends on the causal relationship between the action and the outcome (A-O). With repetition, behavior becomes more automatic and habitual, and actions are no longer sensitive to changes in A-O associations. In learning processes triggered by repeated training trials, priming stimulation of intracellular signaling pathways relevant for synaptic plasticity can affect the extent to which subsequent learning occurs, a process conceptualized as metaplasticity. This might also apply to the impossibility of updating changes in A-O contingencies that characterize inflexible habitual control of behavior. Nonetheless, whether instrumental learning triggers metaplastic processes at corticostriatal synapses on striatal projections neurons (SPNs) of the direct- (dSPN) and indirect (iSPN) pathway remains unexplored.
To test these hypotheses we subjected mice to different training regimes of instrumental conditioning of nose poke for food reward, which promote either goal-directed (short training) or habitual behavior (over training). Training was followed by an omission procedure in which mice have to learn a new causal A-O relation. We found that the inability of reverting A-O association was directly associated with reduced activation of signaling cascades downstream of striatal metabotropic glutamate receptors 1/5 (mGluR1/5), resulting in opposite changes in spike-timing dependent plasticity in dSPNs and iSPNs of the dorsolateral striatum (DLS). Consistent with a metaplastic process, these synaptic and biochemical effects occurred selectively in over trained habitual mice when tested upon omission. Preventing the activation of DLS mGluR5 in-vivo during training restored behavioral sensitivity to omission contingency, and averted biochemical and synaptic changes. Together, our findings point to cell-type-specific mechanisms of metaplasticity at corticostriatal synapses as habit forms, and establish mGluR5-mediated signaling as key molecular substrate for cognitive inflexibility in habitual behavior.

Disclosures: A. Cavaccini: None. V. Paget-Blanc: None. M. Trusel: None. A. Rocchi: None. M. Pennuto: None. A. Contestabile: None. B. Greco: None. R. Tonini: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.02/QQ17

Topic: E.03. Basal Ganglia

Title: Excitatory serotonergic signaling regulates synaptic plasticity at striatal glutamatergic inputs

Authors: *M. GRITTI[1], M. TRUSEL[1], A. GIORGI[2], A. CAVACCINI[1], S. MIGLIARINI[2], A. GOZZI[3], M. PASQUALETTI[2], R. TONINI[1];
[1]Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Universitá di Pisa, Pisa, Italy;
[3]Fondazione Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: The dorsal striatum receives glutamatergic cortical and thalamic afferents, which are extensively modulated by monoaminergic inputs, such as dopamine and serotonin (5-HT). Various models have been proposed regarding the interactions between reward and punishment, with serotonin closely associated with learning of negative events, and countervailing dopamine regulation of corticostriatal circuits and reward. Recent evidence has challenged this view, suggesting that 5-HT signaling can synergize with dopamine signaling to shape reward-guided behavior. However, the molecular and synaptic correlates of this behavioral role of 5-HT at striatal circuits remain to be established.
In the dorsolateral striatum, we found that the chemogenetic inhibition of 5-HT release resulted in a spike-timing-dependent form of long-term depression (t-LTD) at glutamatergic synapses on striatal projection neurons of the direct pathway (dSPNs). These synaptic effects of chemogenetic inhibition of 5-HT release were recapitulated by the pharmacological inhibition of the Gs-coupled 5-HT4 receptor subtype (5-HT4R). t-LTD is independent from endocannabinoid signaling, shows a postsynaptic locus of expression, and it is associated with an increased dendritic Ca^{2+} signal. Additionally, optogenetic interrogation of glutamatergic inputs to dSPNs indicated that this form of t-LTD is expressed at thalamostriatal synapses. Inhibiting *in-vivo* 5-HT4R during instrumental conditioning of nose-poke for food reward affected goal-directed control of behavior, thus supporting the role of striatal 5-HT signaling in mediating aspects of reward-guided behavior.

Disclosures: M. Gritti: None. M. Trusel: None. A. Giorgi: None. A. Cavaccini: None. S. Migliarini: None. A. Gozzi: None. M. Pasqualetti: None. R. Tonini: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.03/QQ18

Topic: E.03. Basal Ganglia

Support: Okinawa Institute of Science and Technology Graduate University

Human Frontier Science Program Grant

Title: Chemogenetic control of striatal cholinergic interneurons in haloperidol treated mice

Authors: *A. ZUCCA, S. ZUCCA, J. WICKENS;
Neurobio. Res. Unit, Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Dopamine antagonists used in treatment of schizophrenia often induces side effects, including a syndrome of muscle rigidity and frozen posture known as catalepsy. Anti-cholinergic drugs are commonly used to relieve the symptoms of catalepsy but their cellular mechanism of action is not fully understood. The striatum is a brain region rich in acetylcholine which is provided mostly by local cholinergic interneurons (CINs). We investigated the hypothesis that lowering activity in the striatal CINs would ameliorate catalepsy, and that increasing CIN activity would worsen the symptoms. We induced catalepsy in transgenic rodents by administering haloperidol, and used ChAT-cre lines and specific expression of designer receptors exclusively activated by designer drugs (DREADDs), to experimentally test the effect of manipulation of CINs on measures of catalepsy. Haloperidol induced catalepsy and impaired locomotion measured with the bar-test and open field test. DREADD-induced increase in CIN firing in haloperidol treated animals did not cause increase of bar time score nor decreased locomotion. In addition, decreasing activity in CINs did not reverse haloperidol-induced catalepsy or increase locomotor activity. These negative results may indicate that anti-cholinergic drugs work by effects on other cholinergic systems that are extrinsic to the striatum, or alternatively, that the DREADD-induced manipulations of CINs were localized to regions that do not mediate cataleptic effects. Further work is needed to characterize the role of CINs in catalepsy.

Disclosures: A. Zucca: None. S. Zucca: None. J. Wickens: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.04/QQ19

Topic: E.03. Basal Ganglia

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

Title: A dual purpose optical system for fluorescence imaging and spectrally resolved fiber array photometry in freely moving animals

Authors: *C. MENG[1], G. CUI[2];
[1]Lab. of Neurobiology/ In Vivo neurobiology group, NIH/NIEHS, Research Triangle Park, NC;
[2]NIEHS/NIH, Research Triangle Park, NC

Abstract: Monitoring the cellular and molecular events in specific types of cells in behaving animals using genetically encoded fluorescent sensors is a powerful approach to decode neural correlates of specific behaviors, and to uncover the mechanisms underlying the psychiatric and neurological disorders. Two types of optical methods are currently available for collecting fluorescence signals from deep brain structures in freely moving animals: the fiber photometry technique and the GRIN lens imaging method. Fiber photometry is a minimally invasive method that collects signals through an optical fiber probe placed above the targeted brain structure. The small size of the probe (125 micrometer OD) and long detection depth (up to 250 -500 micrometer from the tip) can minimize the disturbance of the measured structure. However, fiber photometry method cannot resolve individual cells. The signals collected by a fiber photometry system can only be interpreted as the population activity within the detection cone. When more detailed information on the activity pattern of individual neurons is desired, the GRIN lens-based imaging technique is currently the best option. The major concerns associated with GRIN lens imaging are the relative large tissue damage due to the size (typically 1 mm OD) of the GRIN lens and the low successful rate of the surgeries. Thus, fiber photometry and GRIN lens imaging are two complementary techniques that both have their own strengths and weaknesses, and should be chosen by the need of specific experiments. The current fiber photometry systems and GRIN lens imaging systems are based on completely different platforms; therefore, two separate systems are required to perform photometry and imaging experiments. To integrate the functionality of a fiber photometry system and a GRIN lens imaging system into a single system, we have built a dual purpose microscope that can be easily converted between the GRIN lens-based imaging mode and the spectrally resolved fiber array photometry mode to measure the fluorescence signals in freely moving animals. We were able to resolve individual neurons in the striatum in the imaging mode in mice expressing fluorescent sensors in specific striatal pathways, and simultaneously monitor the neural activity in 8 discrete locations in the dorsal and ventral striatum using the fiber photometry mode.

Disclosures: C. Meng: None. G. Cui: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.05/QQ20

Topic: E.03. Basal Ganglia

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

Title: Simultaneous multi-component measurements in the striatum using spectrally resolved *In vivo* fiber photometry

Authors: *J. ZHOU, C. MENG, G. CUI;
NIEHS/NIH, Research Triangle Park, NC

Abstract: The striatum is the central hub of the basal ganglia that integrates glutamatergic inputs from the cortex and the thalamus and dopaminergic input from the substantia nigra pars compacta (SNc). We have recently reported that the two principal types of striatal projection neurons (SPNs), the D1 dopamine receptor-expressing direct-pathway SPNs and the D2 dopamine receptor-expressing indirect-pathway SPNs, are both activated during action initiation. To further investigate how dopamine modulates these two pathways and facilitates voluntary movements in vivo, we have developed a novel spectrally resolved fiber photometry system that allows for simultaneous monitoring of multiple cellular events from molecularly defined groups of neurons in the local brain circuit using different color fluorescent sensors. Using this system, we are able to simultaneously record glutamatergic inputs and postsynaptic responses in individual striatal pathways using the green glutamate sensor iGluSnFR and the red calcium sensor jR-GECO1a. We are also able to simultaneously record the presynaptic dopaminergic axon terminal activity and the corresponding postsynaptic responses in each pathway using the green calcium sensor GCaMP6f and jR-GECO1a.

Disclosures: J. Zhou: None. C. Meng: None. G. Cui: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.06/RR1

Topic: E.03. Basal Ganglia

Support: NIAAA (grant 2R01AA016022)

Swedish e-Science Research Center

Swedish Research Council

Human Brain Project

Title: Exploration - exploitation at the cellular level

Authors: R. LINDROOS[1], *J. HELLGREN KOTALESKI[2];
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Abstract: The dorsolateral striatum is involved in motor learning and habit formation. During learning the principal cells of striatum, the medium spiny neurons (MSN), attain a stereotypical firing pattern — as the variability of the output is reduced the performance is increased. Dopamine has been proposed to play an active role in regulating this type of learning. However, little is known about how this regulation could take place at the single cell level. We here use computational modeling to investigate the dopaminergic modulation of signal integration in the MSN, specifically comparing signals that are spatially clustered with randomly dispersed input. The assumption is that structurally similar inputs form clusters that are strengthened through learning to generate a deterministic output, while noise or randomly dispersed inputs provide variability to the output. Our simulations predict that clustered inputs give the largest somatic response if triggered in the middle of the dendritic tree, where the axial resistance and the input resistance are balanced. At this point the input can trigger an NMDA spike that is not attenuated too much as it propagates to the soma. When comparing the somatic response resulting from either spatially clustered or randomly dispersed input we see the same type of pattern. The clustered input is more efficient than dispersed in the middle of the dendritic tree while the opposite is true for the more distal parts. In the proximal dendrites no significant difference between the two types of inputs is seen. Finally when repeating the same type of stimulation with concurrent dopamine modulation we see that the relative optimal distance is changed. Under the previously mentioned assumptions our simulations support the idea that dopamine is responsible for regulating the exploration/exploitation balance and provides a plausible mechanism for how this can be regulated at the single cell level.

Disclosures: R. Lindroos: None. J. Hellgren Kotaleski: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.07/RR2

Topic: E.03. Basal Ganglia

Title: Nociceptin/orphanin FQ and dopamine receptors co-localization in the mouse basal ganglia

Authors: *D. MERCATELLI[1], A. RIMESSI[2], P. BOLOGNESI[3], A. CIPPITELLI[4], B. L. KIEFFER[5], L. TOLL[4], M. MORARI[3];
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Abstract: Nociceptin /Orphanin FQ (N/OFQ) and dopamine (DA) interact to regulate motor function through the basal ganglia in vivo. A functional interaction between signaling downstream of the N/OFQ opioid peptide (NOP) receptor and the DA receptors has also been detected in striatal slices. To investigate whether these interactions reflect co-localization of NOP and DA receptors in the basal ganglia, we performed immunohistochemistry (IHC) in striatum, substantia nigra pars compacta (SNc) and reticulata (SNr), and globus pallidus (GP). Due to the lack of validated antibodies for the NOP receptor, we used mice expressing the NOP receptor tagged with eGFP (NOP-eGFP mice; Ozawa et al, *J Neurosci* 35:11682-93, 2014). This study was performed under either basal or NOP activation conditions, to investigate whether NOP receptor activation affects D1 or D2 subcellular localization.
NOP-eGFP mice showed a moderate but consistent (~20%) NOP-D1 membrane co-localization in different portions (dorsal, central and ventral) of the striatum. Conversely, a clear segregation of NOP and D1 receptors was found in SNc (lack of D1 staining) and SNr (lack of NOP staining). Also in GP no NOP receptor signal was observed. In mice treated with the NOP agonist SR-16835, the extent of NOP-D1 receptor co-localization was unaffected in dorsal and central striatum while it was abolished in ventral striatum. To circumvent the failure of directly staining D2 receptors with different commercial IHC antibodies, we attempted to indirectly localize striatal D2 receptors using adenosine A_{2A} receptor antibodies, since A_{2A} receptors selectively localize on the membranes of striato-pallidal medium-sized spiny neurons (MSNs) where they dimerize with D2 receptors. NOP receptors did not localize with striatal A_{2A}-positive neurons in any striatal regions analyzed.
These data indicate that a significant proportion of striatal D1 and NOP receptors co-localize on the membranes of MSNs. This provides a morphological basis to the functionally negative interaction between NOP and D1 receptors observed in striatal MSNs, which might subserve the antidyskinetic action of NOP receptor agonists in a rat model of levodopa-induced dyskinesia (Marti et al, *J Neurosci* 36:16106-19, 2012). NOP-eGFP mice might thus represent a valuable tool to investigate NOP-DA receptors interaction and help shed light on the mechanisms through which N/OFQ regulates motor function in physio-pathological conditions.

Disclosures: D. Mercatelli: None. A. Rimessi: None. P. Bolognesi: None. A. Cippitelli: None. B.L. Kieffer: None. L. Toll: None. M. Morari: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.08/RR3

Topic: E.03. Basal Ganglia

Support: The CHDI Foundation (A-5552)

The William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

The Collaborative Center for X-linked Dystonia Parkinsonism at Massachusetts General Hospital

The Saks Kavanaugh Foundation

Title: Striosome-dendron bouquet formations: a unique striatonigral circuit connection engaging dopamine-containing neurons and their ventrally extending dendrites

Authors: *J. R. CRITTENDEN[1], M. H. RIAD[2], P. W. TILLBERG[2], Y. SHIMA[3], D. E. HOUSMAN[2], S. B. NELSON[3], E. S. BOYDEN[2], A. M. GRAYBIEL[2];
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Abstract: Neurons of striosomes are considered to be the main source of dorsal striatal input to the dopamine-containing neurons of the substantia nigra pars compacta (SNc), both via direct projections to dopamine-containing nigral neurons, and via indirect projections through a pallido-lateral habenula circuit. This striatonigral system is known to target the ventral SNc, and evidence suggests that such ventral dopamine-containing neurons are among SNc neurons projecting back to striosomes. Thus the classic nigro-striato-nigral loop is at least in part based on the striosomal system. This possibility is of great interest, because striosomes are in a position to modulate and transmit signals to the SNc from limbic regions of the neocortex known to be dysregulated in mood disorders in humans. Here, we describe the discovery of an unusual form of synaptic connectivity between striosomal axons and bundled ventrally extending dopamine-containing SNc dendrites entwined and collected together in bouquet-like formations within the substantia nigra. Identifying these striosome-dendron formations was enabled by exploiting new tool-sets. First, we capitalized on our prior discovery of two members of the Ras/Rap signaling cascade that have complementary preferential expression in striosomes (CalDAG-GEFII) and matrix (CalDAG-GEFI) to detect striatonigral inputs in BAC transgenic mice with green fluorescent protein reporters for these genes (Kawasaki et al., 1998, *PNAS*; Gong et al., 2003, *Nature*; Crittenden et al., 2009, *PNAS*). Second, we used an additional, highly specific striosome-reporter mouse line (PiggyBac line P172-mCitrine; Shima et al., 2016, *Elife*) to confirm

striosome targeting of the dendron bouquet formations. Third, we used optimized expansion microscopy (proExM; Tillberg et al., 2016, submitted) to resolve striosomal terminal axons and their dopamine-containing targets with a resolution of 100 nm, along with synaptic markers related to specific types of neurotransmission. With ProExM imaging, we examined inhibitory, excitatory and neuromodulatory synaptic connections within the striosome-dendron plexus formations. We suggest that these dendron bouquet formations are integrative units relating striosomal signals to signaling by glutamate, GABA and acetylcholine for potential control of specific subclusters of nigral dopamine-containing neurons. These results could be critical to understanding nigro-striato-nigral loop function and the human disorders to which dysfunctions of the dopamine system have been related.

Disclosures: J.R. Crittenden: None. M.H. Riad: None. P.W. Tillberg: None. Y. Shima: None. D.E. Housman: None. S.B. Nelson: None. E.S. Boyden: None. A.M. Graybiel: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.09/RR4

Topic: B.05. Transporters

Support: Okinawa Institute of Science and Technology Graduate University

Title: Striatal cholinergic interneurons: their depletion and its progression

Authors: *N. ABUDUKEYOUMU[1], M. GARCIA-MUNOZ[1], O. P. JAIDAR[1,2], G. ARBUTHNOTT[1];
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Abstract: Even before the discovery that Parkinson's was produced by the loss of dopaminergic neurons, this neurological disease was treated with anticholinergic drugs. A balance between cholinergic and dopaminergic activity in striatum is not only important in PD but for the normal function of the nucleus (i.e., behavior, reward, memory and cognitive functions). An important source of striatal acetylcholine (Ach) comes from giant and sparsely distributed cholinergic interneurons (ChI). However, their study has been hampered by a concentration of only 1-3 % of the whole striatal cell population. We performed a stereological systematic random sampling of striatal tissue from 21 days old C57BL/6J male mice. To selectively deplete ChI we performed a stereotaxic injection of saporin ribosome inactivating immunotoxin that targets choline acetyltransferase (0.3µl). Following survival periods of 2, 4 or 6 weeks, animals were sacrificed

and brain sections immunostained against ChAT to identify ChI, or against vesicular acetylcholine transporter (vAChT) to identify synaptic boutons. For each of the three survival periods, we counted and compared the number of ChIs between the intact and the lesioned hemispheres and the change in the number of vesicular acetylcholine transporters (vAChT). Compared to striatal sections from naïve controls and sham injections, we observed a decrease in ChIs according to each survival period of 24.4% (week 2, n=9), 33.74% (week 4, n= 11) and 19.89% (week 6, n=10). In contrast, we observed a percent increase in vAChT positive boutons of 42.3, 21.6 and 28.3% for each of the respective survival periods (n=9, n=11 and n=10). We are investigating whether the increase in vAChT positive terminals is due to an indirect upregulation produced by compensatory axonal sprouting from surviving ChI, or from afferent axonal terminal fields of cholinergic mesopontine neurons.

Disclosures: N. Abudukeyoumu: None. M. Garcia-Munoz: None. O.P. Jaidar: None. G. Arbuthnott: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.10/RR5

Topic: E.03. Basal Ganglia

Support: NIH Grant NS95809

Title: Striatal cholinergic interneuron firing evokes spontaneous synaptic activation of muscarinic receptors

Authors: *A. MAMALIGAS[1,2], C. P. FORD[3];
[2]Neurosci., [3]Physiol. and Biophysics, [1]Case Western Reserve Univ., Cleveland, OH

Abstract: Temporally patterned cholinergic interneuron (ChI) activity is critical for striatal movement and motivated behaviors. The pacemaker activity and broad axonal arborizations of ChIs have been thought to create a background tone of acetylcholine that tonically activates muscarinic receptors on medium spiny neurons (MSNs), the main output neuron of the striatum. Despite the importance of acetylcholine at these synapses, it remains unclear how postsynaptic cholinergic receptors on MSNs directly encode the firing patterns of ChIs in the striatum. To examine this, we used viral overexpression of G-protein activated inwardly rectifying potassium (GIRK2) channels, which efficiently couple to endogenous $G_{i/o}$ coupled receptors, to readout muscarinic receptor activation on MSNs in response to ChI firing. Using a combination of optogenetics and paired recordings between ChIs and MSNs, we found that muscarinic receptors

could encode spontaneous monosynaptic acetylcholine release from the firing of individual ChIs. The resulting inhibitory post-synaptic currents (IPSCs) in GIRK2 expressing MSNs were transient and phasic in nature due to the efficient enzymatic activity of striatal acetylcholinesterase. We found that, despite the synaptic convergence of multiple ChIs on a given MSN, the efficient degradation of acetylcholine allowed for distinct phasic activation of postsynaptic receptors. Our results also show that in native systems not overexpressing GIRK channels, ChI firing was able to decrease collateral GABA release between pairs of direct pathway MSNs as a result of transient muscarinic receptor signaling within 500 ms. The high probability of release and large number of release sites at the ChI-MSN synapse allowed muscarinic receptors to entrain the physiological firing patterns of individual ChIs without failure. Our results show that muscarinic receptors on striatal output neurons respond to ChI firing such that they consistently encode the activity patterns of ChIs.

Disclosures: A. Mamaligas: None. C.P. Ford: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.11/RR6

Topic: E.03. Basal Ganglia

Support: NSERC

Title: Effect of striatal membrane cholesterol removal and replenishment on the dopamine transporter

Authors: *T. P. DIPAOLO[1,2], N. MORIN[1], M. MORISSETTE[1];
[1]Ctr. de Recherche de CHUQ-CHUL, Quebec, QC, Canada; [2]Laval University, Fac. of Pharm., Quebec City, QC, Canada

Abstract: The dopamine transporter (DAT) is abundant in the striatum where it reaccumulates dopamine (DA) molecules into presynaptic nerve terminals. It is the target of drugs of abuse such as cocaine and amphetamine that inhibit the reuptake of DA. It is also the target of antidepressants. There is a loss of DAT associated with the loss of DA neurons in Parkinson disease. The lack of DAT leads to less controlled release of L-DOPA replacement therapy that is implicated in L-Dopa-induced dyskinesias. Membrane cholesterol was previously shown to modulate the outward facing conformation of the DAT and a cholesterol binding site was observed in the crystal structure of the *drosophila* DAT. The present study investigated the effect of cholesterol removal and replenishment *in vitro* in rat total striatal homogenates. DAT protein

levels measures by Western blot showed no change with *in vitro* incubation in all experiments where cholesterol was removed from the membranes with methyl-beta-cyclodextrin (MbetaCD) incubations (1 hour) as well as in *in vitro* incubations (1 hour) to increase membrane cholesterol content. By contrast, significant changes of specific binding to the DAT in the striatum were measured with the radioligand 3-(4-[^{125}I] iodophenyl) tropane-2-carboxylic acid isopropyl ester ([^{125}I]-RTI-121). Non-specific binding was evaluated with binding in the presence of 100 nM of mazindol. A dose-response decrease of striatal [^{125}I]-RTI-121 specific binding was measured with increasing concentrations of MbetaCD (a decrease of 35-50% with 5mM MbetaCD) while the opposite was measured with cholesterol loading (increase of 43% with 1mM cholesterol). Moreover, [^{125}I]-RTI-121 specific binding of striatal membranes depleted of cholesterol with MbetaCD could be restored to their initial DAT content with addition of cholesterol showing the rapid and reversible effect of cholesterol manipulation on DAT binding. Moderate doses of MbetaCD to modulate DAT specific binding maintained the affinity of dopamine for the DAT as measured with dopamine competition for [^{125}I]-RTI-121 specific binding (Ki=5.4 microM in control homogenate). By contrast other striatal dopamine binding sites such as the dopamine receptors 1 and 2 as well as the monoamine vesicular transporter 1 showed no or limited changes by cholesterol manipulations. These results suggest that cholesterol has an important effect on the DAT and this could be taken into consideration for optimization of drug therapies.

Disclosures: T.P. DiPaolo: None. N. Morin: None. M. Morissette: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.12/RR7

Topic: E.03. Basal Ganglia

Support: R25GM069621-11

2G12MD007592

5SC1MH 086070-04

8G12MD007592

Title: Novel glycinergic circuitry in the basal ganglia

Authors: *R. A. PEREZ, R. ORTEGA, Y. P. HUIZAR, E. CASTANEDA, M. MIRANDA; Bio-Science, Univ. of Texas At El Paso, El Paso, TX

Abstract: The basal ganglia controls somatosensory perception, motor movement, emotions, and certain types of memory. Dopaminergic projections stemming from the substantia nigra pars compacta and reticulate (SNc and SNr, respectively) innervate the striatum to modulate GABAergic neuronal activity through activation of dopamine receptors. This study seeks to dissect out the cell type and precise location of glycine transporter 1 (GlyT-1) immunoreactivity in the basal ganglia. The presence of the GlyT-1 transporter has been documented in glial cells; however, recent immune-histochemical studies suggest the presence of this transporter in neuronal cells. Our initial findings using a retrograde tracer (Fluoro-Gold) injected into the Globus pallidus (GP) suggests the existence of several glycinergic nuclei in subcortical regions of the basal ganglia, including the midbrain. Confocal microscopy analysis of GlyT1 immunoreactivity suggests co-localization with several neuronal markers, including NeuN and Map2, in flouro-gold positive cells, consistent with the idea that GlyT-1 is present in neurons. To support our hypothesis, adeno-viral particles delivered into the midbrain, expressing mCherry under control of a neuronal promoter demonstrate reporter expression in GlyT-1 positive cells. Altogether this data confirms the presence of GlyT-1 in neurons in the midbrain that project to the GP, probably contributing to the regulation of activation of neurons in the striatum.

Disclosures: R.A. Perez: None. R. Ortega: None. Y.P. Huizar: None. E. Castaneda: None. M. Miranda: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.13/RR8

Topic: E.03. Basal Ganglia

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) Grant 06653

Fonds de Recherche du Québec Santé (FRQS) Grant

Title: Local action of 17beta-estradiol increases phasic dopamine release in the dorsal striatum.

Authors: *W. SHAMS, M.-P. COSSETTE, P. SHIZGAL, W. G. BRAKE;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Studies using *in vivo* microdialysis provide evidence that 17β-estradiol (E2) increases dopamine (DA) transmission in the dorsal striatum (Becker, 1990; Xiao & Becker, 1994; Becker & Rudick, 1999). For example, both systemic administration of E2 and local infusion into the dorsal striatum rapidly enhance amphetamine-induced DA release (Becker, 1990; Castner, Xiao

& Becker, 1993; Becker & Rudick, 1999). However, it is not known to what degree these effects reflect tonic and/or phasic DA release. In urethane-anaesthetized (1.5mL/kg) female rats, we used fast-scan cyclic voltammetry (FSCV) to study the effects of E2 on phasic, electrically-evoked release of DA in the dorsal striatum. Rats were ovariectomized and implanted with a silastic tube containing 5% E2 in cholesterol, previously shown to mimic low physiological serum concentrations of 18-32 pg/ml. Dopamine release was evoked every 1 min by delivering electrical stimulation to the substantia nigra (500ms trains of biphasic, 2ms pulses, 200 – 400 μA, 60Hz). Local infusions of E2 (244.8 pg/µl) in the vicinity of the dorsal striatal FSCV probe increased the amplitude of the electrically evoked DA transients, thus demonstrating that E2 action in the dorsal striatum specifically enhanced phasic DA release *in vivo*.

Disclosures: W. Shams: None. M. Cossette: None. P. Shizgal: None. W.G. Brake: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.14/RR9

Topic: E.03. Basal Ganglia

Support: Veteran's Administration VISN 22 MIRECC

R01 MH073991

Title: Effect of reduction in GABA function using a modified diphtheria toxin on behavior in mice

Authors: S. CALDWELL[1,2], D. KNOWLAND[1], J. DESLAURIERS[1,2], *S. B. POWELL[1,2], X. ZHOU[1,2];
[1]Univ. of California San Diego, La Jolla, CA; [2]San Diego VA Healthcare Syst., La Jolla, CA

Abstract: Decreased GABA function is a characteristic of schizophrenia and other neuropsychiatric disorders. Thus, being able to target GABA neurons specifically, producing a chronic state of decreased GABA function, will be critical to our understanding of the consequences of this reduced function. Diphtheria toxin which inhibits protein translation and causes apoptosis of cells can be used in an attenuated form to target GABA neurons. The current studies used a modified DTA-YFP fusion protein mutated for reduced toxicity, which inhibits protein translation but is not sufficiently toxic to kill cells, and examined changes in behavior. Specifically, we used an attenuated form of diphtheria toxin A (DTA), DTA tox-176. We generated a YFP-DTA tox-176 fusion protein to further decrease the DTA toxicity. We then

inserted the fusion gene, floxed by double lox-p sites, into an AAV vector which gets expressed in the presence of cre-recombinase and confirmed expression of the control AAV-YFP in striatum of Dlx6a-Cre mice (Cre expression in GABAergic neurons). GAD67 and dopamine D2 receptor expression were measured in striatum via immunohistochemistry to confirm the effects of the modified DTA. In behavioral experiments, AAV-DTA-YFP or AAV-YFP control were infused bilaterally into the dorsal striatum of Dlx6a-cre mice. After 6-8 weeks, mice were tested in locomotor activity, prepulse inhibition of startle, and spontaneous alternation in a t-maze. To probe function of the glutamate system in these mice, locomotor activity was also measured in open field after injection of 50 mg/kg ketamine hydrochloride. Mice infused with DTA tox-176-YFP in the striatum exhibited locomotor hyperactivity in an open field, increased prepulse inhibition of startle, and decreased spatial working memory. Ketamine increased distance traveled in both the control AAV5 Floxed EYFP and in the DTA tox-176-YFP infused mice. Disrupting GABA function by targeting GABA interneurons with DTA in striatum directly affected behavior of mice. Ketamine, which acutely blocks the NMDA receptor and facilitates glutamate release did not have an effect on the distance traveled of mice infused with DTA tox-176-YFP.

Disclosures: S. Caldwell: None. D. Knowland: None. J. Deslauriers: None. S.B. Powell: None. X. Zhou: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.15/RR10

Topic: E.03. Basal Ganglia

Support: DA024689

DA033554

Title: Critical role of striatal d2r signaling in the psychomotor effects of cocaine

Authors: *D. RADL, G. KHARKWAL, R. LEWIS, E. BORRELLI, 92617;
Microbiology and Mol. Genet., Univ. of California Irvine, Irvine, CA

Abstract: The intake of drugs of abuse generates a strong elevation of dopamine (DA) levels (Di Chiara and Imperato, 1988) in brain areas that belong to the limbic system (Di Chiara et al., 2004, Hyman et al., 2006) . DA signaling is thus believed to play a central role in the mechanisms underlying the behavioral effects of drugs of abuse in the central nervous system

(Vallone et al., 2000). The psychomotor effects of cocaine strongly depend on the activation of the major striatal output neurons, the medium spiny neurons (MSNs). In particular MSNs expressing DA D1 receptors (D1R) appear responsible for these effects. Indeed, mice lacking D1Rs fail to respond to cocaine (Xu et al., 1994). Nevertheless, the constitutive ablation of the DA D2 receptor (D2R) in mice also prevents the motor stimulating effects induced by cocaine (Welter et al., 2007). Since D2Rs have a wide and abundant distribution in the brain, it is yet unclear what are the mechanisms responsible for this phenotype. The recent generation of cell-specific D2R mutants allowed addressing the question. For this, we analyzed and compared the behavioral response to cocaine of wild type (WT) versus constitutive D2RKO, MSN-D2RKO and DA-D2RKO mice. MSN-D2RKO and DA-D2RKO are lines in which the specific ablation of D2R has been achieved in MSN, and in mesencephalic DA neurons, respectively. The effect of increasing concentration of cocaine was tested in acute in animals of all genotypes habituated to a novel home cage for 2 h. We hypothesized that since MSN neurons are the only output neurons of the striatum, D2R in these cells is important for the locomotor response to cocaine and other drugs of abuse. Indeed, we observed that MSN-D2RKO mice do not respond to cocaine at any of the dose tested. On the contrary, DA-D2RKO mice respond with an increase of motor activity as WT littermates. We conclude that the absence of D2R specifically in MSNs is responsible for the blunted response to cocaine observed previously in D2RKO mice. Further analyses will be aimed at exploring the molecular mechanisms underlying the phenotype of MSN-D2RKO mice.

Disclosures: D. Radl: None. G. Kharkwal: None. R. Lewis: None. E. Borrelli: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.16/RR11

Topic: E.03. Basal Ganglia

Support: CONACYT 152326

Title: GABAergic input to the globus pallidus externus (Gpe) is depressed by activation of dopamine D4 receptors.

Authors: I. CONDE-ROJAS[1], R. CABALLERO-FLORAN[1], J. ACEVES[1], D. ERLIJ[2], *G. B. FLORAN[1];
[1]CINVESTAV IPN, Mexico DF, Mexico; [2]State Univ. of New York, New York, NY

Abstract: Two major GABAergic afferents modulate the activity of the GPe. One group are the projections from medium spiny striatal neurons the other are the recurrent pallido-pallidal fibers that are collaterals of pallido-striatal fibers. In whole cell patch studies we have confirmed that the evoked inhibitory currents (eIPSCs) produced by release of GABA elicited by electrically stimulating striato-pallidal projections is inhibited by activation of D2Rs. In addition we found that quinpirole depresses the rate of discharge, but not the amplitude, of TTX sensitive GABAergic spontaneous currents (sIPSCs) in GPe neurons. This spontaneous activity has been generally identified as caused by transmitter release by recurrent collateral fibers of pallido-striatal neurons. The quinpirole effect is blocked by L 745,870 a selective D4R antagonist indicating that the response is mediated by D4Rs. In current clamp experiments we found that quinpirole reduces the frequency of spontaneous spiking rate of GPe neurons. This effect is blocked by D4R antagonist L 745,870. Determinations of quinpirole inhibition of [^{3}H] GABA release in K^{+}-depolarized slices show two components; one that is blocked by L 745,870 the other that is not affected by this antagonist. Our findings indicate that sIPSC rate of firing is depressed by activation of D4Rs.
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Disclosures: I. Conde-Rojas: None. R. Caballero-Floran: None. J. Aceves: None. D. Erlij: None. G.B. Floran: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.17/RR12

Topic: F.04. Stress and the Brain

Support: Hillman Family Foundation Grant 109033

NIH Grant R15NS093539

Title: Evidence for cross-hemispheric preconditioning in experimental Parkinson's disease

Authors: *R. K. LEAK, J. N. WEILNAU, N. NOURAEI, D. F. HUTCHISON, K. M. MINER, M. A. CARCELLA;
Pharmacol., Duquesne Univ., Pittsburgh, PA

Abstract: Parkinson’s disease often emerges unilaterally, with motor deficits eventually appearing on the contralateral side only after a considerable delay. It is well established that exposure to mild, subtoxic stress can prepare cells or organisms to survive subsequent toxic

challenges, a phenomenon known as preconditioning, tolerance, or stress adaptation. For example, preconditioning the hindlimbs with a mild ischemic episode has been shown to protect the heart and brain against a second ischemic hit. This type of ischemic stress tolerance acquired at a distant site is termed remote preconditioning, but has not been examined in Parkinson's disease. Given the asymmetrical nature of Parkinson's deficits, we hypothesized that unilateral striatal infusions of the oxidative toxicant 6-hydroxydopamine (6-OHDA) would remotely precondition the contralateral nigrostriatal pathway against the toxicity of a second 6-OHDA infusion in the opposite hemisphere. Consistent with the principles of remote preconditioning, 6-OHDA infusions in one mouse striatum completely abolished loss of the dopaminergic terminal marker tyrosine hydroxylase (TH) in response to a second 6-OHDA infusion in the contralateral striatum. TH^+ cell bodies in the contralateral substantia nigra were also robustly protected against the second 6-OHDA hit, demonstrating complete preconditioning of the entire nigrostriatal pathway in the opposite hemisphere. The results of the cylinder test for forelimb asymmetry were consistent with these histological observations. 6-OHDA infusions increased activation of the phosphokinase ERK2 and expression levels of the antioxidant enzyme CuZn superoxide dismutase (SOD1) in the striata of both hemispheres. In contrast, the phosphokinase ERK1 was only activated on the side ipsilateral to the first 6-OHDA hit. 6-OHDA failed to impact the striatal expression of Mn superoxide dismutase (SOD2), the protein DJ1, the phosphokinase Akt, the growth-associated protein GAP43 (axonal outgrowth marker), or the D1 and D2 dopamine receptors. These findings support the existence of cross-hemispheric preconditioning in experimental Parkinson's disease. Unlike in most preconditioning studies, the preconditioning stimulus was not mild or subtoxic, suggesting that even severely toxic stress can enhance endogenous defenses in the remaining cells. If these results generalize to humans, Parkinson's pathology may progress more slowly on the side opposite to the initial deficits because the contralateral hemisphere is better prepared to combat injury after exposure to the disease-precipitating stressor

Disclosures: R.K. Leak: None. J.N. Weilnau: None. N. Nouraei: None. D.F. Hutchison: None. K.M. Miner: None. M.A. Carcella: None.

Poster

245. Basal Ganglia: Neuromodulation of Striatal Circuits

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 245.18/RR13

Topic: G.03. Emotion

Support: FAPESP Grant 2013/19280-3

FAPESP Grant 2016/05544-7

Title: Influence of aversive stimulation on haloperidol-induced catalepsy in rats

Authors: *A. R. OLIVEIRA[1,2], N. C. B. BARROCA[2,3], M. D. GUARDA[1], N. T. SILVA[1], A. COLOMBO[2,4], A. E. REIMER[2,3,5], M. L. BRANDÃO[2,3];
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Abstract: Catalepsy is characterized by varying degrees of muscle rigidity resulting in an immobile state in which the subject is not able to change externally imposed postures. This condition can be observed in patients treated with haloperidol, a dopamine D2 antagonist widely used in schizophrenia. The pattern of the rigidity of haloperidol-induced catalepsy in rats is very similar to that observed in Parkinson's disease (PD); so this condition makes the haloperidol-induced catalepsy a potential animal model to PD motor impairments. PD seems to have many of its symptoms dependent on emotional state, for example, some immobile patients are able to make quick movements after hearing the cry "fire". On the other hand, anxiety disorders are common in PD. Therefore, it is possible that central mechanisms that regulate emotional and cataleptic states interplay. In this direction, a study suggested that interference in the neural substrates of the inferior colliculus - a midbrain structure involved in auditory and aversive processing and linked to structures of the motor system - can influence haloperidol-induced catalepsy. In addition, in a previous study, we showed that haloperidol reduced alarm calls emission and enhanced freezing response of rats re-exposed to a conditioned aversive context. Now, the current work aims to evaluate the influence of aversive events on the duration of haloperidol-induced catalepsy. For this, male Wistar rats were subjected to tests of catalepsy 15, 30, 45, 60, and 75 min after intraperitoneal administration of haloperidol or saline. To test the influence of aversive states on catalepsy, 30 min after treatment, distinct groups were exposed to open field (OF), elevated plus maze (EPM), confinement in an open arm of EPM, inescapable footshocks, contextual conditioned fear, or remained in the home-cage. Haloperidol-induced catalepsy was verified in all drug-treated animals, regardless their exposure to the aversive situations. Exposure to OF and EPM did not change haloperidol-induced catalepsy, compared to home-cage groups. Confinement to open arm of the EPM, footshocks and conditioned fear increased haloperidol-induced catalepsy 45, 60 and 75 min after drug administration in relation to the same group at 30 min time point. An interesting finding was that exposure to fear conditioning experiment increased haloperidol-induced catalepsy after 75 min compared to the group not exposed at the same time point. Our findings suggest that different threats, that presumably recruit partially distinct defensive circuitry, can influence differently the cataleptic state caused by haloperidol.

Disclosures: A.R. Oliveira: None. N.C.B. Barroca: None. M.D. Guarda: None. N.T. Silva: None. A. Colombo: None. A.E. Reimer: None. M.L. Brandão: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.01/RR14

Topic: E.03. Basal Ganglia

Support: NINDS R01NS078435-04

NINDS F32NS083369-03

Title: Regulation of striatal circuit function by fast-spiking interneurons.

Authors: *S. F. OWEN[1], J. D. BERKE[2], A. C. KREITZER[1];
[1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: The striatum is the primary input nucleus of the basal ganglia, a set of sub-cortical nuclei that are essential for motor planning and action selection. Parvalbumin-expressing fast-spiking interneurons (FSIs) in the dorsal striatum have been linked to decision making during goal-directed behavior, while defects in striatal FSIs have been associated with movement disorders including tics and dyskinesias. Acute slice recordings have demonstrated that FSIs form powerful inhibitory synapses onto Spiny Projection Neurons (SPNs), the principal neurons in the striatum, but cross-correlograms computed from chronic recordings have failed to uncover any clear evidence for this inhibition in vivo. This apparent contradiction raises questions regarding exactly how FSIs influence the striatal network in vivo. Using a combination of acute slice physiology, in vivo physiology, behavioral assays and imaging approaches we are investigating the role of FSIs in striatal physiology and behavior. Surprisingly, in contrast to previous studies using less specific pharmacological interventions, our genetically targeted manipulations of striatal FSIs caused no observable deficit in gross motor behavior. Interfering with striatal FSI function does, however, impair performance on more cognitively demanding basal ganglia-dependent tasks.

Disclosures: S.F. Owen: None. J.D. Berke: None. A.C. Kreitzer: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.02/RR15

Topic: E.03. Basal Ganglia

Support: NH NIDA K99DA038112

Title: Cellular taxonomy of the mouse striatum as revealed by single-cell rnaseq.

Authors: *O. GOKCE[1], G. STANLEY[2], B. TREUTLEIN[4], N. F. NEFF[3], G. J. CAMP[5], R. MALENKA[6], P. ROTHWELL[7], M. FUCCILLO[8], T. SÜDHOF[9], S. QUAKE[10];
[1]Inst. für Schlaganfall- und Demenzforschung (ISD), Lmu-Klinikum Der Univ. München, Muenchen, Germany; [2]Stanford Univ. Med. School, Stanford, A, Howard Hughes Med. Institute,, 94305, CA; [3]Stanford Univ. Med. Sch., Howard Hughes Med. Institute,, Stanford,, CA; [4]Max Planck Inst. for Evolutionary Anthropology, Dept. of Evolutionary Genetics,, Deutscher Platz 6, 04103 Leipzig, Germany; [5]Max Planck Inst. for Evolutionary Anthropology, Dept. of Evolutionary Genetics,, Leipzig, Germany; [6]Nancy Pritzker Laboratory, Dept. of Psychiatry and Behavioral Sci., Stanford, CA; [7]Nancy Pritzker Laboratory, Dept. of Psychiatry and Behavioral Sci., stanford, CA; [8]4Nancy Pritzker Laboratory, Dept. of Psychiatry and Behavioral Sciences,, stanford, CA; [9]Dept. of Mol. and Cell. Physiology, Howard Hughes Med. Institute,, stanford, CA; [10]Departments of Bioengineering and of Applied Physics,, Stanford University Medical School, Stanford, CA

Abstract: The striatum contributes to many cognitive processes and disorders, but its cell types are incompletely characterized. We show that microfluidic or FACS-based single-cell RNA sequencing of mouse striatum provides a well-resolved classification of striatal cell type diversity. Transcriptome analysis revealed 10 differentiated distinct cell types, including neurons, astrocytes, oligodendrocytes, ependymal, immune, and vascular cells, and enabled the discovery of numerous novel marker genes. Furthermore, we identified novel medium spiny neuron (MSN) subtypes with specific markers, many of which are linked to cognitive disorders and addiction. We also describe continuous cellular identities, which increase heterogeneity within discrete cell types. Finally, we identified cell type specific transcription and splicing factors that shape cellular identities by regulating splicing and expression patterns. Our findings suggest that functional diversity within a complex tissue arises from a small number of discrete cell types, which exist in a continuous spectrum of functional states.

Disclosures: O. Gokce: None. G. Stanley: None. B. Treutlein: None. N. F. Neff: None. G. J. Camp: None. R. Malenka: None. P. Rothwell: None. M. Fuccillo: None. T. Südhof: None. S. Quake: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.03/RR16

Topic: E.03. Basal Ganglia

Support: Pilot project grant from the Alcohol and Drug Abuse Center of Excellence, LSU Health Sciences Center, New Orleans

Title: Modeling mechanisms underlying K-ATP mediated bursting in medial SNc dopaminergic neurons.

Authors: *C. J. KNOWLTON[1], J. ROEPER[2], C. C. CANAVIER[1];
[1]Cell Biol. and Anat., LSUHSC, New Orleans, LA; [2]Inst. of Neurophysiol., Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract: We use a computational model of a dopamine neuron to explain how agonists of ATP-sensitive potassium (K-ATP) channels gate bursting selectively in medial substantial nigra (mSN) dopamine neuron and what factors may contribute to the sustained generation of rhythmic bursts as opposed to phasic bursts. Phasic bursts in midbrain dopamine neurons were previously thought to provide homogeneously reward-related signals. Recently, bursts in different subpopulations have been suggested to encode distinct signals. Specifically, bursts mediated by K-ATP channels in medial, but not lateral, substantia nigra (SN) dopamine neurons are thought to signal novelty (Schiemann et al 2012). The same study showed that only calbindin positive (CB+) neurons exhibit a sustained, rhythmic form of burst firing *in vivo*. Dopamine neurons *in vitro* fire in a regular, single spike firing, pacemaking pattern. Our computational model seeks to account for an *in vitro* model of K-ATP mediated bursting in which bath application of an NN414 K-ATP selective channel opener decreases the firing rate, and that of NMDA increases the firing rate, but the co-application of these drugs induced a bursty firing pattern with an even faster average firing rate. Our previous model assumed that the K-ATP channel was activated by the ADP/ATP ratio, which depended upon the cytosolic Ca^{2+} concentration with a delay (introduced by a first order differential equation). NN414 was assumed to increase the sensitivity of the K-ATP channel to the point that the faster spiking evoked by NMDA was periodically silenced, resulting in rhythmic bursting. We have extended these results to show that decreasing the Ca^{2+} buffering, as might be expected in CB- neurons, decreased the burst regularity. Moreover, we developed a new model that includes an explicit dependence on [ADP]. Pump activity converts ATP to ADP driving [ADP] accumulation, which decays exponentially due to ADP uptake. The rate of ADP uptake in the model controls the propensity to burst, providing a possible explanation for why this type of bursting occurs only in the medial and not the lateral SN despite similar levels of K-ATP conductance in both populations.

Disclosures: C.J. Knowlton: None. J. Roeper: None. C.C. Canavier: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.04/RR17

Topic: E.03. Basal Ganglia

Support: K08NS081001

Title: Striatal neurons show aberrant firing in levodopa-induced dyskinesia.

Authors: *M. RYAN[1], A. B. NELSON[2];
[2]Neurol., [1]UC San Francisco, San Francisco, CA

Abstract: As the target of both dopaminergic projections and sensorimotor cortical inputs, the striatum is hypothesized to be a major site of normal motor control and a primary site of pathological activity in Parkinson's Disease. Long-term dopamine replacement therapy is often complicated by the development of abnormal involuntary movements, known as levodopa-induced dyskinesia (LID). The cellular mechanisms underlying LID are unknown, but dopamine-driven aberrant striatal firing is a prevailing hypothesis. The standard model of basal ganglia function posits that the two main cell types in the striatum, the direct and indirect pathway neurons, have opposing responses to dopamine, and this leads to opposing changes in activity in parkinsonism. Reciprocal changes would be predicted in LID, as well. However, these hypotheses have not been tested directly in an animal model of parkinsonism, as labeling these cell types in vivo has proven challenging. In this study we recorded optically identified striatal direct and indirect pathway units before and after levodopa administration in awake, behaving hemi-parkinsonian mice. We found that baseline firing rates in the parkinsonian condition were lower in direct pathway compared to indirect pathway neurons, in line with the standard model of basal ganglia function. In addition, direct pathway neurons increased firing while indirect pathway neurons decreased firing following administration of levodopa, also supporting the standard model. Unit responses to levodopa in both direct and indirect pathway neurons could be sub-classified into those tracking therapeutic (prokinetic) and those tracking pathological (dyskinetic) behaviors. While changes in rate in both pathways correlate with dyskinesia, we hypothesized that increased direct pathway activity was responsible for dyskinesia. To test this hypothesis, we optogenetically stimulated direct pathway neurons in the dorsolateral striatum in vivo. In both normal and parkinsonian animals such stimulation was sufficient to trigger dyskinesia in the absence of levodopa. These results support many tenets of the standard model, and also provide evidence for the hypothesized role of aberrant striatal firing in dyskinesia.

Furthermore, they may point to an opportunity to manipulate the sub-populations of neurons which track dyskinetic behavior to improve treatment for LID and other hyperkinetic disorders.

Disclosures: M. Ryan: None. A.B. Nelson: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.05/RR18

Topic: E.03. Basal Ganglia

Support: NIH Grant K08NS081001

NSF GFRP

Title: Manipulating activated striatal neurons in levodopa-induced dyskinesia.

Authors: *A. E. GIRASOLE[1,2], M. Y. LUM[2], D. NATHANIEL[3], A. C. KREITZER[5,4], A. B. NELSON[2];
[1]UCSF, SAN FRANCISCO, CA; [2]Neurol., UCSF, San Francisco, CA; [3]Neurol., UCSF, SAN FRANCISCO, CA; [4]Physiol., UCSF, San Francisco, CA; [5]Gladstone Inst., San Francisco, CA

Abstract: The basal ganglia are a group of interconnected subcortical nuclei that participate in the control of movement, mood, cognition, and reward processing, and whose dysfunction is linked to movement disorders like Parkinson's Disease. A common side effect of chronic dopamine replacement therapy in Parkinson's Disease is the development of levodopa-induced dyskinesia (LID), in which levodopa triggers abnormal involuntary movements at therapeutic doses**.** While extensive clinical research has characterized LID, the cellular and circuit mechanisms underlying this condition are still unknown. In rodent models of LID, increased expression of immediate early genes, like c-Fos, has been observed in several brain areas, suggesting these regions may develop aberrant activity in the context of dyskinesia. One hypothesis is that aberrant activity generated within the striatum, the primary input nucleus of the basal ganglia, causes LID. This abnormal neuronal signaling may start in the striatum, propagate through the basal ganglia circuit, ultimately leading to involuntary movements. We have sought to test the hypothesis that aberrant striatal activity, as opposed to activity in other brain regions, causes LID. To test our hypothesis, we utilized a novel transgenic mouse tool, Targeted Recombination in Activated Populations (TRAP), which uses activation of c-Fos to gain permanent genetic access to previously activated populations in vivo. In hemiparkinsonian mice, we combined TRAP with optogenetics, behavior, and in vivo and in vitro electrophysiological

recordings to test our hypothesis that aberrant striatal activity causes dyskinesia. Our data demonstrate that c-Fos expression increases in several key regions brain-wide during dyskinesia, including the somatosensory cortex and striatum, and activated neurons can be captured using TRAP. We found that dyskinesia-associated striatal neurons are more likely to be direct pathway neurons. In addition, optogenetic reactivation of TRAPed neurons in the striatum, and not in somatosensory cortex, is sufficient to produce dyskinesia in the absence of levodopa. Optogenetic inhibition of striatal TRAPed neurons reduces levodopa-induced dyskinesia during a dyskinetic attack. Together, these findings support the hypothesis that aberrant activity in the striatum causes levodopa-induced dyskinesia.

Disclosures: A.E. Girasole: None. M.Y. Lum: None. D. Nathaniel: None. A.C. Kreitzer: None. A.B. Nelson: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.06/RR19

Topic: E.03. Basal Ganglia

Support: NS064577

ARRA supplement to NS064577

AA021074

NS051156

GM008441-23

McKnight Foundation

The Brain and Behavior Foundation

Title: Striatal microcircuit mechanisms for the expression of habit

Authors: *J. O'HARE[1,2], K. ADE[2,1], E. GAIDIS[3], H. LI[3], N. KIM[3], H. YIN[3], N. CALAKOS[2,1]; [1]Neurobio., [2]Neurol., Duke Univ. Med. Ctr., Durham, NC; [3]Psychology & Neurosci., Duke Univ., Durham, NC

Abstract: Habits are automated motor programs that can be initiated by environmental cues. This stimulus-response style of behavior is adaptive because it liberates attentional and

computational resources that would otherwise be needed to evaluate an action-outcome relationship. Good habits can make healthy behaviors feel routine. On the other hand, maladaptive compulsions such as drug abuse are hypothesized to share mechanistic features with habit. Therefore, a mechanistic understanding of habit formation is doubly important for its role in health and disease. The dorsolateral striatum (DLS) is required for habit formation and this brain region changes significantly with habit formation. Previously, we reported that habitual lever pressing in mice corresponds with strengthened striatal output to both the direct and indirect pathways as well as a timing bias shift where direct pathway striatal projection neurons (SPNs) fire before indirect pathway SPNs in DLS. Here we examine local microcircuit mechanisms for these effects. We employ two-photon calcium imaging of SPN populations and cellular electrophysiological recordings in naïve and operantly trained mice. Using pharmacological, optogenetic, and chemogenetic approaches, we find that the activity of one class of interneuron in DLS, the parvalbumin-positive fast-spiking interneuron, is sufficient to reproduce striatal circuit features of habit and is necessary for the expression of habitual behavior. These findings reveal a microcircuit mechanism for habit and suggest new strategies for targeting the striatal circuitry to modulate habitual and compulsive behaviors.

Disclosures: J. O'Hare: None. K. Ade: None. E. Gaidis: None. H. Li: None. N. Kim: None. H. Yin: None. N. Calakos: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.07/RR20

Topic: E.03. Basal Ganglia

Support: NIH Grant NS072197

Title: Spike time predictability and noise sensitivity in repetitively firing striatal spiny neurons

Authors: *C. J. WILSON;
Univ. Texas San Antonio, San Antonio, TX

Abstract: An adequate model for any neuron should predict its firing times in response to any input pattern. We previously found that the interspike intervals of a repetitively firing subthalamic nucleus neuron stimulated using current noise could be predicted with high accuracy using a phase model. The phase model was created from a phase resetting curve measured for the same neuron. We have applied a similar approach to prediction of the spike timing of repetitively firing spiny striatal neurons, but this time during stimulation by sine wave currents. Repetitive

firing was maintained in spiny neurons by passage of suprathreshold (250-400 pA) constant currents. Small (20-80 pA peak-to-peak sinusoidal intracellular currents with frequencies from 0.5-40 Hz were superimposed on the constant depolarization. A phase model based on the phase resetting curves measured from the same neuron was integrated to predict the phase of spiking relative to the stimulation current at steady state.The predictability of spike timing was limited by the cell's intrinsic current noise. The influence of the noise in turn depended on the frequency of the sinusoidal stimulus relative to the cell's unperturbed firing rate. For stimulus frequencies close to the neuron's firing rate, firing was phase-locked to the stimulus. In this case the model accurately predicted the phase of firing on each cycle of the stimulus and the changes in phase as stimulus frequency was varied. In this range, the variance of interspike intervals decreased to well below that during unperturbed firing, indicating a reduced sensitivity to the cell's intrinsic noise. Firing was also highly predictable at other frequencies near those commensurate with the cell's unperturbed firing rate. Interspersed with the ranges of predictable firing there were frequencies at which the model predicted a reliable sequence of firing phases, but the cell showed increased spike time variability, indicating a stimulus-induced enhancement of sensitivity to intrinsic noise.The frequencies at which these changes in noise sensitivity occurred were predicted by analysis of the iterative map of the phase of firing predicted by the model. An analysis of the map can explain the changes in spike time predictability and noise sensitivity of spike timing on a cell-by-cell basis. Funded by NIH/NINDS.

Disclosures: C.J. Wilson: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.08/RR21

Topic: E.03. Basal Ganglia

Support: NIH Grant NS072197

Title: Calcium-activated chloride currents regulate spiking in striatal LTS interneurons by generating medium AHPs

Authors: *S. C. SONG[1], J. A. BEATTY[2], C. J. WILSON[1];
[1]Biol., Univ. of Texas At San Antonio, San Antonio, TX; [2]Physiol., Michigan State Univ., East Lancing, MI

Abstract: Striatal interneurons exhibit unique electrophysiological characteristics. Two neurons, the cholinergic interneuron and the low-threshold spike (LTS) interneuron are autonomously

active in slice. We have previously found that LTS interneurons possess a membrane oscillation and membrane resonance that is generated by the combination of L-, N-type calcium channels and calcium-activated chloride channels (CaCCs). These phenomenon were observed under tetrodotoxin to remove action potentials. We wanted to investigate the contribution of CaCCs to the firing properties of LTS interneurons. In our experiments, we used perforated-patch recordings in mice that expressed GFP under the control of the NPY promoter. This allowed for identified recording of LTS interneurons in striatal slices. Both current clamp and voltage clamp recordings were used to measure effects of blocking specific voltage-gated calcium channels and CaCCs. In voltage clamp, we found that the LTS interneuron expressed a mAHP following a spike that was blocked by niflumic acid, a blocker for CaCCs. This mAHP was not blocked by apamin to prevent SK currents, a classic source of mAHPs in various other neurons. The tail current was also unaffected by application of TRAM34, a specific blocker of IK currents. The mAHP was blocked by specific N-type calcium channel blocker ω-contoxin GVIA, showing calcium dependence, but not isradipine. We have previously shown that L- and N-type calcium channels could drive CaCC-dependent membrane oscillations in LTS interneurons. For the mAHP, the depolarization caused by the action potential could be sufficient for enough calcium to enter through the N-type calcium channel, which has a higher voltage range for activation than L-type calcium channels. Finally the mAHP reversed between -60 and -70 mV, indicative of a chloride current and not potassium. The reversal potential as not affected by changes in the external potassium concentration. In current clamp, blockade of the mAHP increased the CV of the interspike interval, which reflects previous findings which showed that mAHPs make spike timing more regular. Blockade of specific calcium channels narrowed the individual spikes while blockade of CaCCs broaden individual spikes. We also investigated whether the membrane resonance mechanism caused the spiking resonance in LTS interneurons. We found that specific calcium channel blockers had no effect on the spiking resonance while blockade of CaCCs increased the strength and frequency selectivity of the spiking resonance in LTS interneurons.

Disclosures: S.C. Song: None. J.A. Beatty: None. C.J. Wilson: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.09/RR22

Topic: E.03. Basal Ganglia

Support: NARSAD Young Investigator Grant

Marie Curie Intra-European Fellowship IEF327648

Fondation pour la Recherche Médicale

Title: Unexpected diversity of striatal Drd2-expressing neurons revealed by cell-type specific mRNA profiling

Authors: *E. PUIGHERMANAL[1], M. MARTIN[1], J. VITRE[1], A. ESTEVE[2], M. GUT[2], E. VALJENT[1];
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Abstract: The striatum, the primary input structure of the basal ganglia, is broadly implicated in motor action and reward-based behaviors, as well as in neurological and psychiatric disorders. Compelling evidence indicate a functional dichotomy between the dorsal striatum and the nucleus accumbens since they receive different afferents and project to distinct brain structures. However, the diversity of molecular markers between these two subregions remains largely unknown. By crossing Ribotag with Drd2-Cre mice, we isolated the mRNAs bound to tagged-ribosomes selectively from striatopallidal cells and cholinergic interneurons in both dorsal striatum and nucleus accumbens. Remarkably, high-throughput RNA sequencing of Drd2-containing neurons revealed almost 6000 genes differentially regulated between these two brain subregions. *In situ hybridization* and immunofluorescence analyses confirmed the vast majority of the differentially expressed genes and showed a diverse topographic distribution within the striatum, suggesting an unsuspected diversity of striatopallidal neurons. The sensitivity of this approach also allowed the identification of novel markers for cholinergic interneurons. Interestingly, Gene Ontology analysis unraveled distinct biological processes, cellular components, and molecular functions of Drd2-expressing neurons depending on their regional pattern. In-depth analysis and classification of differentially expressed genes also revealed a high degree of segregation. Altogether, the present findings indicate that there is a vast diversity of Drd2-containing neuronal subpopulations depending on the striatal subregion where they are located. Future studies will be necessary to determine whether some of the genes differentially regulated define new striatopallidal neuronal ensembles associated with projection-specificity and/or functional properties.

Disclosures: E. Puighermanal: None. M. Martin: None. J. Vitre: None. A. Esteve: None. M. Gut: None. E. Valjent: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.10/DP06 (Dynamic Poster)

Topic: E.03. Basal Ganglia

Support: NIH Grant R01 DA07418

Title: Evidence of synchronous and asynchronous activity between left and right striatum in both direct and indirect paths during goal oriented behaviors in mice

Authors: *R. M. MIKOFSKY[1], J. M. LAWSON[2], H. MACOMBER[2], S. CLARK[2], D. SULZER[2];
[1]Suzler Lab. At Columbia Univ., New York, NY; [2]Neurosci., Columbia Univ., New York, NY

Abstract: Striatal spiny projection neurons (SPNs) regulate movement through two major circuits. The direct pathway SPNs expressing D1 receptors (dSPNs) directly project to the substantia nigra pars reticulata (SNr) and globus pallidus externa (GPe). The indirect pathway SPNs expressing D2 receptors (iSPNs) trigger a multisynaptic circuit, synapsing to the globus pallidus interna (GPi), which then projects to the SNr /GPe. Activation of the direct and indirect paths correlates with enhanced locomotion in mice and dysfunction in both is implicated in the pathology of Parkinson's disease. To our knowledge, previous studies have not delineated the extent of synchronous activity between left and right striatum in the direct or indirect pathways during locomotion. We used time correlated single photon counting (TCSPC) (χ2- 202 ChiSquare Bioimaging), an optical recording method, to record neuronal activity in the direct and indirect paths in both the left and right dorsal lateral striatum. D1-Cre (direct path) and A2A-Cre (indirect path) mice were injected with AAV9 Flex GCaMP6f virus. Imaging fibers were implanted in both the right and left dorsal lateral striatum. Mice were recorded with a high speed video camera framelocked to the sampling rate of the TCSPC machine. This enables simultaneous recording from both the left and right dorsal lateral striatum at millisecond precision, allowing us to correlate locomotor behavior with GCaMP6f changes in fluorescence. Interestingly, in preliminary results we find evidence of both synchronous and asynchronous activity in amplitude and frequency of firing in dorsal lateral striata during goal oriented behaviors in mice.

Disclosures: R.M. Mikofsky: None. J.M. Lawson: None. H. Macomber: None. S. Clark: None. D. Sulzer: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.11/SS1

Topic: E.03. Basal Ganglia

Support: CBR Aotearoa Fellowship

University of Auckland, FRDF Grant

Title: Mapping spatial connectivity between the subthalamic nucleus and substantia nigra in brain slices using optogenetic 'functional mapping'

Authors: *P. S. FREESTONE[1,2,3], K. L. TODD[1,2,3], J. LIPSKI[1,2,3];
[1]Univ. of Auckland, Auckland, New Zealand; [2]Ctr. for Brain Res., Auckland, New Zealand; [3]Brain Res. New Zealand, Auckland, New Zealand

Abstract: The basal ganglia is a sub-cortical network receiving a wide range of inputs from the entire brain. As such, this network is involved in motor control, learning, memory and reward-driven behaviour. The subthalamic nucleus (STN), a major glutamatergic nucleus of the basal ganglia projecting to the substantia nigra pars reticulata (SNr) and pars compacta (SNc) nuclei, plays a crucial role in the *indirect* and *hyperdirect* pathways. We have previously shown this nucleus to be important for modulating SNc dopaminergic activity via an endocannabinoid-mediated attenuation of inhibitory input from the SNr. In the present study, we studied the spatial relationship of the STN and SNr/SNc and the connections between them to better understand the interplay of these three nuclei in regulating basal ganglia function. CD-1 mice expressed channelrhodopsin (H134R) under the CaMKIIα promotor after injection of the viral (AAV5) construct into the STN. Neuronal activity was monitored using whole-cell patch-clamp electrophysiological recordings from horizontal ventral midbrain slices (250 μm) containing the STN, SNr and SNc after 4-8 weeks expression. 'Functional mapping' was conducted using a digital mirror device (*Polygon400*, MightEx) to sequentially illuminate squares (20-80 μm^2) of a grid (800 x 600 μm) in a pseudorandom fashion, while recording post-synaptic currents from a single neuron. Using this approach, we have shown that focal photo-stimulation (6 mW/mm^2, 5 ms) of individual neurons within the STN can evoke excitatory post-synaptic currents in downstream connected SNr neurons. These connections extended over 900 μm between the site of photo-stimulation and the recorded cell body, and the synaptic responses were completely blocked by bath application of ionotropic glutamate receptor blockers (5 μM CNQX and 10 μM MK-801). Focal photo-stimulation within the SNr revealed in some cases several discontinuous 'hot-spots' where excitatory synaptic transmission could be evoked. These hot-spots were located at the cell body and at locations >300 μm distal to the soma. These results confirm the

feasibility of mapping functional connections in ventral midbrain brain slices using optogenetic techniques, and reveal unique patterns of innervation from the STN to neurons in the SNr.

Disclosures: P.S. Freestone: None. K.L. Todd: None. J. Lipski: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.12/SS2

Topic: E.03. Basal Ganglia

Support: CRCNS program, NIAAA R01 AA016022

CRCNS program, NIDA R01 DA038890

Title: Coordinated corticostriatal input produces supralinear calcium in dendritic spines in a spiny projection neuron model

Authors: *D. B. DORMAN, J. JEDRZEJEWSKA-SZMEK, K. T. BLACKWELL;
Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: Spiny projection neurons (SPNs) of the striatum, by integrating cortical, thalamic, and dopaminergic input, underlie goal-directed learning and habit formation. These neurons exhibit state transitions *in vivo* between a hyperpolarized down-state and a subthreshold depolarized up-state that is driven by spontaneous, asynchronous synaptic activity. This activity produces elevations in calcium that control synaptic plasticity when synaptic activity is coupled with back-propagating action potentials (bAP) for proximal synapses, but not distal synapses due to bAP decay. One experimental study (Plotkin et al., 2011) has shown that temporally aligned synaptic stimulation of ~11 adjacent spines on a distal (but not proximal) tertiary dendrite produces a regenerative up-state like plateau potential at the soma, which is dependent on NMDA and voltage-gated calcium channels. This suggests a potential mechanism for associative synaptic plasticity at distally located synapses. To investigate how various patterns of spatially and temporally distributed synaptic input interact and the degree of spine-specific calcium transients, we developed a biophysical, multi-compartment SPN model with a branching dendritic structure and explicitly modeled dendritic spines. Our model uniquely incorporates complex calcium dynamics including buffers, pumps, and diffusion. We tuned the model with experimental data including electrical response to injected current, calcium imaging of action potentials and synaptic inputs, and the generation of plateau potentials by closely aligned synaptic stimulation of distal dendritic spines. Simulation experiments reveal a supralinear calcium elevation in

spines active during a plateau potential when stimulating distal spines on a single tertiary dendritic branch, whereas non-stimulated, neighboring spines exhibit a significantly lower calcium elevation. When stimulating clusters of distal spines on neighboring branches, we find a reduction in the number of spines per branch required to evoke a plateau potential, indicating a small interaction between neighboring branches. When neighboring branches are stimulated with a delay, the spine calcium elevation of the second branch is greater than that of the first branch. Overall our results show that spine calcium exhibits spine specificity and the spine calcium response is sensitive to the temporal order of branch stimulation. As calcium is critical to downstream signaling pathways required for plasticity, these results suggest a mechanism for calcium mediated associative plasticity between either coordinated or sequential synaptic inputs to SPNs of the striatum.

Disclosures: D.B. Dorman: None. J. Jedrzejewska-Szmek: None. K.T. Blackwell: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.13/SS3

Topic: E.03. Basal Ganglia

Support: CRCNS program, NIDA R01 DA038890

CRCNS program, NIAAA R01 AA016022

Title: Calcium based plasticity rule can predict plasticity direction for a variety of stimulation paradigms.

Authors: *J. JEDRZEJEWSKA-SZMEK[1], D. B. DORMAN[1], K. T. BLACKWELL[2];
[1]The Krasnow Inst. For Advanced Study, [2]The Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: The striatum is a major site of learning and memory formation for both sequence learning and habit formation. One of the mechanisms used by the brain for memory storage is synaptic plasticity -- the long-lasting, activity dependent change in synaptic strength. All forms of synaptic plasticity require an elevation in intracellular calcium, though which characteristics of calcium dynamics actually control plasticity are still not completely understood. Our previous research demonstrated that both the amplitude and duration of the calcium transients are required to determine the direction of synaptic plasticity for spike timing dependent plasticity. In this research we extend our previous model spiny projection neuron, with sophisticated calcium

dynamics including calcium diffusion, buffering, and pump extrusion, to include AMPAR desensitization in order to evaluate frequency dependent plasticity paradigms. To further test our two amplitude and duration rule for synaptic plasticity, we use a moderate frequency stimulation of 20 Hz, which has been shown experimentally to elicit LTD, and a theta burst stimulation paradigm (4 pulses at 50 Hz repeated at a theta frequency of 10.5 Hz), which has been shown experimentally to elicit LTP. Our simulations show that, despite the different calcium patterns elicited by frequency dependent stimulation patterns, the same calcium-based weight change rule (plasticity rule) that correctly predicts spike timing dependent plasticity also can explain the change in synaptic weights for these frequency dependent plasticity paradigms. We further show that LTP elicited by the theta burst stimulation paradigm is NMDA receptor dependent and LTD elicited by 20 Hz is independent of L-type calcium channels activation, which is consistent with experimental data. We also investigate the role of dopamine by evaluating synaptic plasticity of direct and indirect pathway spiny projection neurons, both in control conditions and dopamine depleted conditions. Illuminating the role of calcium and dopamine, and their interactions in synaptic plasticity, will allow for better understanding mechanisms of memory storage in health and disease.

Disclosures: J. Jedrzejewska-Szmek: None. D.B. Dorman: None. K.T. Blackwell: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.14/SS4

Topic: E.03. Basal Ganglia

Support: NINDS NS090595

NINDS NS007220

Howard Hughes Medical Institute

European Research Council

Title: Neuronal diversity in substantia nigra pars reticulata delineates specialized basal ganglia output pathways

Authors: *L. E. MCELVAIN[1,2,3], B. LIM[2], D. KLEINFELD[1], R. COSTA[3];
[1]Physics, [2]Neurobio., UCSD, La Jolla, CA; [3]Champalimaud Neurosci. Programme, Lisbon, Portugal

Abstract: The basal ganglia play a central role in motor control, but the circuit mechanisms that mediate their effect on the broader motor system remain poorly understood. We interrogated the largest basal ganglia output, the substantia nigra pars reticulata (SNr), to delineate the organization of basal ganglia efferent signaling. Viral tracing methods were combined with electrophysiological recordings to define the unique projection populations in SNr and their downstream targets in the brainstem and thalamus. Anterograde AAV tracing in mice from GABAergic and parvalbumin-positive SNr neurons revealed major projections to the superior colliculus; the inferior colliculus; the midbrain, pontine, and medullary reticular formations; the pedunculopontine nucleus; the dorsal raphe; and the VM, VA, MD, and Pf thalamic nuclei. To determine whether these regions receive inputs from segregated or overlapping SNr populations, we retrogradely labeled SNr neurons projecting to each structure and assayed their intrinsic electrophysiological properties in vitro. All SNr projection neurons shared several intrinsic features, i.e., spontaneous firing, sustained fast-firing (> 50 Hz) capabilities, and linear firing rate responses to depolarizing currents. However, SNr subpopulations that projected to brainstem regions exhibited specialized intrinsic characteristics and differed significantly from each other in properties that included hyperpolarization-gated currents, firing rate ranges, and passive membrane properties. Brainstem-projecting SNr neurons were topographically organized and, in some cases, morphologically distinct. In contrast, thalamus-projecting SNr neurons exhibited heterogeneous electrophysiological and morphological properties and were distributed throughout the nucleus. In a final set of experiments, intersectional virus strategies mapped the axonal collateralizations from SNr neurons to determine whether their projections to thalamus arose from brainstem-projecting SNr neurons. Our experiments delineate specialized populations of SNr projection neurons and demonstrate their specific and extensive collateralization projection patterns. Further, our experiments establish a framework for future investigations to link individual SNr subpopulations to their motor functions.

Disclosures: L.E. McElvain: None. B. Lim: None. D. Kleinfeld: None. R. Costa: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.15/SS5

Topic: E.03. Basal Ganglia

Support: NIH/NINDS Grant P50NS071669

NIH/ORIP Grant P51OD011132

Title: Ultrastructural features of pallidal GABAergic terminals in the ventral motor and caudal intralaminar thalamic nuclei in normal and MPTP-treated Parkinsonian monkeys

Authors: *A. J. SWAIN[1,2], R. M. VILLALBA[1,2], C. KATONA[1,2], A. GALVAN[1,2], T. WICHMANN[1,2,3], Y. SMITH[1,2];
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Abstract: Parkinson's disease (PD) is characterized by progressive loss of the dopaminergic neurons of the substantia nigra pars compacta. In primates rendered parkinsonian by treatment with the neurotoxin MPTP, parkinsonism is associated with abnormally increased and patterned inhibitory outflow from sensorimotor portion of the internal segment of the globus pallidus (GPi) to the thalamus, targeting preferentially the centromedian nucleus (CM) and the parvocellular part of the ventral anterior nucleus (VApc). While these nuclei receive axon collaterals from the same GPi neurons (Parent and De Bellefeuille, 1983, Brain Res. 278:11), our previous ultrastructural study showed surprising differences in the morphology and pattern of synaptic connectivity of GPi terminals in these nuclei (Sidibe et al.,1997, JCN 382:323). The goal of this study is to compare the ultrastructural features of GPi terminals in VApc and CM of normal and MPTP-treated parkinsonian monkeys, using high resolution electron microscopy (EM) and 3D reconstructed models of individual anterogradely labeled pallidothalamic terminals. Preliminary EM data from an MPTP-treated monkey that received an injection of an adeno-associated viral (AAV5) vector which expressed green florescent protein (GFP) in the GPi show that ~50% GPi terminals in VApc contact mostly medium-sized (0.5-1.0 μm in diameter) dendrites, while ~30% target large (> 1.0 μm diameter) dendrites. In the CM, ~65% of the labeled GPi terminals contacted large dendrites. Ultrastructural observations from single ultrathin sections are consistent with results of our previous study (Sidibe et al., 197, JCN 382:323) such that GPi terminals in VApc are larger in size, have a different morphology, and form more symmetric synapses than those in CM. Additional AAV5 injections in the GPi of normal rhesus monkeys will be performed to complete this analysis. Three dimensional reconstructions of single GPi terminals are in process to further quantify these morphometric characteristics across nuclei and animal groups. Based on data from pallidal terminals in the subthalamic nucleus (Fan et al., 2012, JNS 32:13718) and from multisynaptic GABAergic terminals (ie GPi-like) from the anterior pretectal nucleus in the thalamus (Wanaverbecq et al., 2008, JNS 28:11848), we hypothesize that potential morphological differences and plastic changes of the ultrastructural features of GPi terminals in VApc and CM are linked to their physiological properties.

Disclosures: A.J. Swain: None. R.M. Villalba: None. C. Katona: None. A. Galvan: None. T. Wichmann: None. Y. Smith: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.16/SS6

Topic: E.03. Basal Ganglia

Support: NINDS intramural research program grant NS003134

Title: Morphological study of calbindin-positive and calbindin-negative dopamine neurons located in the substantia nigra pars compacta

Authors: *M. ZHU, R. EVANS, Z. KHALIQ;
NINDS, NIH, Bethesda, MD

Abstract: Dopamine neurons of the substantia nigra pars compacta (SNc) can be divided into cell groups that either express or lack expression of calbindin. In Parkinson's disease, calbindin-negative dopamine neurons of the SNc are vulnerable to degeneration while calbindin-positive dopamine neurons tend to be spared. Therefore, examining the morphological characteristics may help explain why these two subpopulations are differentially vulnerable to degeneration. We analyzed the morphology of 33 calbindin-positive and 47 calbindin-negative SNc dopamine neurons. Dopamine neurons were filled with a red morphological dye, Alexa-594, and high-resolution Z-stack images were taken with a two-photon microscope. Cells were then reconstructed using a semi-automated software and the morphological characteristics were extracted. Relative to calbindin-positive dopamine neurons, we found that calbindin-negative dopamine neurons had larger somas (Calb-neg vs Calb-pos, 26.5 ± 0.64 μm vs 23.1 ± 0.92 μm) and more total dendritic bifurcations (5.08 ± 0.55 vs 2.85 ± 0.4). Using sholl analysis, we examine the amount of dendritic length in relation to the distance from the soma. While the distal (>100um from soma) dendritic lengths were not significantly different between the two subpopulations, calbindin-negative neurons had significantly more proximal (10-100um from soma) dendritic length (527 ± 36.6 μm vs 389 ± 30 μm) due to a larger number of proximal bifurcations. In conclusion, calbindin-positive and calbindin-negative dopamine neurons of the SNc are morphologically unique and these differences may contribute to differences in electrophysiological properties and integration of synaptic inputs between the two subpopulations.

Disclosures: M. Zhu: None. R. Evans: None. Z. Khaliq: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.17/SS7

Topic: E.03. Basal Ganglia

Support: NIH Grant DA038208

VA Grant BX002525

Portland VA PADRECC

Title: AMP kinase regulates ligand-gated K-ATP channels in substantia nigra compacta dopamine neurons

Authors: *S. W. JOHNSON[1,2], Y. WU[2], A. C. MUNHALL[1], K.-Z. SHEN[2];
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Abstract: AMPK is a master enzyme that regulates expression of K-ATP channels in pancreatic beta-cells and cardiac myocytes. K-ATP channels are also expressed in the SNC where they strongly inhibit neuronal activity. However, an AMPK K-ATP interaction in the SNC is unknown. Patch-clamp recordings were used to investigate effects of AMPK on K-ATP currents evoked by diazoxide in SNC dopamine neurons in slices of rat midbrain. In the presence of the AMPK activator A769662, diazoxide current increased 372% when measured 60 min after starting whole-cell recording. But unexpectedly, we found that diazoxide currents also increased over time when pipettes did not contain A769662; although currents increased 200% over control, this was significantly less than the increase observed when A769662 was present ($P < 0.001$). Moreover, superfusing the slice with the AMPK blocking agent dorsomorphin (Compound C) significantly reduced diazoxide current to 38% of control, whether or not A769662 was present. Current-voltage plots showed that the increase in diazoxide current was associated with an increase in whole-cell conductance with a reversal potential near that expected for K+. Control experiments showed that outward currents evoked by the K-ATP channel opener NN-414 also increased over time, but not currents evoked by the GABA-B agonist baclofen. Delaying the application of diazoxide after starting whole-cell recording correlated with augmentation of current, suggesting that the increase in diazoxide current was associated with dialysis of intracellular contents. Diazoxide produced small but significant slowing of spontaneous firing rate using loose-patch recordings. However, superfusion with A769662 significantly augmented the inhibitory effect of diazoxide on firing rate, suggesting that AMPK activation mimics the potentiation of ligand-gated K-ATP current that is seen with whole-cell recording. We conclude that K-ATP channel function is augmented by AMPK, which

is activated during the process of making whole-cell recordings. By potentiating the inhibitory effect of K-ATP channels in SNC neurons, AMPK could significantly alter many dopamine-dependent behaviors and conditions.

Disclosures: S.W. Johnson: None. Y. Wu: None. A.C. Munhall: None. K. Shen: None.

Poster

246. Striatal Cell Biology and Plasticity

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 246.18/SS8

Topic: E.03. Basal Ganglia

Title: Comparison of high-frequency firing mechanisms in mesocortical,mesoaccumbal and mesostriatal dopamine neurons

Authors: *R. A. TARFA[1,2], Z. KHALIQ[3];
[1]NIH, Bethesda, MD; [2]Neurosci., Brown Univ., Providence, RI; [3]NIH/NINDS, Bethesda, MD

Abstract: Midbrain dopamine neurons function in brain circuits mediating reward signaling, motivation, aversion and movement. Because dopamine-dependent circuits vary widely in their function, it is possible that dopamine neurons also display functional heterogeneity. Consistent with this idea, prefrontal cortex projecting dopamine neurons display high firing frequency rates compared to the nucleus accumbens and striatum projecting dopamine neurons (Lammel et al., 2008). However, the mechanism that mediate the differences in firing rates between the subpopulations remain to be elucidated.
We examined spontaneous and high-frequency firing in retrogradely-labeled mesocortical, mesoaccumbal and mesostriatal dopaminergic neurons. As compared to mesostriatal neurons, mesocortical dopamine neurons fired at slightly higher spontaneous rates (MC vs MS: 3.7 ± 0.6 Hz vs 2.4 ± 0.1 Hz, p= 0.003), had significantly narrower action potential widths, shorter spike peaks and more hyperpolarized threshold voltages. Moreover, mesocortical neurons fired at substantially higher maximal firing rates (MC vs MS: 26.8 ± 3.2 Hz vs 11.5 ± 0.6 Hz, p= 0.02). One possible contribution to the difference in high frequency firing among the subpopulations is the difference in the impulse-regulating dopamine (D2) autoreceptors, which is present in mesostriatal dopamine neurons and may slow their firing, but absent in mesocortical dopamine neurons. However, in the presence of sulpiride and Ba^{2+}, to block D2-receptors and G-protein coupled inward rectifying potassium currents (GIRKs), we found that the difference in maximal firing frequencies of both subpopulations was preserved.
Past work has shown that the large-conductance calcium-activated (BK) and delayed rectifier (K_v2) potassium currents are major contributors to spike repolarization in substantia nigra

compacta (SNc) dopamine neurons, however the currents that shape repolarization in mesocortical dopamine neurons remain unknown. Following the block of BK and K_v2 currents, our preliminary experiments demonstrate the presence of a remaining TEA sensitive current in both SNc (4.0 ± 1.1 nA) and VTA (2.3 ± 0.7 nA) dopamine neurons. In response to 1mM TEA, 35.2% of the remaining outward current was blocked in VTA dopamine neurons, and 34.78% in SNc dopamine neurons. We hypothesize that the faster repolarization rates in mesocortical neurons may be as a result of a higher density of fast, high threshold (K_v3) potassium currents, that facilitate their high-frequency firing. We plan to test the contribution of all three potassium currents to action potential repolarization in the mesocortical and mesostriatal subpopulations.

Disclosures: R.A. Tarfa: None. Z. Khaliq: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.01/SS9

Topic: E.04. Voluntary Movements

Support: NIH Grant 1DP2 NS083037

McKnight Foundation

Kavli Foundation

Searle Scholars Program

Simons Foundation

NIH Grant 1F32 NS092350

The Esther A. & Joseph Klingenstein Fund

Title: Time-course of cortical motor preparation during self-paced reaches

Authors: *A. J. ZIMNIK[1], A. H. LARA[1], G. F. ELSAYED[1], J. P. CUNNINGHAM[2,5], M. M. CHURCHLAND[1,2,3,4];
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Abstract: Voluntary movements are believed to be prepared prior to execution. While reaction times (RTs) for visually-guided reaches are typically 300-350 ms, RTs could in principle be ~150 ms given physiological delays. The 'extra' 150-200 ms has been attributed to sluggish preparatory processes (e.g., Riehle and Requin 1993; Churchland et al., 2006). Yet other behavioral experiments argue that preparation can be rapid, completing within 50 ms (Haith et al., 2016). Our recent physiological experiments support this assertion: when reaching under extreme time pressure, preparatory activity leads movement-related activity by only ~30 ms. Conversely, both delayed-reach and decision paradigms reveal that preparation can span 1000 ms or more. This range in preparatory activity durations raises the question: what is the typical time course of preparatory activity before a 'normal', self-paced movement? Is preparation a naturally brief process, becoming extended only when artificially required by laboratory tasks? Or does preparation naturally consume ~150-200 ms as initially proposed? We analyzed the activity of 222 neurons recorded from motor and premotor cortex of monkeys performing a delayed-reach task. We focused on the self-paced return movements made after each trial was complete. These movements returned the hand to the center of the screen, were not time-locked to any particular cue, and involved little time pressure. We leveraged a recent method that separates the population response into preparatory and movement-related components by exploiting their occupancy in orthogonal subspaces (Elsayed et al., submitted). We confirmed that preparatory and movement subspaces (inferred from the outgoing-reach neural data) continued to capture preparatory and movement-related activity for return reaches. We then tracked the time course of preparatory activity. Preparatory activity disappeared by the onset of the outgoing reach, but reappeared before return reaches. The patterns of preparatory activity preceding return and outward reaches made in a common direction were similar (Monkey B: $rho = 0.75$, Monkey A: $rho = 0.79$, $p < 0.01$, *both*). The reappearance of preparatory activity occurred well after the hand became stationary on the target, and began 150-200 ms prior to the onset of the movement-related activity. This time course is briefer than observed with an imposed delay but much longer than observed under time pressure. Thus, while the minimum time needed to prepare may be 30 ms, the 'natural' time course of neural motor preparation is 150-200 ms, matching prior estimates from behavioral data.

Disclosures: A.J. Zimnik: None. A.H. Lara: None. G.F. Elsayed: None. J.P. Cunningham: None. M.M. Churchland: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.02/SS10

Topic: E.04. Voluntary Movements

Support: The McKnight Foundation

The Kavli Foundation

Searle Scholars Program

Simons Foundation

NIH New Innovator Award

The Esther A. & Joseph Klingenstein Fund

The Alfred P. Sloan Foundation

Title: Changes in motor cortex population structure between movement types

Authors: *A. A. RUSSO, B. LONDON, S. PERKINS, M. CHURCHLAND;
Columbia Univ., New York, NY

Abstract: Even a single limb can perform many movement types: throwing a ball, brushing one's teeth, executing a push-up. To investigate the neural basis of switching between movement types, we employed a novel task where monkeys navigate a virtual environment using a hand pedal to cycle for a variety of prescribed distances. Visual cues instruct the monkey to pedal 'forward' (moving away from the body at the top) or 'backward' (the reverse). We reasoned that the motor system must use very different dynamics (internal and feedback) during forward versus backward pedaling. Yet the two directions exhibit mirrored kinematic patterns. Given this, different hypotheses regarding motor cortex function make different predictions. If activity codes kinematics (e.g., velocity), the population response during forward and backward pedaling should be similar but reversed. If activity codes muscle commands, responses should change in parallel with muscle activity. Finally, if motor cortex participates in dynamics that generate muscle activity, the population response should additionally reflect network-level strategies necessary to switch dynamics. To explore these hypotheses we recorded activity from motor and premotor cortex of two monkeys (112 and 108 neurons) and EMG from the major arm muscles (36 and 22 recordings).
Single neurons exhibited striking differences between forward and backward pedaling. Many such differences paralleled those seen in the muscles: profound changes in the 'preferred direction', the depth of modulation, and the temporal response pattern. Indeed, EMG could be successfully decoded from neural activity, with reasonable generalization across directions (generalization R^2= 0.42 and 0.52). In contrast, fits to hand velocity failed to generalize (generalization R^2 was negative). These results support the hypothesis that responses relate to muscle commands, with incidental relationships to kinematics. Yet the neural population response differed from the muscle population response in at least two ways. First, forward and backward responses were separated in neural state space by distances larger than expected from the muscle state space. Second, forward and backward responses occurred in neural subspaces that overlapped less than the corresponding muscle subspaces. Both findings are consistent with a strategy where different dynamics for forward versus backward are achieved by employing

different regions of neural state space. Consistent with this interpretation, the evolution of the neural population was well fit by a single linear dynamical system (R^2 = 0.80 and 0.75) while the evolution of the muscle population was not (R^2 = 0.40 and 0.21).

Disclosures: A.A. Russo: None. B. London: None. S. Perkins: None. M. Churchland: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.03/SS11

Topic: E.04. Voluntary Movements

Support: NSERC

CIHR

Title: Comparison of the modulation of primary motor cortex by dorsal and ventral premotor areas in Cebus apella

Authors: *S. COTE[1], A. HAMADJIDA[1], M. DEA[1], S. QUESSY[1], N. DANCAUSE[1,2]; [1]Neurosciences Department, Univ. of Montreal, Montreal, QC, Canada; [2]Groupe de Recherche sur le Système Nerveux Central, Montreal, QC, Canada

Abstract: The primary motor cortex (M1) plays a crucial role in the control of forelimb movements in primates. The premotor areas of both hemispheres are highly interconnected with M1 and thereby contribute to its corticospinal outputs. Whereas some studies have examined the intrahemispheric influence of premotor areas on M1 outputs, little is known about the influence of premotor areas of the opposite hemisphere. In *Cebus apella*, we investigated the modulatory effects of contralateral dorsal and ventral premotor cortex (cPMd, cPMv) on M1 outputs using paired pulse protocols and compared them to those of ipsilateral PMd and PMv (iPMd, iPMv). After a bilateral craniotomy and durectomy, a conditioning electrode was placed in cPMd, cPMv, iPMd or iPMv to deliver a sub-threshold conditioning stimulus (C). A second electrode was placed in M1 to deliver a supra-threshold stimulus (T). The C and T stimuli were separated by 6 inter-stimulus intervals (ISIs; range: 0-20ms). To quantify the conditioning effects, motor evoked potentials (MEPs) were recorded in 8 forelimb muscles from 4 monkeys (cPMd-M1: n=41, cPMv-M1: n=44, iPMd-M1: n=41, iPMv-M1: n=42). Following cPMd conditioning, facilitation (62%) was more common than inhibition (38%). Facilitatory effects were similarly prevalent across tested ISIs while inhibitory effects were most prevalent with intermediate ISIs (5-10ms). Conversely, cPMv conditioning generated more interhemispheric inhibition (83%) than

facilitation (17%). Inhibitory effects were most prevalent with longer ISIs (15-20ms) and facilitatory effects with intermediate ISIs (5-10ms). In comparison, iPMd-M1 interactions were most often inhibitory (55%) while iPMv-M1 interactions were most often facilitatory (60%). The contrast between the modulatory effects produced by PMd and PMv suggest the existence of a complex premotor-motor dialogue that could reflect the different functions of premotor areas in the production of forelimb movements.

Disclosures: S. Cote: None. A. Hamadjida: None. M. Dea: None. S. Quessy: None. N. Dancause: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.04/SS12

Topic: E.04. Voluntary Movements

Support: NINDS NRSA Fellowship 1F32NS092350-01A1

NIH New Innovator Award

The McKnight Foundtation

The Kavli Foundation

Searle Scholars Program

Simons Foundation

The Esther A. & Joseph Klingenstein Fund.

Title: Preparatory responses in primary motor and premotor cortex are conserved across self-initiated, quasi-automatic and cue-initiated movements

Authors: *A. H. LARA[1], G. F. ELSAYED[1], J. P. CUNNINGHAM[2], M. M. CHURCHLAND[1]; [1]Neurosci., [2]Statistics, Columbia Univ., New York, NY

Abstract: Neurons in primary and pre-motor cortex exhibit preparatory responses during delayed-reach tasks. Most real-world movements, however, are not preceded by a delay period. Movements may be initiated internally (e.g. when reaching for a cup of tea) or quasi-automatically in response to a sudden event (e.g. when catching a falling object). Whether preparatory processing exits in such real-world contexts remains unknown. We trained two

monkeys to make the same set of reaches in three initiation contexts: cue-initiated, self-initiated and quasi-automatic. In the cue-initiated context, a target was presented and a subsequent visual go cue indicated when to initiate the reach (as in the standard delayed-reach task). In the self-initiated context, the decision to move is made internally: waiting longer yielded larger rewards, creating a tradeoff between a small immediate reward and a larger deferred reward. In the quasi-automatic context, reaches had to intercept a rapidly moving target with no time for hesitation. Reach trajectories and patterns of muscle activity were nearly identical across contexts. We used a novel dimensionality reduction approach to isolate preparatory and movement-related processing at the level of the neural population. Leveraging the cue-initiated context, we identified a preparatory subspace and an orthogonal movement subspace of neural activity. We projected the population response for all contexts onto those subspaces. Responses in the movement subspace were nearly identical across contexts consistent with a unified cortical mechanism for producing movement regardless of why it was initiated. All three contexts also had activity in the preparatory subspace, supporting the hypothesis that preparation is an obligatory stage. However, there were marked differences in the timing of preparatory events. In the cue-initiated context, preparation was sustained throughout the delay. In the self-initiated context, preparation was deferred until shortly before movement onset. In the quasi-automatic context, preparation lasted only a few tens of milliseconds before movement-related activity began. Despite these differences, in all three contexts the same neural state was achieved in the preparatory subspace around 70 ms before movement onset (correlation of 0.91 and 0.99 for monkey A and B). We conclude that preparation always precedes the onset of neural dynamics that generate movement. However, the time course of preparation varies according to task demands, and can be remarkably rapid.

Disclosures: A.H. Lara: None. G.F. Elsayed: None. J.P. Cunningham: None. M.M. Churchland: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.05/SS13

Topic: E.04. Voluntary Movements

Title: Emergence of neural representations in premotor (M2) and primary motor (M1) cortices during early motor learning

Authors: *T. VEUTHEY[1], K. GANGULY[2,3];
[1]Ganguly Lab, UCSF, San Francisco, CA; [2]Dept. of Neurol., UCSF, San Francisco, CA; [3]Neurol. and Rehabil. Service, Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: Introduction: Motor learning is a vital capacity impaired in a wide range of psychiatric and neurologic disorders. However, we still do not have a detailed neurophysiological and computational understanding of the neural circuits that enable motor learning. In rodents, recent work indicates that both primary motor and premotor cortex are necessary for learning skilled movements, and that patterns of activity stabilize in each region during skill learning. However, fundamental questions regarding the mechanisms of motor learning remain. Are new movements encoded at single neuron or population levels? Are representations of new movements selected from available movement variations, or do novel representations develop over time and practice? Do primary motor and premotor cortices contribute differentially to new movement learning?
Methods: We studied motor learning in rats learning a single-pellet reach-to-grasp task. We used in vivo electrophysiology methods to chronically record single-neuron spiking activity and aggregate local field potential (LFP) activity simultaneously from both primary motor (M1) and premotor (M2) cortices. We recorded neural signals from the very beginning of motor learning (< 30% reach success) through plateau performance (stable performance above 60% reach success).
Results: During the very earliest phase of motor learning when there was rapid behavioral improvement, single neurons in both M1 and M2 developed strong task-dependent modulation. Interestingly, the emergent patterns appeared to build upon existing patterns, albeit with weak modulation depths. Moreover, modulation of M1 neurons was more temporally precise than that of M2 neurons, potentially reflecting differential encoding of specific movements in M1 versus "planning" of the entire movement sequence in M2.
Conclusion: Our results suggest that single-neuron coding for new movements develops very early during learning and is further strengthened and refined with practice.

Disclosures: T. Veuthey: None. K. Ganguly: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.06/SS14

Topic: E.04. Voluntary Movements

Support: ERC335328

Title: Action planning and action observation in rodent parietal cortex

Authors: *T. TOMBAZ, B. A. DUNN, K. HOVDE, J. R. WHITLOCK;
Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway

Abstract: Our brains continuously plan and execute goal-directed actions, and without any effort we are readily aware of the behavior and actions of others around us. There is a broad consensus that action planning takes place in the brain between parietal and frontal motor cortices, and that these same regions facilitate action understanding via "mirror" neurons, which are activated whether an action is performed or merely observed. The topic of mirror neurons has drawn both considerable excitement and debate over the last two decades and many fundamental questions remain unresolved, such as the biological basis of the mirror mechanism, the utility of mirror neurons, or the existence of mirror neurons in mammals other than primates. These issues stand to be resolved using lower model organisms, such as rodents, for which powerful tools exist to perform anatomical and functional dissections at the circuit level. To determine if mirror neurons exist outside of primates, we are currently conducting *in vivo* calcium imaging in parietal cortex of freely-behaving mice while they perform and observe various goal-directed motor behaviors. A major advantage of this approach is that typical datasets consist of 100-150 neurons imaged simultaneously, which allows us to compare the population coding of actions either when they are executed or observed. I will discuss preliminary results from single-cell and population-level analyses on imaging data collected during performance and observation of a pellet-reaching task.

Disclosures: T. Tombaz: None. B.A. Dunn: None. K. Hovde: None. J.R. Whitlock: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.07/SS15

Topic: E.04. Voluntary Movements

Title: Endogenous motor errors in parietal area 5 but exogenous target errors in area 7 drive adaptation in reaching

Authors: *M. INOUE[1], S. KITAZAWA[1,2,3];
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Abstract: We have recently shown that motor cortical circuits (primary motor cortex and premotor cortex) encode information on end-point errors in reaching, and post-movement microstimulation to these regions caused trial-by-trial increases in errors. The study suggested

that motor cortical circuits provide error signals that drive trial-by-trial adaptation in reaching movement (Inoue et al., *Neuron*, 2016). On the other hand, human imaging studies to date have reported that reaching errors are encoded in parietal association areas as well. We thus aimed at testing whether the error signals in parietal regions are causally related to adaptation in reaching. To this end, we examined neuronal activities of parietal area 5 and 7 while two monkeys made rapid reaching movements toward a visual target that appeared at a random location on a tangent screen. We examined the relationship between neuronal discharges and 1) target position, and 2) visual errors by using information theory. About half of area 5 (59% for target positions and 43%for visual errors) and area 7 (72% for target positions and 49% for visual errors) neurons started to encode information on target positions and/or visual errors with a latency of 100 ms. We then delivered electrical microstimulation after the touch by using the same electrode (area 5: n = 24, area 7: n = 23). Repetitive pairing of reaching movements with microstimulation produced a gradual and significant increase of the endpoint error over 20-30 trials (area 5: 15/24, area 7: 10/23). After stimulation to the area 5, the error increased opposite to the preferred direction of visual errors. After stimulation to the area 7, by contrast, the error increased toward the preferred direction of target position. These results suggest that neurons in the area 5 provide error signals for adaptation to endogenous motor errors whereas those in the area 7 provide error signals for adapting to exogenous target errors.

Disclosures: M. Inoue: None. S. Kitazawa: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.08/SS16

Topic: E.04. Voluntary Movements

Support: Norwegian Research Council 239963

European Research Council Starting Grant 335328

Title: Mapping neural correlates of natural behavior in 3D

Authors: *B. DUNN[1], T. TOMBAZ[1], K. HOVDE[1], G. TAYLOR[2], J. WHITLOCK[1];
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Abstract: More than a century of research on the cortical motor system has led to the view that planning and executing even simple motor behaviors occurs across several regions of cortex, and

it is now appreciated that early stages of movement planning occur in posterior parietal cortex (PPC). Non-human primates have historically served as the premiere system for studying cortical motor physiology, and indeed the vast majority of our current understanding of PPC derives from head-fixed monkeys performing isolated saccadic movements and reaches. However, recent advances in recording techniques for rodents, such as *in vivo* calcium imaging using miniaturized microscopes, have made it possible to sample large-scale ensemble activity in cortex during unrestrained, natural behavior. Here, we imaged activity in 100-150 neurons at a time in superficial PPC while mice performed a variety of spontaneous behaviors, such as foraging for food, eating in place, running on a running wheel, and drinking from a waterspout, in an open arena. To capture the subtleties and recurring patterns in the animals' movements we employed a 9-camera high-speed tracking system to track the major joints, head and limbs of the animals. We are currently building unsupervised deep architectures for time series data to classify dynamic 3D pose and posture, as well as to capture transitions between movement states. Once the behavior is mapped we will test for single neuron and population correlates of ongoing and upcoming movements. By observing multiple spontaneous behaviors, each containing unique and overlapping 3D movements, we aim to shed light on how PPC activity generalizes to the complex and diverse activities of an average day "at home" for a mouse.

Disclosures: B. Dunn: None. T. Tombaz: None. K. Hovde: None. G. Taylor: None. J. Whitlock: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.09/SS17

Topic: E.04. Voluntary Movements

Support: ERC335328

Title: Ensemble coding of self-motion in parieto-frontal circuits in rats

Authors: *B. MIMICA, B. A. DUNN, J. R. WHITLOCK;
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Abstract: Efficient traversals of environments are enabled by a synergy of interconnected brain structures, integrating information about the animals' past and desired future positions. The hippocampus and parahippocampal cortices code space in world-based coordinates, while posterior-parietal cortex (PPC) and premotor cortex (M2) code movement sequences in egocentric coordinate frames; the different frames of reference are combined such that

behavioural sequences can be synthesized to reach spatial goals. Recent work in freely behaving rodents has shown that PPC and M2 both exhibit robust prospective tuning properties in accordance with orienting movements up to and greater than 1 second in advance.. The aim of our current experiments is to characterize the respective contributions of PPC and M2 during the on-line synthesis of movements plans while rats forage for food crumbs in the open field arena. To do so we are conducting simultaneous single-unit recordings in PPC and M2 using silicone probes while tracking the animals' head and spine positions with a 6-camera, infrared 3D tracking system. Our ongoing recordings suggest that cells in both regions show similar types self-motion representation, and we are investigating the relative extent of prospective coding across areas (i.e. whether one area codes movements further ahead in time). In addition, to test whether the properties of prospective coding generalize across tasks, we are recording the same cells in a task where animals repeatedly switch between exploratory and goal-oriented modes of navigational behaviour. The goal of these experiments is to determine whether the temporal properties of action planning are hard-wired or change flexibly according to cognitive demand, and, if possible, to measure how quickly complex movement plans emerge once a goal is established.

Disclosures: B. Mimica: None. B.A. Dunn: None. J.R. Whitlock: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.10/SS18

Topic: E.04. Voluntary Movements

Support: NIH NICHD CRCNS

NIH NINDS

NSF NCS

Neilsen Foundation

BWF

ARCS Foundation

Title: Predicting neural activity in behaviorally-irrelevant dimensions

Authors: *J. A. HENNIG[1], M. D. GOLUB[2,1], P. J. LUND[3,4,1], P. T. SADTLER[5,6,7], K. M. QUICK[5,6,7], S. I. RYU[10,11], E. C. TYLER-KABARA[6,8,9], A. P. BATISTA[5,6,7], B. M. YU[1,2,4], S. M. CHASE[1,4];
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Abstract: The activity of millions of neurons in the motor cortex drives the contraction of hundreds of muscles. Because there are so many more neurons than muscles, a vast number of different population activity patterns would likely generate the same movement. The activity patterns that all lead to the same movement thus define a behaviorally-irrelevant space. How does the brain choose from among these behaviorally equivalent options?
We know that not all neural activity can be generated by the subject due to physiological constraints, such as limitations on minimum and maximum firing, and constraints due to connectivity among neurons. We consider three hypotheses of behaviorally-irrelevant activity, given these constraints. First, the activity may be "uncontrolled," and vary over the allowable range of network states. Second, the motor system may prefer activity patterns that minimize energy usage, in analogy to previous studies of muscle activity. Finally, rather than minimizing energy expenditure, activity in this space may still reflect task demands, despite being behaviorally-irrelevant.
To compare these hypotheses, we analyze data from a brain-computer interface (BCI) center-out task. In this task, Rhesus macaques controlled a computer cursor using their neural activity, recorded in the primary motor cortex using a Utah array. In contrast to traditional approaches (e.g., monitoring neural activity while a monkey performs arm reaches), in a BCI the mapping from neural activity to cursor movement is defined by the experimenter. This allows one to define precisely the set of neural activities that will result in the same cursor movement, i.e., the behaviorally-irrelevant space. Our aim is to assess how well the above hypotheses predict the monkey's selection of neural activity from this behaviorally-irrelevant space.
We find that predictions made by the uncontrolled and energy-minimizing hypotheses fail to match the observed data. Instead, behaviorally-irrelevant activity varies with both task demands and behavioral output. Assessing this activity in the context of two different mappings between neural activity and behavior provides an explanation for this observation: When a monkey learns a new mapping, he chooses from among the same patterns of neural activity that he generated under similar conditions in the previous mapping. Neural activity in the behaviorally-irrelevant space is thus a result of the monkey intervening minimally on the behavior he used in the previous mapping. This finding suggests that the way redundancy is resolved at the level of neural population activity may be different than the way it is resolved at the level of the muscles.

Disclosures: J.A. Hennig: None. M.D. Golub: None. P.J. Lund: None. P.T. Sadtler: None. K.M. Quick: None. S.I. Ryu: None. E.C. Tyler-Kabara: None. A.P. Batista: None. B.M. Yu: None. S.M. Chase: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.11/SS19

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 EY012135

NIH Grant F32 NS076206

Title: Neuronal activity in the posterior parietal cortex encodes arm direction, not saccade direction, during bimanual reaching

Authors: *E. F. MOOSHAGIAN, L. H. SNYDER;
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Abstract: We often orient our gaze to the target of our reaches. Eye-hand coordination may depend in part on circuits in the posterior parietal cortex (PPC). Within the PPC, the spatial locations of saccade and reach goals are preferentially represented by the neurons in the lateral intraparietal area (LIP) and the parietal reach region (PRR), respectively. The stereotyped nature of unimanual coordinated eye-arm movements makes it difficult to dissect the role of these circuits in coordination. Primates, including humans, frequently coordinate two or more limbs at once. For example, we may pick up a pitcher with one hand and a glass with the other. Limb movements can occur more or less simultaneously, but our eyes are yoked together and can move to only one target at a time. As a result, we can move our limbs in parallel but we make eye movements in series, first to one target and then to another. This facilitates a novel means of investigating eye-hand coordination. We recorded neuronal activity in LIP and PRR while monkeys planned a simultaneous reach to two targets, with no constraints on eye movement. PRR neurons coded the direction of the contralateral arm movement irrespective of the direction of the saccade; there was no effect of the direction of the first saccade on PRR activity. Surprisingly, LIP neurons showed a very similar response. That is, LIP neurons did not code the direction of the saccade during the planning period, but instead coded the direction of the contralateral arm movement during bimanual reaches. Only as the saccade was actually executed was a small saccade-specific response seen in LIP. These results challenge a role for LIP in eye-hand coordination.

Disclosures: E.F. Mooshagian: None. L.H. Snyder: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.12/SS20

Topic: E.04. Voluntary Movements

Support: NIH Grant NS079664

Title: Populations of mirror neurons capture similar sequences of hidden Markov states during execution and observation of a reach, grasp, and manipulate task

Authors: *K. A. MAZUREK, M. H. SCHIEBER;
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Abstract: Mirror neurons (MNs) discharge during both the execution and observation of a movement. We applied Hidden Markov Models (HMM) to detect hidden states in populations, asking whether MNs represent behavioral states in addition to the movement.
Two Rhesus monkeys performed a task in which a visual cue instructed the monkey to reach to, grasp, and manipulate one of four target objects: a button, a sphere, a coaxial cylinder, or a perpendicular cylinder. After first performing multiple trials itself, the monkey observed an experimenter performing trials of the same task. Single- and multi-unit activity was recorded from microelectrode arrays implanted in primary motor cortex (M1) and ventral premotor cortex (PMv) during action execution (AE) and action observation (AO) trials.
A recorded unit was considered task related if its firing rate was significantly different after the visual cue as compared to a baseline period 0.5s before the cue. A unit was classified as a MN if it modulated significantly during both AE and AO. For the two monkeys, 16 and 9 MNs were identified, respectively. HMMs were trained using these MN populations to identify between 2 and 5 hidden states. The number of hidden states was selected based on the average HMM state probabilities and their consistency across trials. Although information about the timing of behavioral events—cue presentation, onset of movement, and final hold—was not provided to HMMs during training, the resulting hidden states showed transitions occurring near these behavioral events.
Across all trained HMMs, the baseline state probability fell near the cue presentation. A movement state increased in probability at times that correlated with the onset of movement. A final state transitioned from low to high probability during the final hold. These states were observed in both AE and AO trials, with the state transitions occurring similarly relative to the behavioral events in each trial. Neural activity from AE and AO trials was compared across objects during each type of hidden state to examine how the activity of each MN varied. The HMM state sequences trained on AO trials were similar to those trained on AE trials.

Our results indicate that MNs do not simply respond to the observation of actions. Rather, populations of MNs encode entire sequences of observed behavioral events.

Disclosures: K.A. Mazurek: None. M.H. Schieber: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.13/SS21

Topic: E.04. Voluntary Movements

Support: CIHR MOP84454

FRQS Postdoctoral Fellowship (NB)

Title: Neural correlates of motor skill learning in dorsal premotor cortex (PMd) reflect mainly predictive feedforward compensation rather than online error correction.

Authors: N. BHUTANI[1], *J. F. KALASKA[1], R. SHADMEHR[2], A. M. GREEN[1];
[1]Univ. De Montréal, Montréal, QC, Canada; [2]BME, Johns Hopkins Univ., Baltimore, MD

Abstract: As we practice a new motor skill, we learn by making errors. Error feedback signals help correct the movements in real time, and also guide incremental changes in the motor system to permit predictive improvements in performance in subsequent movements (Kawato & Gomi 1992; Thoroughman & Shadmehr 2000; Li et al. 2001; Padoa-Schioppa et al. 2004). However, the neural processes that lead to this transformation remain poorly understood. We recorded neural activity in the dorsal premotor cortex (PMd) of a Rhesus monkey (*Macaca mulatta*) during reaching movements made while holding onto a torquable robotic manipulandum. PMd neurons are implicated in the early preparatory activity for the movement (Crammond and Kalaska 1996, 2000; Padoa-Schioppa et al. 2004). Each neuron's preferred reach direction (PD) was identified while the monkey made unperturbed reaches in 8 directions in a visually-guided instructed-delay reach task. Each neuron was then studied while the monkey made movements in unpredictable clockwise (CW) and counterclockwise (CCW) velocity-dependent "curl" force fields that pushed on the monkey's arm perpendicular to the direction of movement. Most PMd neurons showed weak feedback-error responses to these unpredictable perturbations. To examine the contribution of PMd to predictive compensatory changes, the monkey was then re-trained each day in one curl field (CW or CCW) while reaching in a direction perpendicular to the neuron's PD. As the monkey learned to compensate for the force field, PMd neurons showed gradual response changes primarily during the delay period before movement onset, but showed

much less modification of their movement-related activity. This contrasted with many primary motor cortex (M1) neurons, which responded strongly at 100-250ms latencies to the unpredictable perturbations and whose predictive compensatory changes in activity during reaches in the predictable curl field were seen mainly during the peri-movement period after the end of the delay period. These findings suggest that PMd contributes mainly to predictive feedforward compensation for task dynamics and less to feedback-mediated error correction, whereas M1 is implicated in both real-time error feedback-mediated corrections for unpredicted perturbations and to feedforward compensation for predictable perturbations.

Disclosures: N. Bhutani: None. J.F. Kalaska: None. R. Shadmehr: None. A.M. Green: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.14/SS22

Topic: E.04. Voluntary Movements

Support: German BMBF grant 01GQ1005C

DFG grants CRC-889

DFG grants GA1475-B2

Title: More active neural dynamics during movement control in monkey dorsal premotor cortex compared to parietal reach region

Authors: *H. GUO[1], S. KUANG[1,2], A. GAIL[1,3,4];
[1]German Primate Ctr., Goettingen, Germany; [2]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China; [3]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany; [4]Georg-August-Universität Göttingen, Goettingen, Germany

Abstract: The dorsal premotor cortex (PMd) and the parietal reach region (PRR) both belong to the frontoparietal reach network in monkeys and are involved in the planning and control of visually guided reach movements. A recent study showed that sustained motor goal encoding in PRR during movement planning predominantly reflects the physical rather than the visual goal of impending reaches conducted under reversed vision (1). Also, preliminary statistics on the numbers of motor-goal encoding neurons in area PMd compared to PRR suggested high similarity in both areas during the planning period of a movement. Yet, pre-selecting and grouping subsets of neurons based on their selectivity as function of spatial task parameters can mask single-neuron heterogeneity since neural response patterns might be partially driven by

internal network dynamics rather than external drive. Here we test if, despite the similarity of PMd and PRR during steady-state motor planning, both areas exhibit similar or different spatial encoding properties and neural dynamics during movement execution. Monkeys conducted memory-guided delayed center-out reaches under normal or reversed vision. First, we assessed the predominance of either physical or visual motor goal encoding in the population dynamics by computing a 'vector of selectivity' (VOS) in the high-dimensional state space of all neurons activity as a function of time. The VOS was defined as vector connecting the state space trajectories of right- and left-side cued trials separately in each viewing context (normal vs. reversed). Opposing VOS between the two viewing contexts, for example, indicate preferential encoding of physical rather than visual goals. During movement planning, PMd exhibited predominant physical goal encoding, but less predominantly than PRR. Second, in both areas physical goal encoding strongly predominated during the movement period. Finally, the neural dynamics during transition from reach planning to reach execution revealed larger dynamical changes in PMd compared to PRR. These state space dynamics suggest more compound encoding in PMd compared to PRR during motor planning, followed by richer intrinsic dynamics in the transition from planning to movement in PMd. We conclude that PMd during motor planning seems to exhibit more diversity in spatial encoding, i.e. less predominance of a single frame of reference, which then leads to larger dynamical changes from planning to movement, because physical goal representation dominate during reach execution in both areas. (1) Kuang et al. 2015 Cereb Cortex DOI: 10.1093/cercor/bhu312

Disclosures: H. Guo: None. S. Kuang: None. A. Gail: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.15/SS23

Topic: E.04. Voluntary Movements

Support: CIHR 274317

GSK/CIHR 288733

Title: Deactivation of PMd and A5 in non-human primates impairs feedback responses to mechanical disturbances of the limb.

Authors: *T. TAKEI[1], S. G. LOMBER[2], D. J. COOK[1], S. H. SCOTT[1];
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Abstract: Recent studies highlight that neural responses to mechanical disturbances of the limb are quickly transmitted throughout sensory and motor cortices in non-human primates. However, the causal relationship between the neural activities of these areas and the generation of the feedback response to the mechanical perturbations remain unclear. Here we investigated the contribution of dorsal premotor cortex (PMd) and parietal area 5 (A5) to feedback control of the upper limb by deactivating these areas with a reversible cortical cooling technique. We trained a male rhesus monkey to perform a unilateral arm postural control task. While the monkey maintained the finger tip in a circular target (0.8 cm radius), a mechanical step-torque perturbation was applied on his elbow and shoulder joints and the monkey was required to return to the target within 500 ms. During the task, we cooled PMd, A5 or both with chronically implanted probes (cryoloops). We quantified the speed ('return time') and accuracy ('endpoint error') of the corrective response before, during and after cooling. Results showed that the PMd cooling increased both of the return time and the endpoint error relative to the control condition (sham cooling). On the other hand, the A5 cooling increased the endpoint error, but did not change the return time. In addition, when we cooled the areas simultaneously, the cooling impaired both of the return time and the endpoint error and the effects were predictable by a linear summation of the individual cooling. These results indicated that these areas have unique contributions to the feedback control of voluntary limb movements.

Disclosures: T. Takei: None. **S.G. Lomber:** None. **D.J. Cook:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN technologies.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.16/SS24

Topic: E.04. Voluntary Movements

Support: HKRGC-GRF grant 14119214

SRFDP-HKRGC Joint Grant M-CUHK409/13

Title: Local circuits in reshaping of motor cortex projection neurons during motor training

Authors: *D. C. CHAN[1], H. KO[3], Y. KE[2], W. YUNG[2];
[1]The Chinese Univ. of Hong Kong, Hong Kong, China; [2]Sch. of Biomed. Sci., The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong; [3]Dept. of Med. and Therapeut., Li Ka Shing Inst. of Hlth. Sci., Hong Kong, Hong Kong

Abstract: The recruitment of primary motor cortical neurons in the formation of motor memory is not well understood. While extensive work has been done in literature regarding the intrinsic encoding of motor primitives such as limb kinematics as well as changes to ensemble dynamics in the learning of a novel motor task, the circuit mechanisms that mediate the conversion of individual primitive-encoding neurons to task-encoding neurons remain unclear. Previous work in our laboratory using in vivo microelectrode array recordings in rats learning a forelimb reaching task observed the formation of a subset of temporally-coupled neurons from a wider neuron pool, with differential long-term potentiation profiles between different dendritic layers of layer 5B projection neurons. We therefore hypothesize that the local circuits in specific cortical layers mediate the reshaping of the roles of individual neurons.We approach this problem using chronic in vivo two-photon imaging while head-fixated mice are trained to perform a stereotyped forelimb motion on a manipulandum not normally found in the animal's motor repertoire. Functional calcium recordings were taken from both layer 2/3 and layer 5 of the contralateral caudal forelimb region of the primary motor cortex, and local connectivity was inferred via histological reconstruction. We then explore the causal role of local inputs in the final task-relevance outcome of the projection neurons.

Disclosures: D.C. Chan: None. H. Ko: None. Y. Ke: None. W. Yung: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.17/SS25

Topic: E.04. Voluntary Movements

Support: FQXI Grant 2013-111430 (4661)

Title: Strong neuron-to-body coupling implies weak neuron-to-neuron coupling in motor cortex.

Authors: *P. KELLS[1], S. GAUTAM[2], W. L. SHEW[2];
[1]Physics, [2]Univ. of Arkansas, Fayetteville, AR

Abstract: Cortical neurons can be strongly coupled to the network in which they are embedded, firing in sync with the majority, or they can fire more independently. Both these scenarios - strong and weak population coupling - have potential computational advantages when considering the information output from a cortical network. In motor cortex, for instance, if the information output to the body is conveyed by a strongly coupled population of neurons, one might expect that the information would be more robustly conveyed, benefiting from strength in numbers. On the other hand, a motor code with greater information capacity could be

implemented by neurons that fire more independently. To determine which of these scenarios is employed, here we directly measured information transmission from motor cortex neurons to bodily movement in awake, freely moving rats, as well as the population coupling for these neurons. First, we found that neurons in motor cortex exhibit a wide range of different population coupling, which was tunable by manipulating inhibitory signaling in the motor system. All neurons with high population coupling had low information transmission from cortex to body movement. Conversely, low population coupling was a necessary condition for high information transmission from motor cortex to body movement. Inhibitory tuning of population coupling did not change these functional roles. Our results suggest that the population of neurons in motor cortex is divided into an 'internal processing' group with high population coupling and an 'external output' group devoted to high capacity information output to the body.

Disclosures: P. Kells: None. S. Gautam: None. W.L. Shew: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.18/SS26

Topic: E.04. Voluntary Movements

Support: NIH: R01-NS047356

NIH: R01-NS050256

NIH: RC1-NS070311

DARPA: W911NF-06-1-0053

DARPA: N66001-10-C-4056

DARPA: N66001-12-C-4027

Title: Dynamic representation of reach speed in the motor cortex

Authors: *S. B. SUWAY[1], J. ORELLANA[2], A. B. SCHWARTZ[1];
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Abstract: In primate motor cortex (M1), single neurons are broadly tuned to reach direction, with each cell discharging maximally for a particular direction. The speed of the reach also affects neural activity, contributing both a multiplicative and additive influence on firing rates. Early studies reported a "representation" of reach speed in single units and in population

analyses. However, such studies commonly restricted analysis to the trial period in which the arm was moving. It was subsequently suggested that representation of speed is less robust in the firing rate fluctuations that continue after movement ceases. Recently, our group identified distinct epochs in the activity of M1 neurons spanning the reaction time through the target-hold period of a reaching trial. Directional tuning of single neurons is robust and stable within an epoch, but preferred direction may change from one epoch to the next. We wondered if speed encoding might also vary between epochs. We recorded spiking of M1 neurons from three monkeys during a center-out reaching task. Firing rates of each neuron were analyzed using two models: a direction-only tuning model, and a velocity-speed tuning model. Both models were fitted during each epoch, and the goodness-of-fit of each model was assessed. We found clear evidence of dynamic coding, with the velocity-speed model fitting rates of many neurons markedly better during the early epochs. Using the population vector algorithm (PVA), we reconstructed reach trajectories using firing rates from only the speed-related epochs. We found the decoded trajectories were highly accurate, and the time course of their magnitude was strikingly similar to the recorded reach speeds. Given that velocity can be extracted from the population with high fidelity by selectively utilizing segments of firing, we wondered if a simpler computation, using all the data, could yield similar results. This would be useful for example in neural prosthetics because it would allow us to estimate arm velocity despite the ongoing changes in single unit tuning during a reach. We developed a simple regression method to find a linear combination of single unit firing rates that closely matches the measured arm velocity. The method identifies an axis in the n-dimensional population firing rate space. When the population activity is projected onto this axis, we find robust and accurate velocity tuning while the hand is moving. When the arm comes to a stop, and despite continued fluctuations in firing of most neurons, activation along this axis drops nearly to zero. This method may be advantageous in neural prosthetics, which often fail to afford subjects control of output speed.

Disclosures: S.B. Suway: None. J. Orellana: None. A.B. Schwartz: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.19/TT1

Topic: E.04. Voluntary Movements

Support: NINDS Grant R01 NS079664

Title: Neural dynamics of reach-to-grasp: sequential location-potent and then object-potent projections of state-space trajectories.

Authors: A. G. ROUSE, *M. H. SCHIEBER;
Neurol. and Neurosci., Univ. of Rochester, Rochester, NY

Abstract: In reach-to-grasp movements, reaching with the arm and grasping with the hand typically are thought to proceed concurrently. Recent evidence suggests, however, that neural activity in motor and premotor cortex may control location early and grasp shape later in the same movement. We therefore examined the neural state-space trajectories of primary motor cortex (M1) neuron populations to determine whether location-potent projections were distinct from object-potent projections, and if so, whether the state-space trajectories projected simultaneously or sequentially onto location-potent and object-potent planes.
We analyzed data recorded from two Rhesus monkeys, each of which performed a reach-grasp-manipulate task that dissociated the location to which the hand reached from the object the hand grasped. Populations of M1 neurons were recorded in multiple sessions from microelectrode arrays implanted in each monkey. For each session, we examined the state-space in which the firing rate of each neuron constitutes an orthogonal state-space dimension. In this space the simultaneous firing rate of all sampled neurons constitutes a state which moves in time, forming a trajectory. We identified two 2-dimensional planes: one on which the projection of firing-rate trajectories segregated best according to reach location, another on which the trajectories segregated best according to the object grasped. The location-potent plane and object-potent plane in a given session were distinct.
Next, we followed the time course of the trajectories. Prior to movement onset the trajectories moved in state-space directions that projected substantially onto the location-potent plane, but were relatively null in the object-potent plane. After the onset of movement, however, the direction of the neural trajectories changed, coming to project substantially onto the object-potent plane while being relatively null in the location-potent plane. Additional analyses showed that the location-potent motion and object-potent motion occurred in overlapping neural sub-spaces, i.e. the firing rates of many individual neurons modulated during both the location-potent phase and the object-potent phase of the same movement.
Our findings demonstrate how distinct latent output features may be represented in the joint activity of a neural population. In theory, multiple latent features could be represented simultaneously. But during the present reach-to-grasp movements the direction of neural trajectories changed such that first one feature and then the other could be controlled sequentially by the same population of neurons.

Disclosures: A.G. Rouse: None. M.H. Schieber: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.20/TT2

Topic: E.04. Voluntary Movements

Support: NIH Grant 1F31NS089376-01

NIH Pioneer 1DP1OD006409

DARPA REPAIR N66001-10- C-2010

Howard Hughes Medical Institute

Title: Decoding kinetic information from PMd and M1 during reaching

Authors: *E. TRAUTMANN, S. STAVISKY, J. KAO, S. RYU, K. SHENOY;
Stanford Neurosciences, San Francisco, CA

Abstract: Several experiments have characterized the tuning properties of neurons PMd and M1 when loads are applied to the arm [Kalaska 1989, 2005], though less is understood about the relationship between population-level neural dynamics and reach kinetics. Here, we report how neural dynamics in PMd and gyral M1 adapt to external loads to control force, demonstrating that the arm's output force along the vertical axis can be decoded from neural activity before and during movement.

Two rhesus macaque monkeys, implanted with two 96 electrode arrays in PMd and M1, made delayed reaches in a vertical workspace to eight targets with three levels of added weight: 0g, 100g, or 200g. Weights were attached to the top of the wrist for randomly selected blocks of 250 trials. At the start of a trial, monkeys held their arm at the center, resisting gravity against the different weights.

For monkey J, 109 of 192 (monk R: 50/171) electrodes' multiunit activity displayed tuning to the applied weight, while 170/192 (80/171) displayed target tuning. While statistically significant, the applied weights modulated firing rates only modestly. Despite this, we asked whether it is possible to discriminate the level of weight attached from the neural activity before movement. Classification accuracy between the 0g and 200g weight levels was 75.3% (58.9%), using a multi-class SVM with 10-fold CV.

We used the SVM to define a neural Gravity Force Axis (GFA) that best discriminates applied weight before movement, and ask whether this axis contains information about the arm force generated in the vertical dimension during the reach. Targets at the top and bottom of the workspace require different force profiles to accelerate the arm with or against gravity. We modeled the required force profile for each target, and compared this to the trial-averaged force

decoded from the projection of the neural state along the GFA. The correlation coefficient between decoded force and modeled force was .67 (.26), suggesting that this axis can serve as a readout of behavioral kinetics along one dimension of physical space.
Curiously, the percentage of neural variance captured by the GFA was only 0.7% (0.59%). The top principal components of neural activity are dominated by the large changes in firing rate during the movement period, which are largely independent of the total force. Despite this, we show that kinetic information is available in the population activity in gyral PMd and M1, amenable to recordings using multielectrode arrays, and potentially important as an additional channel of information for controlling neural prosthetic devices.

Disclosures: E. Trautmann: None. S. Stavisky: None. J. Kao: None. S. Ryu: None. K. Shenoy: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.21/TT3

Topic: E.04. Voluntary Movements

Support: NIH R01N545853-01

Title: Neural activity in primary motor cortex correlates with previous trial performance during adaptation of reaching movements to a visuomotor rotation

Authors: *A. J. SUMINSKI, J. KOSER;
Electrical Engin. and Computer Sci., Milwaukee Sch. of Engin., Milwaukee, WI

Abstract: It is well understood that the adaptation of reaching movements to unpredictable environmental perturbations flows from the nervous systems ability to update motor commands based on performance information from only the most recent movement attempts. The neural mechanisms supporting reach adaptation remain unclear as previous studies non-human primates have demonstrated that neurons in primary motor cortex (M1) show learning related changes in response to constant perturbations over the course of many reaches. However, these studies have not addressed changes in neural activity that occurs on a much shorter timescale (i.e. from one trial to the next). Here we investigate whether M1 participates in this short term adaptive process. Two rhesus macaques were implanted with a 100 micro-electrode array (Blackrock Microsystems) that was used to record the spiking activity of neural ensembles in M1 while they reached from a central target to one of eight peripheral targets (6cm) using a two degree of freedom, planar robotic manipulandum. Experiments contained three phases. During the pre and

post adaptation phases, the monkeys made at least 5 unperturbed movements to each of the peripheral targets, which were pseudo-randomly selected prior to each trial. During the adaptation phase, all movements were directed to the 90 degree target and were perturbed by a counter-clockwise rotation of the cursor position about the central target that varied in magnitude pseudo-randomly from trial-to-trial. Movement error was calculated as the peak deviation orthogonal to the line connecting the center and peripheral target. Consistent with the results of previous human subject experiments, we observed a linear relationship between movement error and rotation magnitude and that current trial movement errors were best predicted by an autoregressive model incorporating error and rotation information from the most recent movement attempts. We found a significant correlation between the time series of binned firing rates (on a trial-by-trial basis) of a subset of M1 neurons and the measured reaching errors and rotation magnitudes from previous movement attempts. Furthermore, we found that the binned firing rates of a separate subset of neurons were also significantly correlated with the expected rotation on the upcoming trial predicted by the autoregressive model. These results suggest that M1 incorporates information from previous movement attempts into the planning and execution of subsequent movements when there is environmental uncertainty.

Disclosures: A.J. Suminski: None. J. Koser: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.22/TT4

Topic: E.04. Voluntary Movements

Support: EUROHEADPAIN grant 602633

Title: Cortico-cerebellar integration in the motor thalamus.

Authors: *C. B. SCHAEFER, S. V. GORNATI, F. HOEBEEK;
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Abstract: Relating sensory information about the surroundings with the current posture of the body and the upcoming motor plans is essential for executing coordinated movements. Sensorimotor integration has been studied separately in the cerebellum and in the cerebrum, but how these two networks converge remains to be elucidated. Within the motor system the most suitable candidate nucleus for cortico-cerebellar convergence is the thalamic ventro-lateral nucleus, as it receives direct synaptic input from cerebellar nuclei neurons but also feedback from primary motor cortex, in particular from layer VI neurons. Cerebellar input in the thalamus

has been shown to exhibit driver properties, in that microstimulation in the superior cerebellar peduncle can evoke postsynaptic action potential firing, whereas the synaptic properties of the input from layer VI of the primary motor cortex remains to be defined. We hypothesize that synapses from layer six of the motor cortex converge with cerebellar synapses upon single thalamic relay neurons and modulate the thalamic response to cerebellar input. Therefore, we established an optogenetic dual-channel photostimulation approach by expressing channelrhodopsin2 (ChR2) in cerebellar nuclei and a flexed version of red-shifted ChR2-construct in layer VI of the motor cortex of Ntsr1-Cre mice. Using isolated photostimultion of both axonal populations synapsing in the thalamus we found that information from both cerebellar nuclei and motor cortex layer VI converges on individual neurons in the ventral-lateral nucleus. Furthermore, our electrophysiological recordings revealed that motor signals from cortical layer VI show paired-pulse fascilitation, which together with the presence of a metabotropic receptor mediated current indicates that these inputs show modulatory properties. In contrast, higher frequency stimulation of cerebellar synapses resulted in paired-pulse depression indicative of driver properties. These results support a role of relay neurons in ventro-lateral thalamic nucleus in integrating cortical and cerebellar information. The properties of input coming from layer VI of motor cortex are suggestive of a modulatory role that might shift the thalamic membrane potential from a hyperpolarized to a more depolarized state. We speculate that this gating signal induces a switch in the thalamic responses to cerebellar input from burst firing in absence of layer VI input to tonic thalamic firing in presence of layer VI input. These findings may shed light on how thalamic relay cells respond to high-frequency excitatory cerebellar nuclei input that converges with modulatory excitatory cortico-thalamic inputs.

Disclosures: C.B. Schaefer: None. S.V. Gornati: None. F. Hoebeek: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.23/TT5

Topic: E.04. Voluntary Movements

Support: NIH Pioneer 1DP1OD006409

DARPA REPAIR

Stanford Graduate Fellowship

Title: Electrical microstimulation in primate premotor cortex hijacks local neural activity

Authors: *D. J. O'SHEA[1], K. V. SHENOY[2];
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Abstract: Intracortical electrical microstimulation (ICMS) is a powerful tool for probing neural circuit function and organization. Evidence from behavioral studies and intrinsic, functional MR, and calcium imaging suggest that the activity evoked by high frequency ICMS is spatially and temporally complex. However, the responses of local neural populations to ICMS are typically challenging to record directly due to the large electrical artifact. Consequently, it is unclear how ICMS engages local neural populations and how these activation patterns interact with the dynamics of normal task-related activity. Here, we developed an artifact removal method to directly record the local effects of high frequency ICMS delivered to dorsal premotor cortex (PMd) in a rhesus macaque engaged in a reaching task. We find that neural activity near the stimulation site no longer reflects pre-existing task-related activity.

We stimulated in PMd (60 ms, 333 Hz biphasic pulses) and verified that twitches of the arm and shoulder were evoked above threshold. Within 1 mm of the stimulating electrode, we acutely recorded neural activity using a linear multielectrode array (Plexon V-probe). During the task, we stimulated on a subset of interleaved trials (3 distinct timepoints, 5 reach directions). For currents below 40 uA, the recorded artifacts did not saturate the amplifier. We developed a method to remove the artifact by exploiting the common structure of the artifact over electrodes, pulses, and trials, enabling us to detect spiking activity during and after the stimulation. 59 / 77 neurons' firing rates were altered by stimulation ($p < 0.05$).

For each of 61 neurons exhibiting significant modulation during the reaching task ($p < 0.05$, ANOVA), we summarized the contribution of the underlying task-related neural activity to during-stimulation activity using an index ranging from 0 (no task contribution) to 1 (purely additive interaction). The median index across the population was 0.25 (0.15-0.47, 95% CI), indicating that ICMS-evoked activity mostly replaced, rather than superimposed with, task activity. Immediately after stimulation, many neurons were suppressed for > 150 ms; the index recovered to pre-stimulation level only 180 ms following stimulation. These results provide direct neural evidence for the hypothesis that ICMS can "hijack" local neural circuitry (Griffin et al., 2011), and contrasts with earlier reports that optogenetic stimulation interacts additively with task-activity (O'Shea et. al, SFN 2013) in PMd. The results highlight that combining stimulation with recording and artifact rejection methods can provide precise, causal insight into neural computation.

Disclosures: D.J. O'Shea: None. K.V. Shenoy: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.24/TT6

Topic: E.04. Voluntary Movements

Support: DARPA HDC HR011-14-C-0102

DARPA N66001-11-1-4205

Simons SCGB 325548

NEI R01-EY024067

Title: Multi-scale electrophysiology in macaque motor cortex during reaching

Authors: *A. L. ORSBORN[1], C. WANG[2], K. CHIANG[2], N. C. BOLES[1], J. VIVENTI[2], B. PESARAN[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Biomed. Engin., Duke Univ., Durham, NC

Abstract: Neural computations governing behavior span many spatial scales, from individual neurons to large-scale networks within and across brain areas. Similarly, electrophysiology can monitor neural activity from microns, to millimeters, and up. Relationships between these different measures are poorly understood. Moreover, how each scale of neural computation relates to behavior is unclear. Connecting neural computations across spatial scales will require simultaneous multi-scale measurements during behavior. We therefore developed a flexible platform to combine multiple measurement scales and used it to study motor cortex during reaching. The platform can integrate subdural micro-electrocorticography (μECoG) and a microdrive of independently movable electrodes to measure action potentials, local field potentials (LFP), and surface potentials (μECoG) within the same volume of tissue. We embed a μECoG array within a silicone artificial dura (AD), providing chronic subdural access and precise alignment between the μECoG array and penetrating microdrive. We implanted this multi-scale system in one macaque (Macaca Nemestrina, male). A custom-shaped chamber (2.6cm inner diameter) was implanted over the primary motor cortex, using MRI-based targeting (BrainSight software, Rogue Research). A 244-contact μECoG array (50 μm LCP with 0.3 μm thick gold traces; 200 μm diameter contacts with a 0.15 μm platinum coating; 0.75 mm contact spacing) was implanted, followed by a 32-electrode microdrive (Gray Matter Research; glass-coated tungsten electrodes, 125 μm diameter; 1.5 mm spacing). Spiking, LFP, and μECoG activity were recorded during rest, full-field light-flash visual stimulation, and a reach-to-grasp task. Visual stimulation-triggered responses were used to assess day-to-day variability in signal

recordings. Reaching behavior was monitored with motion tracking (Motion Analysis Inc.) of the arm and hand in 3-dimensions. The subject performed a naturalistic reach and grasp task in which he grasped a small square with a power grip presented in random locations in the workspace. We present analysis of spike, LFP, and μECoG signals' relationships to behavior (decoding analyses) and relationships between measurement scales. By using an artificial dura, our system provides chronic, flexible brain access for multi-scale, multi-modal measurements. Future work will extend our approach to incorporate transparent μECoG for optical imaging and stimulation access. Such multi-scale recording and interrogation of neural circuits will be critical for dissecting neural circuits and their relationships to behavior.

Disclosures: A.L. Orsborn: None. C. Wang: None. K. Chiang: None. N.C. Boles: None. J. Viventi: None. B. Pesaran: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.25/TT7

Topic: E.04. Voluntary Movements

Support: NSG GRFP (SDS, JCK)

NSF IGERT 0734683 (SDS)

DARPA N66001-10-C-2010 (KVS)

DARPA W911NF-14-2-0013 (KVS)

NINDS R01NS076460 (KVS)

NIH 8DP1HD075623-04 (KVS)

NIMH 5R01MH09964703 (KVS)

Title: Error-related motor cortical activity transforms from output-null to output-potent dimensions

Authors: *S. D. STAVISKY[1], J. C. KAO[2], S. I. RYU[2,6], K. V. SHENOY[2,3,4,7,5]; [1]Neurosciences Grad. Program, [2]Electrical Engin. Dept., [3]Neurobio. and Bioengineering Departments, [4]Bio-X Program, [5]Stanford Neurosciences Inst., Stanford Univ., Stanford, CA; [6]Palo Alto Med. Fndn., Palo Alto, CA; [7]Howard Hughes Med. Inst. at Stanford Univ., Stanford, CA

Abstract: The sensorimotor system must continuously monitor afferents so as to detect movement errors, compute a response, and then output this command to muscles. This constrains the system: error-related computation must be decoupled from causing movement until it has progressed enough to generate the appropriate correction. We investigated how this separation is achieved in the primary (M1) and dorsal premotor (PMd) cortex, areas in which activity can drive movement and strongly reflects sensory inputs signaling errors. We found that the 'output-null hypothesis' (Kaufman et al., 2014, Druckmann & Chklovskii 2012) is a mechanism by which error processing can occur in motor cortex without prematurely "leaking out" as output. This proposed mechanism takes advantage of the many-to-few mapping between motor cortex and its downstream movement-generating targets: because of this redundancy, there are many ways in which cortical neurons' activities can change while still "canceling out" as far as the downstream readout is concerned. For a simplified linear model of the network, this is equivalent to restricting motor cortical changes to the null space of the matrix mapping firing rates to movement.

To study neural dynamics following errors, we recorded from M1 and PMd of two rhesus macaques as they performed a 2D cursor task to which we added random mid-trial 'cursor jump' perturbations. In the first experiment, the monkeys controlled a computer cursor with their hand. We approximated the relationship between cortex and movement with a linear mapping, W, between firing rates and hand velocity. When we projected perturbation-evoked firing rate changes into the output-null and output-potent spaces of W, we observed that an output-null response preceded (putatively) output-potent changes by as much as 55 ms. While this result is consistent with our hypothesis, we recognize that W is only a rough estimate of the relationship between motor cortical activity and muscles.

To address this limitation, we performed a second experiment in which the monkeys controlled the cursor via a brain-machine interface (BMI). This paradigm removes all intermediaries between motor cortex and its effector: the cortical output command is solely determined by the recorded neural population via a mapping, M, that we have full knowledge of. We observed output-null changes as early as 66 ms after perturbation, followed by (definitively) output-potent changes after 103 ms. This result shows that motor cortex can indeed isolate early error-related activity through an internal output-null mechanism. It also suggests that sensory-evoked cortical activity need not interfere with ongoing BMI use.

Disclosures: S.D. Stavisky: None. J.C. Kao: None. S.I. Ryu: None. K.V. Shenoy: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.26/TT8

Topic: E.04. Voluntary Movements

Support: CIHR Grant MOP97944, MOP142220

GRSNC-FRQS

Title: Dorsal premotor (PMd) neurons code reaching movements preferentially in a space of action choices, not the salient sensory-property space of instructional cues.

Authors: *C. MONTANEDE, J. F. KALASKA;
Neurosciences, Univ. of Montreal, Montreal, QC, Canada

Abstract: Coallier & Kalaska (2015) recorded PMd neural activity while monkeys did a Choose-and-Go (CG) task. Two colored (Blue, Yellow) spatial cues (SC) appeared at two potential reach target locations, followed after a delay by a checkerboard-like decision cue (DC) containing different numbers of B and Y squares. The color bias of DCs, the difference in numbers of B and Y squares, varied from net-100 to net-4 between trials. The monkeys had to decide on the DC's dominant color and reach to the color-matched SC as quickly as possible. One monkey's response times (RTs) increased as DC color bias decreased. The rate of growth of directional activity of many PMd neurons was modulated by each DC's color bias, but few responded differentially to its dominant color. However, any color-related activity while the monkeys assessed the DC and chose a target during the RT period before reach onset may have been masked by activity related to initiation of the chosen reach. This motivated two new tasks. 1) CG task with delay (CGD): the DC appeared for an imposed delay period before the Go signal. This could allow expression of color-related activity during the sensory decision and action selection without movement-related confounds. 2) Match-to-Sample (MS) task: temporally-inverted CG task; the DC appeared first for a delay period, followed by the SCs and Go (Wang et al SfN 16). This could allow the monkey to make a sensory decision about the DCs' dominant color before knowing the spatial locations of the colored SCs. The RTs of a new monkey trained in all 3 tasks were shorter for net-20 and net-4 DCs in the CGD and MS tasks than the CG task, indicating that it made a sensory decision during the DC delay periods. The rate of growth of PMd directional activity was modulated by DC color bias during the DC delay period of the CGD task, but color per se had little effect. In the MS task, many PMd neurons showed little response to the DCs during the initial DC delay period but responded strongly after the SCs appeared. Other neurons responded to the DC, but showed little differential response to either its color or color bias, even though the monkey was assessing its color content. Our results show that PMd neurons weakly encode a critical sensory property of the DCs, their color. Instead, they express the DCs' salient sensory properties in terms of their support for different specific known actions defined by the color-location conjunctions of the SCs. The monkey had this information before the start of the DC delay period of the CGD task, but not during the initial DC delay period of the MS task. PMd neurons could not map the DCs' sensory properties onto specific actions in the MS task until after the SCs appeared to end the DC delay period.

Disclosures: C. Montanede: None. J.F. Kalaska: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.27/TT9

Topic: E.04. Voluntary Movements

Support: Howard Hughes Medical Institute

NIH Pioneer 1DP1OD006409

National Defense Science and Engineering Graduate

K99-NS092972-1

Fundacao para a Ciencia e Tecnologia

Title: Dorsal premotor cortex activity reflects a candidate decision variable during the action selection epoch of an abstract perceptual decision-making task

Authors: *M. WANG[1], C. CHANDRASEKARAN[2], D. PEIXOTO[3,5], W. T. NEWSOME[3,6], K. V. SHENOY[2,6,3,4];
[1]Neurosciences Program, [2]Electrical Engin., [3]Neurobio., [4]Bioengineering, Stanford Univ., Stanford, CA; [5]Champalimaud Neurosci. Inst., Lisbon, Portugal; [6]Stanford University/HHMI, Stanford, CA

Abstract: Neuroscientists have often studied decision-making using tasks in which decisions about a stimulus are made concomitantly with the action selection to report the outcome. These two processes, perceptual decision-making and action selection, may be independent or may occur together as one broader sensorimotor process. Here, we use psychophysics and electrophysiology to compare the role of dorsal premotor cortex (PMd) when these processes are concurrent or temporally dissociated. We trained rhesus monkey T to report the dominant color of a red-green checkerboard by reaching to a corresponding colored target (Wang et al., SfN 15; Montanède and Kalaska, SfN 16), under two task blocks, within session. The target locations are fixed, but the colors are assigned randomly. In the Target First (TF) task, targets are presented before checkerboard onset, so that decision-making (determining checkerboard color) and action selection (determining reach direction) are concurrent. In the Stimulus First (SF) task, this presentation order is reversed. The animal cannot plan a reach when the checkerboard alone is present, since the target colors are not yet available. In addition, a blank screen is introduced between the two events to further dissociate decision-making and action selection. In the SF task, psychophysical thresholds are higher and reaction times (RT) longer overall. RT are weakly negatively correlated with checkerboard strength. We used single electrodes and linear electrode arrays to record 159 units (54 single, 105 multi) in PMd. 1) Firing rates (FR) during the TF task

are consistent with previous reports: initially there is no response to the targets, and after checkerboard onset, FR increase in a manner that correlates with reach direction, checkerboard strength, and RT (Chandrasekaran et al., SfN 13, 14, 15). 2) In the SF task by contrast, FR do not change in response to checkerboard onset. 3) Rather, FR modulate only after the target onset in the SF task, in a manner that is correlated with reach direction, checkerboard strength, and RT. This is surprising because the putative decision epoch has already passed. These SF task data are consistent with two possible interpretations. One possibility is that the perceptual decision is made before target onset and is represented elsewhere but not in PMd; other cognitive elements involved in action selection are then reflected in neural activity after target onset. Alternatively, decision-making may be intentionally delayed until target onset, and the stimulus-dependent activity that follows target onset could reflect integration of the noisy stimulus representation retrieved from working memory.

Disclosures: M. Wang: None. C. Chandrasekaran: None. D. Peixoto: None. W.T. Newsome: None. K.V. Shenoy: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.28/TT10

Topic: E.04. Voluntary Movements

Support: Wellcome Trust Grant 106149

Title: Conserved patterns of neural coordination across sleep, movement and brain-control training

Authors: *A. JACKSON, F. DE CARVALHO, J. TULIP, T. M. HALL;
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Abstract: Understanding how skilled behaviour emerges from the coordinated activity of distributed neural populations is a central problem in modern neuroscience. Previous studies of synchronous or sequential activity in waking and sleeping states have typically examined whether such patterns encode information about *extrinsic* factors (e.g. sensory stimuli, spatial location or movement parameters) during or after a specific behaviour. However neural coordination may also arise from *intrinsic* constraints that limit network dynamics to a low-dimensional manifold within the neural state space, in which case these patterns should be preserved across diverse behavioural states and direct the learning of new skills towards neural solutions that lie within the manifold. Here we use long-term neural recordings from monkey

motor cortex to reveal conserved coordination patterns across awake behaviour and slow wave sleep. Neurons active sequentially during movements fired in the same sequence within the slow oscillation cycle in sleep, while neurons coactive at low frequencies during movements also fired synchronously with fast sleep spindles which were modulated by the slow oscillation phase. Moreover, taking both synchronous and sequential coordination patterns into account improved prediction of the neural strategies that emerged as animals learned abstract 'brain-control' tasks over several weeks. By contrast, the learning process did not cause task-specific changes to coordination patterns in sleep over the same period. We conclude that intrinsic network constraints shape low-frequency neural coordination patterns in behaviour, and these are manifested at higher frequencies in sleep due to cross-frequency coupling between sleep rhythms.

Disclosures: A. Jackson: None. F. de Carvalho: None. J. Tulip: None. T.M. Hall: None.

Poster

247. Reaching: Neurophysiology

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 247.29/TT11

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 DA037229-02

Title: spatial tuning for both manual and oculomotor movement in dorsal anterior cingulate cortex neurons

Authors: *S. YOO[1], S. PIANTADOSI[2], B. HAYDEN[2];
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Abstract: Several studies have revealed that the dorsal anterior cingulate cortex (dACC) is responsible for associating action with reward. Until recently, however, only a few studies have addressed the question of whether specific movement variables like direction selectivity are encoded in dACC. Even the studies that have explored whether the dACC encodes action information only addressed a specific type of movement: oculomotor movement. Here we show that firing rates of dACC neurons are sensitive to direction of movement by the physiological data from the dynamic-interactive-reward pursuit (DIRP) task. The task is conceptually similar to a simplified Pac-man game. Compared to more conventional laboratory tasks in which responses are constrained to one modality, our current task enabled the subject to continuously generate movement with a high degree of freedom in multiple possible directions for both oculomotor and manual modalities. We find that dACC neurons encode not only the direction of

oculomotor movement as previously reported but also the direction of manual movement. Furthermore, tuning direction in the two domains was often distinct.

Disclosures: S. Yoo: None. S. Piantadosi: None. B. Hayden: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.01/TT12

Topic: E.05. Brain-Machine Interface

Support: FIU Graduate School Presidential Fellowship

FIU Coulter Eminent Scholar Endowment

Title: Development of a rootlet interface to localize cutaneous stimuli applied to specific regions of the rat hindlimb

Authors: *I. BLACK[1], J. ABBAS[2], A. THOTA[1], R. JUNG[1];
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Abstract: Mapping of mammalian dermatomes has demonstrated a relationship between the rostral-caudal point of emergence of dorsal roots from the spinal cord and the specific cutaneous zones innervated by them, and studies have shown similar relationships to be preserved at the rootlet level. In rodent models, high signal-to-noise ratio recordings of bladder and cutaneous afferents have been obtained using dissected portions of lumbar roots implanted in microchannel electrode arrays. However, the somatotopic arrangement of rootlets has not been used to preferentially target and record neural signals arising from stimulation of specific cutaneous zones. This work reports the development of a rootlet interface (RI) implanted on teased lumbar dorsal rootlets to localize stimuli applied to different skin regions of the rat hindlimb.
Custom microchannel electrode arrays were developed by gluing together 8mm long silicone tubes having inside diameters of 310um. Tubes were slit longitudinally and opened along their entire length by pulling on flanges connected to both sides of the array to facilitate nerve insertion and reduce trauma. Pt/Ir and stainless steel recording electrodes were inserted into each channel. Silver wires at both ends of the array were used as a reference electrode for all channels. L4-L6 dorsal roots were exposed by removing the dorsal and lateral processes of T13-L3 vertebra in Sprague-Dawley rats and a portion of each root (i.e., “rootlet”) was dissected and implanted into the microchannel array. A variety of cutaneous stimuli were applied using an

instrumented von Frey device. The leg joints were also moved independently and simultaneously. Raw signals were amplified (10,000x), band-pass filtered (0.3-10kHz) and sampled at 50kHz. An audioscope was used to monitor real-time activity in each channel. Preliminary results demonstrate that activity in specific channels corresponded to stimuli applied to specific skin regions. As expected, the more rostral the emergence of the recorded "rootlet", the more proximal the skin zone that activated it. Leg movement produced activity in the majority of recordings in which responses to cutaneous stimuli were detected.
The RI concept appears to be a viable method to localize stimuli applied to different regions of the hindlimb. Methods to separate proprioceptive signals from those of cutaneous origin may be required. Ongoing work is focused on further characterization of the organization of afferents across the rootlets and on microfabrication of rootlet interfaces with greater channel counts and smaller channel cross sectional areas.

Disclosures: I. Black: None. J. Abbas: None. A. Thota: None. R. Jung: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.02/TT13

Topic: E.05. Brain-Machine Interface

Support: ESPRC UK EP/M025977/1

Title: Detection of sensory information in whole nerve recordings using a multi-contact cuff

Authors: *E. K. BRUNTON[1], C. BLAU[2], K. NAZARPOUR[1];
[1]Electrical and Electronic Engin., Newcastle Univ., Newcastle Upon Tyne, United Kingdom;
[2]Fac. of Med. Sci., Newcastle Univ., Newcastle, United Kingdom

Abstract: The performance of neuroprostheses that stimulate muscles to generate controlled and functional movement of paralysed limbs has been shown to be significantly enhanced when sensory feedback is provided. For sensory feedback to be provided, robust detection of the type and strength of sensory signals is required. Previously it has been shown that different types of sensory information can be decoded using a single channel cuff electrode. Here we report on the use of a multi-contact cuff electrode for recording and decoding sensory information from peripheral nerves.
All animal care and procedures were performed under appropriate licenses issued by the UK Home Office under the Animals (Scientific Procedures) Act (1986) and were approved by the Animal Welfare and Ethical Review Board of Newcastle University. Commercially available

nerve cuffs with 16 electrodes, comprising of 4 rings of 4 electrodes were implanted around the sciatic nerve of Sprague Dawley rats under terminal general anaesthesia, the electroneurogram signal (ENG) was recorded in response to sensory stimulation applied to the ipsilateral foot. Five different sensory stimuli were used: ankle flexion, ankle extension, outer toe pinch, thumb pinch, and stroking the dorsum of the foot. Each stimulus was repeated at least five times, and the beginning and culmination of the stimulus were indicated on the recording. The ENG signals were analogue filtered between 0.3 and 7.5 kHz and subsequently digitally filtered between 250 Hz and 5 kHz. During post-processing the mean absolute value (MAV) of the recorded signal was measured over 200 ms during the time that the stimulus was applied to the foot. This gave at least 5 measures for each stimulus type.
Clustering of data points showed that the ratio of MAV on pairs of electrodes located at different positions on the nerve cuff could be used to identify different sensory signals. Additionally, the results showed that the amplitude of the MAV recorded may be used to decode the strength of the stimulus applied.
The results of this study indicate that multi-contact nerve cuffs can be used to decode sensory signals recorded from whole nerves using features with low computation times that could be implemented in real time. Future research is still required to determine whether sensory information can still be obtained in awake and moving animals.

Disclosures: E.K. Brunton: None. C. Blau: None. K. Nazarpour: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.03/TT14

Topic: E.05. Brain-Machine Interface

Support: CALBrain Grant 350285

NSF Grant 1533589

Title: Artificial sensations through cortical stimulation of human somatosensory cortex with subdural mini-ECoG grids

Authors: *M. ARMENTA SALAS[1], D. R. KRAMER[2], B. LEE[2], D. BROWN[1], T. DOBREVA[1], C. KLAES[1], C. Y. LIU[3], R. A. ANDERSEN[1];
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Abstract: In recent years, we have seen an extraordinary advance in BMI systems control (Aflalo et al., 2015; Collinger et al., 2013; Hochberg et al., 2012), however there are still numerous open questions concerning the restoration of sensation, and the sensory feedback given to subjects controlling these BMI systems. We are interested in characterizing the somatic sensations elicited through cortical electrical stimulation, and the information these stimuli can deliver. We designed a proof of concept study, aimed at generating artificial sensation through cortical stimulation in humans. We recruited six epilepsy patients (4 female, 24-55 yrs.) undergoing Phase II electrocorticography (ECoG) monitoring, and implanted a 64-electrode mini-ECoG subdural grid (Adtech FG64C-MP03) over the somatosensory hand area on the ipsilateral side of the epilepsy mapping grid. The stimulation grid along with the standard subdural grid were connected to a clinical electroencephalography machine. We used the Grass Technologies S12X Cortical Stimulator to deliver the electrical stimuli. We mapped the somatotopy of the hand for all six patients and found self-reported sensations which ranged from electric "tingling" to "moving" of the fingers. Next, we tested the effect different stimulation parameters (frequency, current amplitude, pulse width, and waveform polarity) had in the perceived sensations. Finally, subjects used these artificial sensations to complete three behavioral tasks. Two were target acquisition tasks, where the patient was asked to move his/her hand over hidden targets and notify the experimenters when the target was found. The target locations were indicated through electrical stimulation on the mapped hand region. The last task was a discrimination one, where subjects had to differentiate between "natural" and "artificial" sensations. Our results show that, with standard parameters, we were able to stimulate distinct regions of the hand with the mini-subdural grid. The sensations described ranged from distinctly artificial sensations to more natural sensations of finger and hand movements. Each sensation also had variable intensities as we modified waveform parameters. On target localization trials, subjects had 100% accuracy and consistently reported the stimulation sensations. Furthermore, on discrimination trials, the subjects correctly detected the artificial sensation in all trials. This initial study is encouraging, given the consistency of the stimulation, reliability and speed of detection by subjects, and opens the possibility to combine touch timing and topographical localization to create finer and more complex movement control.

Disclosures: M. Armenta Salas: None. D.R. Kramer: None. B. Lee: None. D. Brown: None. T. Dobreva: None. C. Klaes: None. C.Y. Liu: None. R.A. Andersen: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.04/UU1

Topic: E.05. Brain-Machine Interface

Support: DARPA grant W911NF-15-2-0016

Title: Electrical stimulation of the cervical dorsal root ganglia for sensory restoration in upper-limb amputees

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Abstract: Sensory feedback greatly enhances the embodiment, acceptance, and also the ease of use of a prosthetic device. Electrical stimulation of the peripheral and central nervous system is the focus of extensive research as a means to provide this sensory feedback. In our study, we attempted to target the dorsal root ganglion (DRG) as the site of electrical stimulation. The DRG is a uniquely advantageous site for introducing sensory feedback as it is the most peripheral site that provides clear separation between the sensory and motor pathways. There are also multiple minimally invasive techniques for surgical access to the DRG. Further, it has been shown that DRG stimulation could help alleviate phantom limb pain. In animal studies, we have previously shown that sensory afferents from nerves throughout the hind limb can be recruited selectively by stimulating at the DRG. Here, we present observations from human psychophysics experiments performed while stimulating the C5-C8 DRG in two upper-limb amputees using FDA-approved spinal cord stimulation (SCS) leads. Study participants were implanted with three 8-contact SCS leads (Boston Scientific) in the lateral epidural space near the cervical spinal cord. Multi-polar current-steering was used to improve the focality of sensory percepts. Feedback about the modality, location, and intensity of perceived sensations was provided by the subject through a structured reporting setup. Preliminary testing showed that sensory percepts, primarily in the form of paresthesia, could be evoked via selective stimulation through different SCS lead contacts. The sensations reported by the subjects include focal percepts localized to the amputated shoulder, arm, hand, wrist, palm, and fingers. In one subject, these sensations were stable for more than two weeks of testing. Continuous modulation of stimulus amplitude was perceived as intensity changes and did not affect the overall location of the percepts. DRG stimulation using current-steering approaches can generate focal sensory percepts in specific regions of the missing limb in long-term amputees.

Disclosures: S. Chandrasekaran: None. A. Nanivadekar: None. E. Helm: None. M. Boninger: None. J. Collinger: None. R. Gaunt: None. L. Fisher: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.05/UU2

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX program, Contract No. N66001-15-C-4014

VA Merit Review Award #I01 RX00133401

NSF Grant No. DGE-1451075

NIH Grant T32AR007505

Title: Fascicular organization affects tactile sensation evoked from peripheral nerve cuff stimulation

Authors: *E. L. GRACZYK[1], M. A. SCHIEFER[1], H. P. SAAL[2], B. P. DELHAYE[2], S. J. BENSMAIA[2], D. J. TYLER[1];
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Abstract: Electrical nerve stimulation has restored sensation for amputees and can be a powerful tool to investigate the neural coding of touch. We performed psychophysical experiments on the perceived magnitude of sensations evoked from peripheral nerve stimulation and developed a computational model to describe the relationship between stimulation parameters and evoked sensations. We hypothesize that the underlying neuroanatomy can predict afferent recruitment from stimulation and that population activity estimated by computational modeling can predict tactile intensity. Two trans-radial amputees, chronically implanted with nerve cuff electrodes on their median, ulnar, and radial nerves, participated in the study. On multiple electrode contacts, the subjects performed intensity discriminations (n=7), perceived magnitude estimations (n=4), and mechanical indentation matching ratings (n=5) with stimulation that varied in pulse width (PW), pulse frequency, or both. A biophysical model of the human median nerve surrounded by a cuff electrode was constructed based on cadaveric cross sections. Populations of neurons were simulated within the fascicles and the activation of each neuron was determined using a linear approximation method. Fiber recruitment was plotted as a function of stimulation PW and approximated with a linear function. The thresholds and slopes were compared based on fascicle cross-sectional area and electrode-to-fascicle distance. From the experimental data, we found that perceived magnitude of sensation is predicted by the “activation charge rate” (ACR), which can be calculated from stimulation parameters. While the relationship between ACR and sensation magnitude was constant for each contact, it differed across contacts in a way that was

predicted by the computational model. The model showed that the threshold and slope of afferent recruitment differ between fascicles. Based on their distance from the stimulating contact, fascicles can be divided into two groups with different activation thresholds (two-sample t test, $p<0.001$). When the distance between the contact and fascicle is > 0.45 mm, the recruitment function is significantly shallower (two-sample t test, $p<0.001$) and does not depend on fascicle area. For shorter distances, slopes are steeper and increase linearly with fascicle area. Nerve anatomy helps explain the variability in perceived magnitude scaling. Future work in clinical subjects with known fascicular organization will be used to validate the model. Understanding the relationship between nerve anatomy, afferent recruitment, and subjective perception will enable us to improve stimulation paradigms.

Disclosures: E.L. Graczyk: None. M.A. Schiefer: None. H.P. Saal: None. B.P. Delhaye: None. S.J. Bensmaia: None. D.J. Tyler: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.06/UU3

Topic: E.05. Brain-Machine Interface

Support: TÜBİTAK 113S901

Boğaziçi University BAP 15XD2

Title: Psychophysical correspondence between vibrotactile stimulation of glabrous skin and intracortical microstimulation of the primary somatosensory cortex in the rat

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[1]Inst. of Biomed. Engin., Bogazici Univ., Istanbul, Turkey; [2]Dept. of Biomed. Engin., Namık Kemal Univ., Tekirdağ, Turkey

Abstract: Intracortical microstimulation (ICMS) is a promising technique for delivering somatosensory feedback from neuroprostheses. ICMS parameters are typically adjusted by matching psychophysical performance obtained from mechanical stimulation of the skin and the electrical stimulation of the cortex (Berg et al., IEEE Trans. Neural Syst. Rehabil. Eng., 21:500-7, 2013). Here, we specifically matched the probability of correct decisions in a yes/no detection task. 3 male Wistar rats were trained to detect bursts of mechanical sinusoidal vibrations (frequency: 40 Hz, zero-to-peak amplitude: 200 μm, duration: 0.5 s) delivered to the volar surface of their hindpaws. Subsequently, they performed the detection task at 6 intensity levels

(3-200 μm) and 3 frequencies (40 Hz, 60 Hz and 80 Hz). Each condition was repeated 4 times. Psychometric functions were obtained based on hit rates corrected for false alarms. For each rat, the psychometric functions were fitted by a mathematical model based on intensity and frequency variables (R^2 values are 0.88, 0.87 and 0.92 for individual rats). Later, rats were implanted with microwire arrays in the hindpaw representation of the somatosensory cortex and trained to detect trains of biphasic, charge-balanced current pulses (cathodic phase leading, phase duration: 600 μs, frequency: 40 Hz, duration: 0.5 s). Similar yes/no detection tasks were performed with ICMS intensity levels of 2.5-40 μA, and mathematical models were constructed for the corrected hit rates (R^2 values are 0.73, 0.86 and 0.92 for individual rats). By using the models from vibrotactile and ICMS experiments, we calculated psychometric correspondence functions at 5 frequencies (40 Hz, 50 Hz, 60 Hz, 70 Hz and 80 Hz) for each rat. These functions establish a map between vibrotactile and electrical stimulation amplitudes which produce the same corrected hit rate. The psychophysical correspondence was validated in an additional detection task with probe trials containing either a tactile or an ICMS stimulus with some frequencies and amplitudes not tested before. Kolmogorov-Smirnov statistic was used to test the null hypothesis that vibrotactile and ICMS stimuli produce similar psychometric curves in the validation experiments. According to the results, we could not reject the null hypothesis (all p values >0.3 for five frequencies and three rats). Therefore, the approach based on psychophysical correspondence functions seems to be useful for determining the amplitude parameters of ICMS in a cortical neuroprosthetic application.

Disclosures: I. Devecioglu: None. B. Güçlü: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.07/UU4

Topic: E.05. Brain-Machine Interface

Support: NHMRC, Project Grant RG1087224

Title: High frequency electrical stimulation: effect of spatial location on retinal ganglion cell responses *In vitro*

Authors: *J. W. MORLEY[1], C. Y. YANG[2], D. TSAI[3], T. GUO[2], G. J. SUANING[2], S. DOKOS[2], N. H. LOVELL[2];
[1]Med., Western Sydney Univ., Sydney, Australia; [2]Univ. of New South Wales, Sydney, Australia; [3]Columbia Univ., New York, NY

Abstract: Retinal prostheses allow electrical stimulation of surviving retinal ganglion cells (RGCs) following photoreceptor degeneration due to diseases such as retinitis pigmentosa. Recently, high frequency stimulation (HFS) has been used to excite or inhibit responses of ON and OFF RGCs in the retina by modulating the stimulus amplitude. It is a promising technique for achieving preferential RGC activation that may enhance the quality of phosphene perception in clinical applications. HFS-based preferential activation has relied on careful placement of small electrodes close to the RGC soma. It was unclear how well the technique would scale-up for clinical settings, due to the inability in an implant to precisely place electrodes relative to individual RGCs. In this study, we placed our stimulating electrode at locations around the soma and examined how RGCs responded to HFS. Retinae from wild-type C57BL/6 female or male mice aged 4–8 weeks were used, and RGC responses were recorded with whole-cell patch clamp recording. All procedures were reviewed and approved by the UNSW Animal Care and Ethics Committee. The stimulating electrode was 25µm diameter, and HFS pulses were delivered with cathodic-first, charge-balanced biphasic waveforms at 2kHz for 300ms. Cathodic and anodic pulse duration was 100µs, with cathodic-anodic inter-phase duration of 140 µs and anodic-cathodic inter-pulse duration of 160µs. The stimulation amplitudes ranged from 5µA to 120µA, in steps of 5µA. We also simulated the experiments using morphologically- and biophysically-accurate computational models, performed and analyzed in NEURON 7.2 and Matlab R2010a (Mathworks). In the in vitro experiments we observed the characteristic non-monotonic response curves of RGCs to increasing amplitude of HFS. This response was maintained even when the stimulating electrode was moved away from the axon initial segment of the RGC under study. When the stimulating electrode was moved away from the RGC soma the amplitude required to reach maximum spike rate increased, and when positioned on the side of the soma opposite the axon, the stimulus amplitude required to reach maximum spike rate was significantly higher compared to when the electrode was located on the axonal side of the soma (Kruskel-Wallis, $P<0.0001$). The computational models also produced non-monotonic response curves similar to those observed in the in vitro experiments. Our results show that HFS-based stimulation of RGCs results in characteristic response curves that are not overtly sensitive to electrode placement relative to the axonal initial segment, suggesting the generalizability of the HFS-based preferential activation technique.

Disclosures: J.W. Morley: None. C.Y. Yang: None. D. Tsai: None. T. Guo: None. G.J. Suaning: None. S. Dokos: None. N.H. Lovell: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.08/UU5

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01EY019678

NIH Grant F32EY025542

Title: Exploration patterns in a visual prosthesis simulation

Authors: ***N. J. KILLIAN**[1,3], M. J. KYADA[4], J. S. PEZARIS[2,3];
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Abstract: Understanding the patterns of visual exploration used in the reduced acuity vision available through a visual prosthesis provides important insights for device design, and improving accessibility to visual information for recipients. As part of the preparations for implanting a visual prosthesis, we have trained three monkeys (*Macaca mulatta*) to recognize letters of the Roman alphabet using simulated artificial vision, using a task that we have previously shown humans are highly skilled at without any training. Here, we have examined the eye movement patterns of the three monkeys during the cue exploration phase of the simulated artificial vision task. We found that the animals' overall performance on the two-alternative forced-choice task improved as they honed their usage of eye movements to explore cue images. In particular, saccade rates increased during learning and higher saccade rates were correlated with greater performance throughout most of the learning time course. Each animal employed unique global patterns of eye movements that were established at the outset of training, suggesting the distinct exploration styles were a result of innate biases rather than high-level strategies. Each animal had their own preferential viewing regions for each individual letter, such as vertices and openings in curves that were largely independent of the viewing condition (letter size, number of phosphenes being simulated). Viewing of these specific regions was detected on all conditions down to the lowest phosphene densities and at all but the smallest font size, suggesting that the animals recognized individual letter forms even in the hardest conditions where performance above chance was barely detectable. The animals performed better when making more saccades within the cue image region and when fixations had higher microsaccadic jitter. Furthermore, the fastest-learning animal used an exploration style characterized by a high saccade rate and high microsaccadic jitter. Our results suggest that training visual prosthesis recipients to explore with a high saccade rate, high jitter style, or artificially mimicking this style through modifications of stimulation patterns, may assist in learning to use phosphene vision for symbol recognition.

Disclosures: N.J. Killian: None. M.J. Kyada: None. J.S. Pezaris: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.09/UU6

Topic: E.05. Brain-Machine Interface

Title: The potential for evoking visual perception using epicortical microelectrodes: micro-stimulation of primary visual cortex in nonhuman primates

Authors: *D. OSWALT[1], D. ZHOU[2], D. TOBEY[2], P. DATTA[2], N. TALBOT[2], R. GREENBERG[2], L. RIETH[3], R. SHARMA[3], P. HOUSE[4], B. GREGER[1];
[1]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; [2]Second Sight Med. Products, Sylmar, CA; [3]Blackrock Microsystems, Salt Lake City, UT; [4]Univ. of Utah, Salt Lake City, UT

Abstract: Electrical stimulation of primary visual cortex is a potential means to restore limited vision to those with profound blindness. Research on restoring vision by cortical stimulation has historically focused on stimulation via epicortical macroelectrodes or intracortical microelectrodes. Epicortical macroelectrodes, those with diameters greater than 2mm, required high levels of current to evoke a visual percept, yielding unnatural sensations and a concern for initiation of seizure activity. Intracortical microelectrodes allowed for greater spatial resolution and lower stimulation thresholds. However, the intracortical placement of a microelectrode array presents surgical challenges and may engender a greater tissue response than epicortical placement. Epicortical microelectrodes may strike a balance between spatial resolution, surgical access, and longevity that maximizes utility to patients.
A 96-channel intracortical microelectrode array (Blackrock Microsystems) and a 47-channel epicortical microelectrode array (Second Sight) were implanted in a nonhuman primate at the right occipital pole and sagittal fissure respectively. The epicortical array was designed with 200um diameter disk electrodes organized into 14 banks of four concentric rings. Inter-electrode spacing was 100um within each bank and 0.5mm to 1.5mm spacing between banks. Photic stimuli were presented to the nonhuman primate at 15 retinotopic points while recording cortical activity. Electrical stimulation consisted of 200ms trains of 0.2ms biphasic pulses. Stimulation was applied monthly via the intracortical array and daily via the epicortical array.
Electrode impedances in the intracortical array fluctuated following implantation and with stimulation (80kΩ-800kΩ). Excluding a few aberrant measurements, electrode impedances on the epicortical array have remained within tolerance (8kΩ-11kΩ) on single disk electrodes. Previously visual percepts have been evoked with simulation as low as 18uA via intracortical microelectrodes (Torab 2011), however, stimulation of a few hundred micro-amps via several electrodes was routinely required (Davis 2012). In the current study, preliminary results indicate the nonhuman primate may perceive a visual sensation with 150uA-300uA electrical stimulation

using epicortical microelectrodes.
Epicortical microstimulation of primary visual cortex may evoke visual percepts within safe levels of current injection, and potentially provide a more practical alternative to intracortical arrays.

Disclosures: D. Oswalt: None. D. Zhou: None. D. Tobey: None. P. Datta: None. N. Talbot: None. R. Greenberg: None. L. Rieth: None. R. Sharma: None. P. House: None. B. Greger: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.10/UU7

Topic: E.05. Brain-Machine Interface

Title: Addition of pupil diameter measurements increases brain computer interface performance.

Authors: *K. A. FERCHO[1], E. BURNISON[2], E. K. HANSON[3], L. A. BAUGH[1];
[1]Basic Biomed. Sci., [3]Communication Sci. and Disorders, [2]Univ. of South Dakota, Vermillion, SD

Abstract: When patients suffer from severe motor disabilities, such as those that often accompany amyotrophic lateral sclerosis (ALS), alternative forms of communication may be required to maintain well-being and offset the loss of verbal and/or gesture communication. Although great progress has been made in the development of brain computer interfaces (BCI), improvements are still necessary in the usability, accuracy, and speed of these systems before they can be widely adopted within patient populations. Previous research has shown that pupil diameter not only modulates in response to levels of light and emotionally arousing events, but also in response to a wide range of mental functions including decision making. More recently, the use of pupil diameter has been shown effective as a form of simple no/yes communication within patients with locked-in syndrome. In the present study, we hypothesized that the inclusion of pupil diameter, as recorded through an eye-tracking camera, would increase the accuracy and reduce the classification time associated with the use of a P300 BCI speller. Twenty-five healthy participants identified target letters presented in random sequence while both 9-channel electroencephalography and binocular eye-tracking were performed. As expected, pupil diameter varied as a function of the presence of the target letter, with a mean response of approximately 2mm of pupil dilation occurring during the presence of the target letter. Offline classification algorithms correctly identified target trials with a mean accuracy of 65%, based on P300 data alone. As hypothesized, classification accuracy significantly increased when moment-to-moment pupil diameter was included in the algorithm. This study verifies the utility of including pupil

diameter to create a hybrid brain computer interface application, a particularly appealing option as many alternative communication devices include basic eye-tracking equipment that can readily access this novel source of information. Furthermore, as pupillary response is preserved in patients in a complete locked-in state, when combined with traditional EEG-based BCIs, increased accuracy and ease of use may be possible in cases of severe motor disability. This is especially important as current alternative communication methods require an increase in usability before widespread adoption within patient populations is possible.

Disclosures: K.A. Fercho: None. E. Burnison: None. E.K. Hanson: None. L.A. Baugh: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.11/UU8

Topic: E.05. Brain-Machine Interface

Support: NEI Grant R01EY022931

NEI Grant R01EY022931-S1

Research to Prevent Blindness

NSF Grant EEC-0310723

Title: Threshold manipulation of retinal ganglion cells using precondition anodic stimulation.

Authors: *Y.-C. CHANG, J. D. WEILAND;
Biomed. Engin., USC, Los Angeles, CA

Abstract: Retinal prosthetic implants have shown potential to restore partial vision to patients blinded by retinitis pigmentosa or age-related macular degeneration, via a camera-driven multielectrode array (MEA) that electrically stimulates surviving retinal neurons. Commercial epi-retinal prosthesis use charge-balanced symmetric cathodic-first biphasic pulses to depolarize retinal ganglion cells (RGCs) and bipolar cells (BCs), resulting in the perception of light in blind patients. However, the potential for asymmetric pulse and the order of both phases has not been fully studied. To investigate RGC activation in response to epi-retinal stimulation, we developed an adeno-associated viral (AAV) vector incorporating the genetically encoded calcium indicator GCaMP6f for labeling a majority of RGCs in mice. The virus was administered through intravitreal injection and the optimal time window for in vitro electrophysiological studies is determined 3-4 weeks later by observing fluorescence of retinal cells in situ, through a

customized fundoscope. At the optimal point, the retina was dissected and maintained in vitro as a wholemount. We then used calcium imaging to record the neural activity from RGCs at single cell resolution in wholemount retinas while applying the electrical stimulation through a MEA with transparent indium tin oxide electrodes. Results show that the spatial pattern of RGC activation can be influenced by the choice of pulse paradigms. Compared with supra-threshold charge balanced symmetric biphasic pulses with cathodic first phase that excite most of RGC in the effective region, symmetric pulses with anodic first phase can inhibit the activation of most RGCs. However, an asymmetric biphasic pulse consisting of an anodic first phase of relatively long duration and low amplitude and a cathodic second phase of short duration and high amplitude has a lower activation threhosld, compared to a biphasic, symmetric, cathodic first pulse. Extending the duration of anodic phase with sub-threshold amplitude slightly hyperpolarizes the membrane potential of RGCs. This has the effect of making the cells highly sensitive to stimulation, thus amplifying the depolarizing effect of a cathodic pulse. These findings support the possibility to manipulate the electrical stimulation thresholds and responses of RGCs through asymmetric pulses.

Disclosures: Y. Chang: None. J.D. Weiland: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.12/UU9

Topic: E.05. Brain-Machine Interface

Support: NSF GRFP Grant DGE-1247842

DARPA Award N66001-10-C-4056

Title: Neuronal responses in the primary somatosensory cortex during reach-to-grasp movements using native and robotic arm

Authors: *H. MAO[1], S. N. FLESHER[2], R. A. GAUNT[3], A. B. SCHWARTZ[4];
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Abstract: Primary somatosensory cortex (SI) receives afferent input from the peripheral sensory apparatus and responds with high fidelity according to characteristics of the stimulus such as location and modality. In addition to this well-recognized afferent driving of SI, we are interested in the possibility of non-peripheral activation of SI cells during movement. While operating a

robot arm via a brain-computer interface (BCI), movement commands from motor cortex are used to control the device and interactions between the robot hand and environment are monitored via visual feedback, which might have the potential to drive SI responses. Thus, this paradigm provides a unique opportunity to study SI activity during motor control but without peripherally driven somatosensory feedback. Two sets of microelectrode arrays were implanted in two hemispheres of a non-human primate. Each set had one array (88 electrodes) placed in the upper arm area of the primary motor cortex (MI) and one array (32 electrodes) in the hand and finger representation of SI. The subject was first trained to perform a reach-to-grasp task using his native arm. Action potentials from arrays contralateral to the performing arm were recorded for this hand-control (HC) experiment. In the BCI experiment, neural activity from the MI array was used to control the robot arm to perform the same reach-to-grasp task. Simultaneously recorded SI activity (in the same hemisphere as the MI array) and kinematics of the robotic arm were saved for offline analysis. An accelerometer, attached to the arm contralateral to the recorded units, was used to determine whether movement of the arm might be generating peripheral sensation during the task. Trials contaminated by movements of the native hand were excluded from analysis for the BCI experiment. In the HC experiment, the overall activity of SI neurons was depressed during reaching, began to increase before object contact, and sharply peaked shortly after contact. In the BCI experiment, 9 out of 29 SI neurons showed modulation around object contact. These included 7 cutaneous and 2 proprioceptive units with receptive fields in the fingers. Most of these units had a peak in activity around object contact or during grasping shortly after initial contact. One, a cutaneous unit, was most active when the robot hand started to close around the object at the end of reaching. Object-related activity peaks during the BCI tasks were broader than those of the same neurons in the HC tasks. These results show that SI can be modulated during motor control by both peripheral and non-peripheral input.

Disclosures: H. Mao: None. S.N. Flesher: None. R.A. Gaunt: None. A.B. Schwartz: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.13/UU10

Topic: E.05. Brain-Machine Interface

Support: DARPA Contract: N66001-10-C-4056

Title: Comparing perceptual latency of microstimulation in human somatosensory cortex to peripheral sensory input

Authors: *J. M. WEISS[1,2], M. KRYGER[1], S. FLESHER[2,3], D. J. WEBER[2,3], J. L. COLLINGER[1,2,3,4], R. A. GAUNT[1,2,3];
[1]Physical Med. and Rehabil., [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [3]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [4]DVA, Pittsburgh, PA

Abstract: We have recently demonstrated that intracortical microstimulation (ICMS) of human primary somatosensory cortex (S1) can evoke tactile percepts with naturalistic qualities, suggesting that ICMS could be an effective way to provide somatosensory feedback. We expect that the perceptual latency of ICMS needs to be similar to that of natural somatosensory input to be useful for motor control. While animal work has suggested that these latencies are indistinguishable [Romo 2000], these results have yet to be verified in humans. Here we compare the simple reaction times (RTs) of a human participant in response to ICMS in S1 and electrical and mechanical stimulation of the hand.

Under an Investigational Device Exemption, a person with a C5/C6 spinal cord injury was implanted with four microelectrode arrays, including two arrays in S1. Reaction times were measured for single-electrode ICMS of S1 and compared to that of vibrotactile or surface electrical stimulation of the hand in a region with spared sensation. Stimulation amplitudes and electrode/vibrotactor locations were matched to produce perceptually similar experiences from ICMS and peripheral stimulation. After a variable delay, the subject was randomly presented with either ICMS or peripheral stimulation and responded using a bite switch as quickly as possible. All stimuli were presented at 100 Hz for 500 ms. Catch trials in which no stimulus was delivered were randomly interleaved.

RTs to ICMS and peripheral stimulation were similar but highly variable. A total of 176 trials were acquired over three sessions comparing ICMS and vibrotactile RTs. Across the sessions, the median ICMS RT was 28 ms longer than the vibrotactile RT ($p < 0.01$, Wilcoxon rank sum). However, vibrotactile RTs were highly variable between days, ranging from 55 ms shorter than ICMS to 129 ms longer. An additional 144 trials over three sessions were acquired comparing ICMS and electrical RTs. Across the sessions, the median ICMS RT was 21 ms longer than the electrical RT ($p < 0.05$), and ranged from 69 ms longer than electrical to 9 ms shorter. Considering RTs from all six sessions and stimulus modalities (ICMS vs. peripheral stimulation), a multi-factor ANOVA revealed that day-to-day variability was significant ($p < 0.001$), but stimulus type was not ($p = 0.168$). Across all sessions, the participant failed to respond to 2.2% of stimulus trials and erroneously responded to 3.1% of catch trials.

These data suggest that the difference in RT between single-electrode ICMS and natural sensory input is small, although additional data are required to account for the day-to-day variability.

Disclosures: J.M. Weiss: None. M. Kryger: None. S. Flesher: None. D.J. Weber: None. J.L. Collinger: None. R.A. Gaunt: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.14/UU11

Topic: E.05. Brain-Machine Interface

Support: NIH-R01-EB008578

Title: A sensory substitution system for providing grasping force and hand opening feedback from a sensorized myoelectric hand

Authors: ***A. E. PENA**[1], L. RINCON-GONZALEZ[1], D. AGUILAR[1], J. ABBAS[2], R. JUNG[1]; [1]Florida Intl. Univ., Miami, FL; [2]Arizona State Univ., Tempe, AZ

Abstract: Current myoelectric prosthetic limbs are limited in their ability to provide direct sensory feedback to their users. Increased reliance on visual cues and attentional demand by the amputee can impact their ability to perform several daily life activities. We are investigating a non-invasive approach for providing sensory feedback through vibrotactile stimulation. Previous studies have taken advantage of the "referred sensations" in the residual limbs, placing tactors directly on the locations where the sensation is felt as if it was coming from the phantom fingers. While this approach can be effective, it requires costly prosthetic socket modifications to embed the tactors and not all amputees experience referred sensations. Though many sensory substitution studies have been done, none have shown significant performance improvements in control of myoelectric hands. For this study we have developed a wearable system that delivers vibratory patterns mapped to the sensor outputs of an instrumented myoelectric hand to deliver grasping force and hand opening (position) information. The feedback system uses a single tactor to convey force and an array of five tactors to convey position.
Initial studies with 6 able-bodied subjects were performed to validate the feedback system. During these studies, the tactors were placed on the lateral surface of the upper arm. We investigated whether subjects could discriminate and identify 5 different levels of force and position independently. In 3 blocks of randomized stimulation trials, subjects were instructed to report the level of force and position perceived while receiving no visual feedback from the hand. Performance was assessed using success rate during the third block. On average, subjects had a success rate of 79±10.9% and 84±12.7% at identifying discrete force and position levels, respectively. These results suggest that this sensory substitution system could be used to deliver grasping force and position feedback. Future work will investigate the effect of substitute sensory feedback on the users' ability to control force and position outputs of a myoelectric hand.
Supported by NIH-R01-EB008578

Disclosures: A.E. Pena: None. L. Rincon-Gonzalez: None. D. Aguilar: None. J. Abbas: None. R. Jung: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.15/UU12

Topic: E.05. Brain-Machine Interface

Support: DARPA contract number N66001-10-C-4056

NSF Grant No DGE-1247842

Title: Intracortical microstimulation of human somatosensory cortex elicits cutaneous percepts

Authors: ***S. N. FLESHER**[1], J. L. COLLINGER[2], S. T. FOLDES[2], J. M. WEISS[1], J. E. DOWNEY[1], E. C. TYLER-KABARA[3], S. J. BENSMAIA[5], A. B. SCHWARTZ[4], M. L. BONINGER[2], R. A. GAUNT[2];
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Abstract: Dexterous object manipulation requires cutaneous sensory feedback. In its absence, simple tasks are very difficult. In prosthetic limbs controlled through brain-computer interfaces (BCIs), providing somatosensory feedback could be an important step to improving function as vision provides impoverished cues about object interactions. Intracortical microstimulation (ICMS) of primary somatosensory cortex (S1) is a potential method to restore this sensory channel, particularly in people that cannot benefit from stimulation of the peripheral nervous system. Under an Investigational Device Exemption, a 28 year old participant with a chronic spinal cord injury was implanted with 2 intracortical microelectrode arrays (MEAs) in motor cortex and 2 MEAs in area 1 of S1. Locations of the S1 MEAs were based on presurgical imaging with the goal of eliciting percepts that project to the fingers of the right hand. Electrodes were stimulated at supraliminal intensities and the participant described the locations and qualities of evoked percepts. Projected fields were located on the proximal pads of digits 2-5. Sensations were evoked on 59 of 64 electrodes, and no painful sensations or paresthesias were reported. We tested whether the subject could use this spatial information to identify which of four fingers on a robotic limb was touched by converting robot finger torque to stimulus intensity. The load-bearing finger was identified with 84.3% accuracy (54 trials).
We measured detection thresholds using a two-alternative forced choice task and found the

median detection threshold to be 34.9μA, with upper and lower quartiles at 60.0 and 24.8μA, respectively. Thresholds were generally stable over 11 months. Of the 32 electrodes with 3 or more measured thresholds 7 changed significantly over time, 3 of which had a negative slope, suggesting thresholds were not globally increasing.
We measured the discriminability of ICMS trains differing in amplitude and found the just noticeable differences to be 15.4 ± 3.9 μA (mean ± s.d.) and independent of the magnitude of the reference stimulus. In magnitude estimation experiments, we found that perceived intensity increased linearly with stimulation amplitude (R2 = 0.98) for 5 electrodes tested.
In summary, percepts were evoked at somatotopically appropriate locations with intensities that scaled linearly with amplitude over a wide range. These properties of evoked percepts can be used to convey location and intensity of object contact, key types of information for guiding interactions with objects. Providing artificial somatosensory feedback to BCI users could improve the user's control and experience with the prosthetic device.

Disclosures: S.N. Flesher: None. J.L. Collinger: None. S.T. Foldes: None. J.M. Weiss: None. J.E. Downey: None. E.C. Tyler-Kabara: None. S.J. Bensmaia: None. A.B. Schwartz: None. M.L. Boninger: None. R.A. Gaunt: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.16/UU13

Topic: E.05. Brain-Machine Interface

Support: European Union (FP7-611687, NEBIAS)

Title: Investigation of peripheral nervous system interfaces for motor decoding and somatosensory stimulation.

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Abstract: Until recently, neural interface research for (bidirectional) prosthetic use has focused to a large extent on direct interactions with the brain. An alternative approach is to interface at the peripheral nervous system (PNS) instead. Transverse Intrafascicular Multichannel Electrodes (TIMEs) are thin film, polymide, electrode arrays that are inserted transversally through the nerve. These allow the recording and stimulation of individual fascicles in the peripheral nerves. To investigate if these PNS interfaces are suitable for bidirectional prosthetic use, we implant

multi-channel TIME electrodes in the medial and ulnar nerve in the upper limb of a rhesus monkey (Macaca mulatta) and assess if we can decode motor activity from them and stimulate the nerve in order to evoke somatosensation.
Purpose-bred macaque monkeys (*Macaca mulatta*) are trained in a delayed grasping task to grasp a wide range of different objects (42 objects with equal weight but different size and shape). Simultaneously activity of the median and ulnar nerve is recorded as well as the hand and arm kinematics with a 27 DOF electromagnetic hand tracking system. Decoders are trained to decode both grip type (Bayesian decoder) and hand kinematics (Kalman filter) from the neural activity.
For the somatosensory discrimination task, a rhesus macaque is trained to perform a two-alternate-forced choice task in which it receives vibrational or electrical stimulation to the median and ulnar side of the hand, respectively, by applying vibration to the index or little finger, or electrical stimulation to the median or ulnar nerve. It is required to indicate, by pressing a button, which side of the hand was stimulated more intensely. Observing the monkey's behavior will show how strong the electrical stimuli are perceived compared to the vibrational stimuli.
A short-term implantation experiment showed that the implantation technique of TIME electrodes in the PNS of rhesus macaques is feasible, including the placement of a subcutaneous cable and a cranial connector; no signs of loss of hand function were observed post-surgery. Neural activity was recorded over a period of two weeks, in which single unit spikes have been observed, and it was shown that the electromagnetic field, generated by the hand tracking system, did not affect the signal quality, neither concerning the noise level nor the ability to detect spike information. This makes us believe that hand tracking is possible during neural PNS recordings. Investigation of TIMEs in non-human primates provides valuable information regarding the recording and stimulation capabilities of these PNS interfaces prior and in addition to human studies.

Disclosures: J.M. Buil: None. H. Scherberger: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.17/UU14

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01GM111293

Taubman Medical Institute

Title: Quantification of tactile sensory content in multielectrode array recordings from finger area of macaque motor cortex

Authors: *K. E. SCHROEDER[1], Z. T. IRWIN[1], A. J. BULLARD[1], D. E. THOMPSON[6], J. BENTLEY[7], P. G. PATIL[7,8,1], C. A. CHESTEK[1,2,3,4,5];
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Abstract: Challenges in the control of dexterous upper-limb brain-machine interfaces (BMIs) have prompted renewed interest in the amount and nature of sensory information encoded in primary motor cortex (M1). Signals used for motor decoding also contain sensory information, which can interfere with prosthetic performance in contexts where sensation is most important, such as object manipulation. Previous single unit studies in monkeys showed M1 is responsive to tactile stimulation, as well as passive and active movement of the limbs. Recent work in this area has quantified proprioceptive responses of chronically recorded neurons, so here we examined to what extent tactile somatosensation of the hand and fingers is represented in M1 array recordings. We recorded multi- and single units from macaque M1 while gently brushing individual finger pads at 2 Hz. Units displaying significant differences in firing rates between individual fingers ($p<.05$) were found in four animals. Many units exhibited cosine-like tuning across multiple digits, similar to motor tuning profiles. Enough sensory information was present in M1 to correctly decode stimulus position from multiunit activity significantly above chance levels (ranging from 60-90% correct across four animals for a 1 of 3 classification). Additionally, preliminary examination of unit tuning during tactile and proprioceptive inputs indicates cells are often tuned differently in different contexts. The depth and widespread presence of sensory tuning found in these M1 populations implies that more careful and comprehensive training of decoders in the presence of sensory stimuli will be needed for the refinement of BMI for dexterous grasping.

Disclosures: K.E. Schroeder: None. Z.T. Irwin: None. A.J. Bullard: None. D.E. Thompson: None. J. Bentley: None. P.G. Patil: None. C.A. Chestek: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.18/VV1

Topic: E.05. Brain-Machine Interface

Support: NIH National Research Service Award F32-DC013486

NIH Grant R00-NS065120

NIH Grant DP2-OD00862

NIH Grant R01- DC012379

Ester A. and Joseph Klingenstein Foundation

NSF Grant 1144247

Use of NERSC Center - US DOE Contract No. DE-AC02-05CH11231

Title: Neural speech recognition: Continuous phoneme decoding using spatiotemporal representations of human cortical activity

Authors: *D. A. MOSES[1,2,3], N. MESGARANI[2,3], M. K. LEONARD[2,3], E. F. CHANG[1,2,3]; [1]Bioengineering, UC Berkeley - UC San Francisco, San Francisco, CA; [2]Neurolog. Surgery, [3]Ctr. for Integrative Neurosci., UC San Francisco, San Francisco, CA

Abstract: The superior temporal gyrus (STG) and neighboring brain regions play a key role in human language processing. Previous studies have attempted to reconstruct speech information from brain activity in the STG, but few of them incorporate the probabilistic framework and engineering methodology used in modern speech recognition systems. In this work, we describe the initial efforts toward the design of a neural speech recognition (NSR) system that performs continuous phoneme recognition on English stimuli with arbitrary vocabulary sizes using the high gamma band power of local field potentials in the STG and neighboring cortical areas obtained via electrocorticography. The system implements a Viterbi decoder that incorporates phoneme likelihood estimations from a linear discriminant analysis model and transition probabilities from an n-gram phonemic language model. Grid searches were used in an attempt to determine optimal parameterizations of the feature vectors and Viterbi decoder. The performance of the system was significantly improved by using spatiotemporal representations of the neural activity (as opposed to purely spatial representations) and by including language modeling and Viterbi decoding in the NSR system. These results emphasize the importance of modeling the temporal dynamics of neural responses when analyzing their variations with respect to varying stimuli and demonstrate that speech recognition techniques can be successfully leveraged when decoding speech from neural signals. Guided by the results detailed in this work, further development of the NSR system could have applications in the fields of automatic speech recognition and neural prosthetics.

Disclosures: D.A. Moses: None. N. Mesgarani: None. M.K. Leonard: None. E.F. Chang: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.19/VV2

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX, N66001-15-C-4014

VA Merit Review #I01 RX00133401

NSF #DGE-1451075

Title: Muscle contraction is significantly associated with proprioception restored with electrical nerve stimulation with a Flat Interface Nerve Electrode (FINE)

Authors: *M. SCHIEFER[1,2], I. CUBEROVIC[1,2], E. L. GRACZYK[1,2], D. J. TYLER[1,2]; [1]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH; [2]Louis Stokes Cleveland Dept. of Veterans Affairs Med. Ctr. (LSCDVAMC), Cleveland, OH

Abstract: Amputees lack a sense of proprioception of the prosthetic limb, requiring visually monitoring of limb motion and object interactions. A subject with a trans-radial amputation was implanted with a sensory restoration system. The system has 8-channel Flat Interface Nerve Electrodes (FINEs) positioned around the median, radial, and ulnar nerves in his residual arm. FINE leads are routed to a percutaneous site through which we interface our programmable stimulator with his nerves. The subject undergoes experiments during which he reports the perception in his phantom hand, including its position, during electrical nerve stimulation. We observed that muscle contraction accompanies a sensation of movement in the phantom hand. We investigated the effect of electrical stimulation on the perceived position of the thumb and index finger. To assess phantom digit position, the subject was fitted with a calibrated CyberGlove, which measures the angle of the fingers' joints. When presented with electrical stimulation, the subject mirrored his phantom hand's position with his contralateral hand. Surface electrodes were positioned over the locations in his residual arm where muscle contraction was observed and electromyograms (EMGs) were recorded. EMGs were filtered, rectified and integrated, and normalized by the maximum voluntary contraction. Finally, the subject described the sensation to each stimulus that was presented. During one set of trials, biphasic stimuli with constant amplitude (0.7 mA) and frequency (100 Hz) were delivered to the median nerve. The pulsewidth (PW) of the stimulus had one of four envelopes: flat; a linearly increasing PW followed by a linearly decreasing PW; a linearly increasing PW that plateaued; a linearly increasing PW followed by abrupt cessation of stimulation. The stimulus duration varied between 1-5 seconds. During a second set of trials, the flat PW envelope was applied while PW varied between 120 μs (sensory threshold) and 255 μs. Results support initial observations that

proprioception occurs only when EMG was observed ($p<0.001$). As duration and PW increased, EMG and perceived motion increased. In 25% of trials, EMG was recorded while no movement was reported. In 75% of trials, both EMG and proprioception were concomitant. The shape of the PW envelope was an excellent predictor of joint motion. Results suggest that proprioceptive sensation is due to muscle contraction, but because 25% of trials had EMG alone, muscle contraction may not be the sole driver of proprioception. This is further supported by results from a second subject who has never reported proprioception and in whom muscle contraction has never been observed.

Disclosures: M. Schiefer: None. I. Cuberovic: None. E.L. Graczyk: None. D.J. Tyler: None.

Poster

248. Neuroprosthetics: Sensory Processing

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 248.20/VV3

Topic: E.05. Brain-Machine Interface

Support: EU grant FP7-602547 EPIONE

Title: Intraneural implants enable long-term bidirectional control of hand prostheses

Authors: ***S. RASPOPOVIC**[1], F. PETRINI[1], E. D'ANNA[1], G. VALLE[1], I. STRAUSS[1], G. GRANATA[2], R. DI IORIO[2], D. GUIRAUD[3], T. STIEGLITZ[4], S. MICERA[1];
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Abstract: Recently, several groups reported the possibility to effectively restore the natural hand sensory information in trans-radial amputees, by using intraneural, epineural and wire peripheral neural interfaces. In particular, we showed that intraneural peripheral stimulation could provide sensory information about grasping force, object shape, and stiffness to an amputee during the real-time control of a dexterous hand prosthesis in a one-month trial implemented by using non-portable devices for recording and stimulation. Here we show the results of a six-month implant of transversal intraneural multifascicular electrodes (TIMEs) in median and ulnar nerves of a trans-radial amputee. Moreover, we developed a fully portable system for recording and stimulation, which enabled the user to perform ecological tasks in unstructured environments. During the stimulation sessions, the map of the different sensations elicited in the phantom hand and palm was obtained by changing the stimulation parameters (amplitude, pulse width and

frequency) for all 56 contacts of the TIMEs. Sensations were mainly reported as touch-like pulsations, vibrations, and electroparesthesia. Moreover, when different channels were coupled together it was possible to obtain different, more complex sensations. The physiological plausibility and homologous nature of elicited sensations have been confirmed by EEG recordings. The information acquired with the sensation map was used to implement a bidirectional control of hand prosthesis. This setup has been used then, to evaluate the capability of the user to produce different force levels, to perform manipulation tasks (e.g., a blindfolded box and block test) and everyday activities tasks. The participant showed good ability to produce high and low force levels, and performed very well during blindfold manipulation tasks.Finally the users was able to step out from the laboratory and perform different tasks using the induced feedback.The next steps towards the close-to-natural replacement of lost hands are to extend the types of sensations, which can be elicited and to develop a fully implantable device.

Disclosures: S. Raspopovic: None. F. Petrini: None. E. D'Anna: None. G. Valle: None. I. Strauss: None. G. Granata: None. R. Di Iorio: None. D. Guiraud: None. T. Stieglitz: None. S. Micera: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.01/VV4

Topic: E.08. Respiratory Regulation

Support: R21 NS087257 (PI: Del Negro)

R01 HL104127 (PI: Del Negro)

Title: Determining the role of TRP channels in inspiratory burst generation

Authors: K. E. DORST, *M. C. D. PICARDO, C. A. DEL NEGRO;
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Abstract: We aim to understand the neural origins of breathing in mammals. The neurons that generate the breathing rhythm are located in a region of the medulla called the preBötzinger Complex (preBötC). These rhythmic neurons in the preBötC arose from progenitors that have expressed the embryonic transcription factor Dbx1. Dbx1-derived preBötC neurons also generate Ca^{2+}-activated non-selective cationic current (I_{CAN}), which is hypothesized to give rise to inspiratory drive potential, i.e. the envelope of depolarization underlying inspiratory bursts. The inspiratory drive potential of preBötC rhythmic neurons can be altered through manipulations

affecting I_{CAN}, such as blocking the current by external flufenamic acid (FFA) or preventing its activation via intracellular BAPTA dialysis. However, the underlying molecular component that is involved in inspiratory burst-generation in these neurons remains unknown. Transient receptor potential (TRP) ion channels are likely candidates of I_{CAN} generation in the preBötC based on previous evidences. For many years, we focused our efforts on the Trpm4 as the putative ion channel responsible for generating the inspiratory drive potential. Trpm4 has been previously shown to be present in the preBötC, and possesses properties that best suit the channel underlying inspiratory drive potentials.
However, recent data acquired from single-cell RNA sequencing (RNAseq), immunohistochemistry, and electrophysiological experiments suggest that Trpm4 may not be the main ion channel that underlies inspiratory behavior. Whole-cell *in vitro* experiments using newborn mouse brainstem slices revealed that the selective Trpm4 antagonist, 9-phenanthrol, produced minor to no attenuation of inspiratory drive potential in Dbx1-derived preBötC neurons. In comparison, experiments using FFA have shown a greater extent of inspiratory drive potential attenuation in these neurons. RNAseq results show that the Trpm4 gene is not as highly expressed as a few other TRP genes. Immunohistochemical staining have also indicated the relatively minimal expression of Trpm4 proteins in Dbx1-derived preBötC neurons. These findings lead us to suggest that Trpm4 may not be a major contributor in generating inspiratory drive potential, and that there is another ion channel of interest. Further electrophysiological and immunohistochemistry experiments are needed in order to determine if our new candidate channel is the molecular component that we seek to identify.

Disclosures: K.E. Dorst: None. M.C.D. Picardo: None. C.A. Del Negro: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.02/VV5

Topic: E.08. Respiratory Regulation

Support: CIHR Grant 130306

AIHS

CFI

WCHRI

Title: Ca^{2+}-activated K^+ (BK) channels contribute to the ATP excitation of inspiratory rhythm generating networks *In vitro*

Authors: V. JALUBULA, Y. ZHANG, A. K. KATZELL, V. RANCIC, *A. L. REVILL, K. BALLANYI, G. D. FUNK;
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Abstract: The hypoxic ventilatory response is biphasic. An initial carotid-body mediated increase is followed by a secondary, centrally-mediated respiratory depression that can be life-threatening in premature infants. During hypoxia ATP is released in the ventral respiratory column, including the preBotzinger Complex (preBötC, key site of inspiratory rhythm generation), where it evokes a $P2Y_1$ receptor-mediated increase in inspiratory frequency that attenuates the secondary depression. In other systems, $P2Y_1$ receptors are coupled to the Gαq-signaling pathway, which involves phospholipase C- and inositol trisphosphate-mediated release of Ca^{+2} from intracellular stores. My objectives were to test whether the $P2Y_1$ receptor-evoked frequency increase depends on release of Ca^{+2} from intracellular stores and then to identify the downstream ion channel(s) that mediate the actions of $P2Y_1$ receptors in the preBötC. Rhythmically-active medullary slices from neonatal rats were loaded with the Ca^{+2} indicator Fluo-4 (via pressure injection) and fluorescence monitored with multiphoton microscopy. Inspiratory neurons, identified by rhythmic Ca^{+2} fluorescence oscillations in phase with inspiratory-related bursts recorded from the XII nerve, responded to the $P2Y_1$ receptor agonist, MRS2365 (100 μM, 10 s), with 2-3 fold increases in intracellular Ca^{+2}. Depletion of intracellular Ca^{+2} stores via bath and local application of SERCA blockers, Thapsigargin (200 μM) and Cyclopiazonic acid (100 μM), blocked the MRS2365-evoked increase in intracellular Ca^{+2} and reduced the MRS2365-evoked frequency increase by 58 ± 9% and 28 ± 7% of control, respectively. I then tested involvement of ion channels known to be modulated by $P2Y_1$ receptors or intracellular Ca^{+2}. I compared MRS2365-evoked frequency increases before and after bath or local application of $BaCl_2$, Apamine, Iberiotoxin and trimethylamine to block G protein-coupled inwardly rectifying potassium channels (GIRK), SK channels, and BK channels, respectively. None of these drugs significantly attenuated the MRS2365 frequency increase. In contrast, Paxilline, a type 2 BK channel blocker, applied at 20 and 1 μM attenuated the MRS2365 response by 46 ± 11% and 32 ± 5% of the control response, respectively. Multiphoton Ca^{+2}-imaging revealed that Paxilline did not affect intracellular Ca^{+2}. Thus, it is unlikely that the effects of Paxilline were due to potential off-target actions on SERCA. These data suggest that $P2Y_1$ receptor mediated increases in inspiratory related frequency in vitro depends partly on release of Ca^{+2} from intracellular stores and activation of BK channels. Supported by CIHR, AIHS, CFI, WCHRI

Disclosures: V. Jalubula: None. Y. Zhang: None. A.K. Katzell: None. V. Rancic: None. A.L. Revill: None. K. Ballanyi: None. G.D. Funk: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.03/VV6

Topic: E.08. Respiratory Regulation

Support: CIHR

AIHS

CFI

WCHRI

ALA

Title: ATP excites the preBötzinger Complex network *In vitro* via activation of a $G_{\alpha q}$-signaling pathway

Authors: *Y. ZHANG[1], T. ALVARES[1], A. V. GOURINE[4], S. KASPAROV[5], G. D. FUNK[1,2,3]; [1]Dept. of Physiol., [2]Women and Children's Hlth. Res. Inst., [3]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada; [4]Dept. of Neuroscience, Physiol. & Pharmacol., Univ. Col. London, London, United Kingdom; [5]Sch. of Physiol. & Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: The ventilatory response to hypoxia comprises an initial increase in ventilation followed by a secondary depression that can be life-threatening in premature infants. Astrocytes sense hypoxia and release ATP. ATP released within the ventral respiratory column, including the preBötzinger Complex (preBötC, critical site for inspiratory rhythm generation), during hypoxia attenuates this depression. Our working hypotheses are that ATP released from preBötC astrocytes during hypoxia 1) excites local inspiratory neurons directly via $P2Y_1$ receptor (R)-dependent activation of $G_{\alpha q}$-signaling pathway and 2) acts in an autocrine/paracrine manner to enhance gliotransmission, which further depolarizes inspiratory neurons. Using 700 μm rhythmically-active medullary slices from neonatal rats, we first tested the effects of U73122 (phospholipase C inhibitor), 2-APB (inositol triphosphate receptor inhibitor) and chelerythrine (protein kinase C inhibitor) on the $P2Y_1R$ excitation of the inspiratory network. Bath application and local injection into the preBötC of U73122 (20 μM) shortened the duration of MRS2365 ($P2Y_1R$ agonist, 100 μM)-evoked frequency increase of inspiratory-related activity recorded from XII nerve rootlets by 42.8 ± 11.8% (n=9), but did not affect the peak frequency. In contrast, bath application of 2-APB (100 μM) and chelerythrine (10 μM) reduced the MRS2365-evoked frequency increase by 32.6 ± 8.8% (n=5) and 31.1 ± 4.2% (n=8) respectively. We then compared ATP (5 mM) currents evoked in inspiratory neurons before and after intracellular dialysis of the

same drugs. Intracellular dialysis of U73122 (2 μM, n=7), 2-APB (50 μM, n=5) and chelerythrine (10 μM, n=8) attenuated ATP-induced inward currents in inspiratory neurons by 38 ± 4.3%, 32.6 ± 12.6% and 20.6 ± 4.6%, respectively. Intracellular dialysis of endoplasmic reticulum Ca^{2+}-ATPase blocker thapsigargin (4 μM, n=10) and cyclopiazonic acid (20 μM, n=9) also attenuated the ATP currents by 22.6 ± 5.2% and 21.6 ± 3.5% respectively. Our second objective was to determine if the ATP excitation of inspiratory neurons includes an astrocytic glutamate component. We compared ATP currents evoked in inspiratory neurons before and after bath application of glutamate R antagonists AP5 (100 μM) and CNQX (10 μM) (in 0.5 μM TTX). AP5 and CNQX had no effect on ATP currents (n=8). These data suggest that i) ATP excitation of the preBötC network is produced, at least in part, via activation of a $G_{\alpha q}$-signaling pathway in inspiratory neurons and ii) neuronal excitation by ATP does not include a glial-derived glutamatergic component.

Disclosures: Y. Zhang: None. T. Alvares: None. A.V. Gourine: None. S. Kasparov: None. G.D. Funk: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.04/VV7

Topic: E.08. Respiratory Regulation

Title: Optogenetic dissection reveals principles underlying respiratory frequency control

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Abstract: Central neural networks operate continuously throughout life to control respiration, yet mechanisms regulating ventilation frequency are not understood. Inspiratory rhythm is generated by a *purely excitatory* network within the preBötzinger complex (preBötC) of the ventrolateral medulla. Working models predict that preBötC burst frequency is controlled by a balance of excitatory and inhibitory synaptic inputs, where excitation increases and inhibition decreases inspiratory rate. We tested assumptions of this model using an optogenetic approach. We found that in the absence of inhibitory synaptic transmission, excitatory network activity can generate rapid inspiratory bursting, with an upper frequency limit of ~35 min^{-1}. Interestingly, selective stimulation of excitatory Phox2b+ neurons generated inspiratory rates reaching twice this frequency—as high as ~70 min^{-1}—suggesting that inhibitory neurons are critical for rapid inspiratory bursting. In support of this, we found that blocking inhibitory synaptic transmission

completely abolished *Phox2b::ChR2* mediated increases in inspiratory frequency. To investigate inhibitory mechanisms that might contribute to rapid inspiratory bursting, we selectively stimulated Vgat+ neurons and found that phasic stimulation of inhibitory neurons can actually drive rapid inspiratory rates through post-inhibitory rebound excitation. Blocking post-inhibitory rebound abolished *Phox2b::ChR2* mediated increases in inspiratory frequency. To examine the source of phasic inhibition which generates rapid inspiratory bursting, we tested the hypothesis that Phox2b+ neurons initiate expiratory bursting, but via inhibitory synaptic coupling, cause inspiratory bursting through post-inhibitory rebound excitation. Indeed, we found that stimulation of Phox2b+ neurons resulted in late expiratory bursting with ~110 ms latency, followed subsequently by inspiratory bursting (~390 ms latency). These results demonstrate that—at least at high respiratory frequencies—inspiratory rhythm is generated by dynamic inhibitory interaction between two or more medullary nuclei rather than the dominant excitatory pacemaker activity of the preBötC. Additionally, these data indicate that inhibitory neurons are essential for generation and maintenance of fast inspiratory rhythms.

Disclosures: J. Cregg: None. K. Chu: None. T.E. Dick: None. L.T. Landmesser: None. J. Silver: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.05/VV8

Topic: E.08. Respiratory Regulation

Support: KAKENHI on Innovative Areas (Comprehensive Brain Science Network) from MEXT

KAKENHI (25430012, 16K07003)

Title: Optogenetic analysis of neuronal network of medullary respiratory center in brainstem-spinal cord preparations from transgenic newborn rats expressing Archaerhodopsin in Phox2b positive cells

Authors: *H. ONIMARU[1], K. IKEDA[2], M. OGAWA[3], K.-I. IHARA[4], K. KOBAYASHI[4], K. KAWAKAMI[5];
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Abstract: Preinspiratory (Pre-I) neurons in the parafacial respiratory group (pFRG) compose one of the respiratory rhythm generators in the medulla of the newborn rat. A subgroup of pFRG/Pre-I neurons express the transcription factor Phox2b. To analyze detailed neuronal mechanisms of respiratory rhythm generation using optogenetics, we made transgenic (Tg) newborn rats in which Phox2b positive cells expressed Archaerhodopsin-3 (Arch). Phox2b_tTA-2A-Cre Rec BAC rats were mated with CAG-loxP-Stop-loxP-Arch/Rosa BAC rats. We found specific expression of Archaerhodopsin-3 in Phox2b positive cells when CAG-loxP-Stop-loxP-Arch/Rosa BAC male rats were mated with Phox2b_tTA-2A-Cre Rec BAC female rats, whereas the expression was non-specific in reverse pairings. Brainstem-spinal cord preparations were isolated from 0-4 days old Tg newborn rats deeply anesthetized with isoflurane and were superfused at a rate of 3.0 ml/min with the artificial cerebrospinal fluid, equilibrated with 95% O_2 and 5% CO_2, pH 7.4, at 25-26°C. The preparations were cut transversely at a level just rostral to the anterior inferior cerebellar artery. To examine photostimulation to the pFRG (left side), the medulla was further cut to remove a right half of the pFRG in the most experiments. Photostimulation of the ventral medulla was performed by green laser light (532 nm) through an optic fiber with 1 mm outer diameter. Inspiratory C4 ventral root activity was monitored and membrane potentials of Pre-I or inspiratory neurons were recorded in the pFRG. Continuous photostimulation up to 90 s of the rostral ventral medulla covering the pFRG induced decrease of respiratory rate or complete cessation of respiratory rhythm accompanied with membrane hyperpolarization of pFRG-Phox2b positive Pre-I neurons, whereas the inhibitory effects tended to decline during the continuous photostimulation more than 30 s. We confirmed that this method is useful for analysis of the local circuits of respiratory rhythm generation in the en bloc newborn rat preparation.

Disclosures: H. Onimaru: None. K. Ikeda: None. M. Ogawa: None. K. Ihara: None. K. Kobayashi: None. K. Kawakami: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.06/VV9

Topic: E.08. Respiratory Regulation

Support: NIH/NCCAM R01 AT008632

IRP of NIH/NINDS

Title: Optogenetic stimulation of pre-Bötzinger complex and Bötzinger complex inhibitory neurons reveals a major role of inhibition in respiratory central pattern generation

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Abstract: The architecture of respiratory central pattern generator (CPG) circuits remains not fully understood. Different components of the normal three-phase rhythmic respiratory pattern are postulated to be generated by different neuronal populations distributed within the pre-Bötzinger complex (pre-BötC) and Bötzinger complex (BötC) regions, which contain core circuit components of the CPG. Interacting inhibitory microcircuits in these compartments are proposed to be critical for generating the three-phase rhythmic pattern, but this has not been demonstrated definitively. We applied optogenetic techniques to manipulate activity of pre-BötC or BötC inhibitory neurons to test for perturbations of respiratory neural activity. We employed a transgenic mouse line expressing Cre-recombinase in neurons promoted by the vesicular GABA transporter (VGAT) for inhibitory neuron specific expression of Channelrhodopsin-2 (ChR2). VGAT is expressed in both GABAergic and glycinergic inhibitory neurons. With precisely positioned optical cannulae bilaterally in adult transgenic mouse *in situ* perfused brainstem-spinal cord preparations, we laser (473 nm) stimulated inhibitory neurons in pre-BötC or BötC regions, while recording phrenic and vagus nerve respiratory motoneuronal outflows. Continuous photo-stimulation (20 Hz, pulsed trains) of BötC inhibitory neurons prolonged the duration of the expiratory phase in a laser power dependent manner (0.5-2 mW), and at highest laser power eliminated respiratory oscillations in both phrenic and vagus nerves. Extracellular recordings showed suppression/silencing of inspiratory activity in pre-BötC during the laser stimulation of BötC inhibitory neurons. Continuous photo-stimulation of the pre-BötC increased respiratory frequency by reducing the duration of expiration in a laser power dependent manner (0.5-2 mW). Extracellular recordings demonstrated suppression/silencing of activity of post-inspiratory neurons in BötC during photo-stimulation of pre-BötC inhibitory neurons. We quantitatively reproduced these perturbations in our computational model describing interactions between pre-BötC and BötC circuits by selective graded stimulation of the corresponding inhibitory populations. Our experimental data together with computer simulations indicate a major role of BötC and pre-BötC inhibitory neurons in controlling respiratory phase durations and three-phase respiratory pattern generation in a manner consistent with mutual inhibitory interactions.

Disclosures: H. Koizumi: None. W. Barnett: None. M.F. Tariq: None. B. Mosher: None. T. John: None. R. Zhang: None. I.A. Rybak: None. Y.I. Molkov: None. J.C. Smith: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.07/VV10

Topic: E.08. Respiratory Regulation

Support: NIH Grant R01-HL104127 (PI: Del Negro)

Title: Optogenetic inhibition of Dbx1 preBötzinger complex neurons suppresses breathing in mice

Authors: ***N. C. VANN**, F. D. PHAM, C. A. DEL NEGRO;
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Abstract: Breathing is an essential and incessant behavior for humans and all terrestrial mammals. Inspiratory breathing movements emanate from neural rhythms generated in the preBötzinger complex (preBötC) of the ventral medulla, wherein interneurons derived from Dbx1-expressing precursors (i.e., Dbx1 neurons) are hypothesized to comprise the core oscillator circuit. Here we test this Dbx1 core hypothesis using intersectional mouse genetics. We expressed the proton pump Archaerhodopsin (Arch) in Dbx1 neurons to transiently inhibit those in the preBötC via bilateral 589-nm laser pulses, while simultaneously monitoring respiratory rhythm in vitro or breathing behavior in vivo. In a slice model that spontaneously generates inspiratory rhythm and motor output, Arch activation resulted in a 6 mV hyperpolarization of Arch-expressing Dbx1 neurons in patch-clamp recordings as well as ~25 s cessations of inspiratory motor output. In vagus-intact adult mice, Arch activation reduced respiratory frequency and prolonged inspiratory cycle time. Furthermore, Arch activation caused apneas lasting as long as ~18 s in anesthetized mice. These data are consistent with the Dbx1 core hypothesis, specifically indicating that Dbx1 neurons comprise the core oscillator for inspiratory breathing movements. Breathing behavior is unique in that the genetic origin of a key rhythmogenic population is known, which provides a well-defined target for detailed investigations that elucidate the cellular, synaptic, and molecular-level mechanisms underlying respiration in mammals.

Disclosures: N.C. Vann: None. F.D. Pham: None. C.A. Del Negro: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.08/VV11

Topic: E.08. Respiratory Regulation

Support: CIHR

AIHS

CFI

WCHRI

ALA

AHS/FoMD

Title: Adenosine inhibits the preBötzinger complex inspiratory network during the first two weeks of postnatal development

Authors: *R. J. REKLOW, G. D. FUNK;
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Abstract: Acute exposure to moderate hypoxia evokes a biphasic ventilatory response, comprising of an initial increase in ventilation within the first minute followed by a secondary depression over the next several minutes that can be life-threatening in premature infants. The initial increase is primarily attributed to activation of the carotid body chemoreceptors while the secondary depression is believed to be primarily central in origin. The hypoxic depression of breathing is strongly implicated in life-threatening apneic events that occur in premature infants, cardiovascular diseases that accompany obstructive sleep apnea and may be a factor in SUDEP (sudden unexplained death in epilepsy). Despite its clinical significance, underlying mechanisms are poorly understood. The increased level of extracellular adenosine (ADO) during hypoxia is implicated in the ventilatory depression but data are ambiguous, likely reflecting variability in the magnitude of the hypoxic stimulus, as well as developmental and species differences in the extent of the secondary depression and its underlying mechanisms. In adult sheep and rats, a suprapontine A2a receptor-mediated activation of GABAergic neurons is implicated. In neonatal rats, a direct A1 receptor-mediated inhibition of the brainstem respiratory network also appears to contribute. The ADO inhibition in rats disappears at 3 days postnatal (P3), suggesting that this may contribute to the greater depression in neonates. The aim of this study was to test the hypothesis that the ADO sensitivity of preBötC network decreases during postnatal development. ADO was locally applied to the preBötC (functionally identified by a strong Substance P evoked

frequency increase) of rhythmically-active medullary slices isolated from mice ranging in age from P0-12. At P0-5, ADO (500 μM, 30 s) inhibited inspiratory-related frequency by approximately 30%. Similar inhibitory effects were observed between P6-12. The $P2Y_1$ receptor agonist, MRS2365 (100 μM, 10 s), increased frequency by 2-3 fold at all ages (P0-12), indicating that the sensitivity of the respiratory network to $P2Y_1$ receptor-mediated excitation also persists over this developmental window. These data suggest that ADO-mediated inhibition of the preBötC inspiratory rhythm generating network may contribute to the hypoxic respiratory depression during the first two weeks of development. In addition, if extracellular ADO is derived from the degradation of extracellular ATP that is released during hypoxia, the secondary depression will be determined by the interaction between the excitatory effects of ATP at P2 receptors and inhibitory effects of ADO at P1 receptors.

Disclosures: R.J. Reklow: None. G.D. Funk: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.09/VV12

Topic: E.08. Respiratory Regulation

Support: NIH/NCCAM R01 AT008632

IRP of NIH/NINDS

NIH/NINDS R01 NS069220

Medical Research Council MR/L02661/1

Biotechnology & Biological Sciences Research Council BB/L019396/1

Title: Glycinergic neurons in the pre-Bötzinger complex are crucial for eupnea: an optogenetic study

Authors: *Y. I. MOLKOV[1], A. P. L. ABDALA[2], B. LIU[2], D. J. MORAES[3], S. KASPAROV[2], W. H. BARNETT[1], J. C. SMITH[4], J. F. R. PATON[2];
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Abstract: Respiratory movements in mammals are produced by the brainstem respiratory central pattern generator (CPG). Core structures of the respiratory CPG are the pre-Bötzinger (pre-BötC) and Bötzinger (BötC) complexes whose network interactions control respiratory oscillations. The normal breathing rhythm (eupnea) includes three phases of the cycle: inspiration, post-inspiration (post-I), and the second phase of expiration (E2). Synaptic inhibition in the CPG circuits has been proposed to be essential for generating this eupneic pattern. However, the importance of inhibitory interactions in the respiratory CPG as well as types of inhibitory neurotransmitters involved has been established based on pharmacological and modeling studies. As a first step in deciphering the CPG inhibitory circuits, we employed lentiviral vector-based gene transfer to express either channelrhodopsin (ChR2) or archaerhodopsin (Arch) photo-sensitive channels in glycinergic neurons of pre-BötC in Wistar rats (confirmed using immunocytochemistry *post hoc*), and we used optical control for either ChR2-mediated excitation (480 nm) or Arch-mediated inhibition (530 nm) to stimulate/inhibit these neurons, respectively, while recording from multiple respiratory motor outputs simultaneously in the *in situ* perfused juvenile rat brainstem-spinal cord preparation. Optogenetic excitation of pre-BötC glycinergic neurons significantly increased respiratory frequency due to the disappearance of E2. Optogenetic inhibition also increased the respiratory frequency, but unlike optical excitation, activation of Arch suppressed the post-I phase. We used a well-established computational model of the respiratory CPG to mechanistically explain these increases in respiratory frequency and perturbations of the three-phase pattern. In the model, we found that similar perturbations could be induced by altering the activity of the inhibitory population of pre-BötC inspiratory neurons. Simulated excitation of this population up to tonic spiking completely suppressed the activity of BötC E2 neurons and, thus, augmented oscillation frequency. In contrast, inhibiting this population disinhibited both post-I and E2 expiratory populations causing tonic discharge, thus eliminating phasic post-I activity and creating conditions for endogenous bursting of excitatory inspiratory neurons at a higher respiratory frequency. In summary, we explicitly demonstrated the functional importance of glycinergic interneurons in the pre-BötC for generation of eupnea and provide mechanistic insights for these responses using mathematical modeling.

Disclosures: Y.I. Molkov: None. A.P.L. Abdala: None. B. Liu: None. D.J. Moraes: None. S. Kasparov: None. W.H. Barnett: None. J.C. Smith: None. J.F.R. Paton: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.10/VV13

Topic: E.08. Respiratory Regulation

Support: FAPESP

CNPq

CAPES/PROEX

Title: Breathing responses produced by selective optogenetic activation of rostral ventrolateral medullary catecholaminergic neurons are dependent on the integrity of PreBotzinger complex

Authors: M. R. MALHEIROS-LIMA[1], L. T. TOTOLA[1], M. V. G. LANA[2], E. CONSTANZI-STRAUSS[3], B. E. STRAUSS[2], A. C. TAKAKURA[4], *T. S. MOREIRA[5];
[1]Physiol. and Biophysics, [2]Cancer Inst., [3]Cell and Develop. Biol., [4]Pharmacol., [5]Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Optogenetic stimulation of the catecholaminergic C1 neurons produces vigorous cardiorespiratory stimulation. We also demonstrated that depletion of C1 neurons attenuates breathing responses induced by hypoxia. The Pre-Bötzinger complex (PBC) is a specific group of neurons located in the ventrolateral medulla that is critical for respiratory rhythmogenesis. Our hypothesis is that the PBC contributes to breathing responses caused by selective activation of C1 neurons. To explore the specific contribution of the C1 neurons to breathing control, we used an optogenetic approach to activate these cells *in vivo*. PRSx8-ChR2-eYFP, a lentiviral vector that expresses channelrhodopsin-2 (ChR2) under the control of the catecholaminergic neuron-preferring promoter PRSx8, was injected into the rostral ventrolateral medulla (RVLM: 2-3 unilateral injections; 100-150 nl/site) of the male Wistar rats (240-280g). After 3 weeks, mean arterial pressure (MAP) and electromyography recording of diaphragm (Dia_{EMG}) were evaluated in urethane anesthetized and artificially ventilated rats. Photostimulation of ChR2-transduced RVLM neurons (473 nm, 20 Hz, 10 ms, 9 mW) was performed before and after unilateral injection of the ionotropic glutamate antagonist, kynurenic acid (kyn: 100 mM - 50 nl) in the PBC. After 3 weeks, ChR2 was largely confined to TH-expressing neurons (87%). The ChR2-expressing neurons were non-GABAergic and non-glycinergic. Photostimulation of ChR2-transduced RVLM neurons increased MAP (143±27, vs. baseline: 131±25 mmHg) and Dia_{EMG} freq (45±3, vs. baseline: 37±3 bpm). Unilateral injection of kyn in the PBC blocked the increase in Dia_{EMG} freq (0±0 vs. saline + laser: 24±6%) without changing the increase in MAP (18±5, vs. saline + laser: 12±4 mmHg) elicited by photostimulation of C1 neurons. Our results suggest that the increase in breathing produced by photostimulation of RVLM-C1 neurons can be caused by a direct glutamatergic activation of PCB. These results provide the most direct evidence that the C1 neurons have a role on breathing control

Disclosures: M.R. Malheiros-Lima: None. L.T. Totola: None. M.V.G. Lana: None. E. Constanzi-Strauss: None. B.E. Strauss: None. A.C. Takakura: None. T.S. Moreira: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.11/VV14

Topic: E.08. Respiratory Regulation

Support: NIH Grants NS72211

HL 70029

China Scholarship Council (CSC) Joint-Training Program

Title: Defining the respiratory role of preBötzinger Complex Dbx1-derived and somatostatinergic neurons *In vivo*

Authors: *Y. CUI[1,2], K. KAM[1], D. SHERMAN[1], W. A. JANCZEWSKI[1], Y. ZHENG[2], J. L. FELDMAN[1];
[1]UCLA, Los Angeles, CA; [2]Sichuan Univ., Chengdu, China

Abstract: To fully understand the neural control of breathing, the functional roles of distinct neural subpopulations of preBötzinger Complex (preBötC), a medullary nucleus critical for generation of respiratory rhythm, must be identified. Normal breathing in rodents requires activity of glutamatergic Dbx1-derived ($Dbx1^+$) preBötC neurons expressing somatostatin (SST). We combined *in vivo* optogenetics and pharmacology in channelrhodopsin 2 (ChR2)-expressing transgenic mice to determine firing patterns of preBötC $Dbx1^+$ and SST^+ neurons and the effects of precisely phase-timed photoactivation of these neurons on breathing. In transgenic adult mice expressing ChR2 in $Dbx1^+$ neurons (Dbx1-ChR2), photostimulation: i) during expiration evoked ectopic inspiratory bursts, and; ii) during early inspiration augmented inspiratory bursts; these effects were associated with excitation of preinspiratory and inspiratory neurons. In transgenic mice expressing ChR2 in SST^+ neurons (SST-ChR2), photostimulation: i) during early inspiration evoked augmented inspiratory bursts, and; ii) during expiration delayed the subsequent inspiration; these effects were associated with excitation of preBötC inspiratory and postinspiratory neurons. Inhibitory photoresponses were largely eliminated by blocking synaptic inhibition within preBötC or by local viral infection to limit ChR2 expression to preBötC SST^+ neurons. We propose that preinspiratory preBötC $Dbx1^+$ neurons are essential elements of the inspiratory rhythmogenic kernel and that inspiratory preBötC $Dbx1^+$ and SST^+ neurons primarily serve a premotor patterning role by activating downstream premotoneurons to shape inspiratory motor output. Moreover, in confirmation of published data, we conclude that postsynaptic inhibition within the preBötC is not essential for rhythmogenesis, but is critical for fine tuning breathing by broadening the dynamic range of inspiratory burst amplitude, stabilizing breathing

rhythm in the presence of significant perturbations, and, when inhibitory neurons are acutely activated, inducing apneas, such as needed during swallowing and breathholding.

Disclosures: Y. Cui: None. K. Kam: None. D. Sherman: None. W.A. Janczewski: None. Y. Zheng: None. J.L. Feldman: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.12/VV15

Topic: E.08. Respiratory Regulation

Support: NIH Grant R21-NS087257 (PI: Del Negro)

NIH Grant R01-HL104127 (PI: Del Negro)

Title: Imaging of dendritic calcium transients in preBötzinger neurons in organotypic slice cultures.

Authors: *W. S. PHILLIPS[1,2], C. A. DEL NEGRO[2], J. C. REKLING[1];
[1]Univ. of Copenhagen, Kobenhavn N, Denmark; [2]Dept. of Applied Sci., Col. of William & Mary, Williamsburg, VA

Abstract: The rhythm for breathing is generated by a network of excitatory interneurons in the preBötzinger complex (preBötC) of the ventral medulla. Rhythmic build-up of excitatory activity within this network results in an envelope of synaptic drive that is integrated into characteristic firing patterns recorded at the soma of preBötC neurons. Postsynaptic membrane properties are capable of amplifying excitatory synaptic input, but the extent to which dendrites actively or passively remodel EPSPs prior to their arrival and integration at the soma - and whether such a process selectively favors a more robust response to synchronized input - remains unclear. Here we use a novel organotypic brainstem-slice culture preparation, which retains the preBötC, to indirectly investigate voltage responses within dendrites of rhythmically active preBötC neurons. These cultured brainstem slices maintain a bilaterally synchronous pattern of inspiratory-like activity, and preBötC neurons exhibit electrophysiological properties consistent with those recorded and documented using acute slice preparations—a well-established experimental model for 25 years. Reduced light scattering and slice depth conferred via culturing has allowed us to image fluorescent calcium dynamics along extended dendritic segments during both rhythmic behavior and in response to current injection. Using wide-field fluorescence microscopy and whole-cell patch-clamp recording, we observed invasive dendritic calcium transients detectable

over 150 µm from the soma during inspiratory-like bursts of preBötC network activity. By comparing the dynamics of network-evoked dendritic calcium activity to that evoked by current injection under various conditions we aim to elucidate the role of dendrites in synaptic integration serving inspiratory burst generation in the preBötC.

Disclosures: W.S. Phillips: None. C.A. Del Negro: None. J.C. Rekling: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.13/VV16

Topic: E.08. Respiratory Regulation

Support: DFG - AOBJ: 614602

Title: The consequence of antiepileptic drug treatment on the respiratory activity generated in the PreBötzinger Complex

Authors: *H. KOCH[1,2], J. BRANDES[2,1], L. RÜSCHSTROER[2,1], H. LERCHE[2,1];
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Abstract: Sudden Unexpected Death in Epilepsy (SUDEP) is the major source of death in patients suffering from therapy-resistant epilepsy. Although the mechanism of action is not entirely understood, respiratory dysfunction after generalized tonic-clonic seizures seems to play a major role. Risk factors include polytherapy with several antiepileptic drugs (AEDs). Therefore, we hypothesized that AEDs critically alter the central respiratory response to hypoxia, as induced by periictal respiratory disturbances, and contribute to fatal apnea in SUDEP patients. As a model, we used isolated transversal brain stem slices containing the pre Bötzinger Complex of neonatal and juvenile mice (P3-P14). This nucleus is essential for inspiration and contributes to respiratory rhythm generation. In vitro, it continues to generate rhythmic activity which closely resembles eupneic breathing. We recorded the activity of the PreBötzinger Complex (preBötC) under normoxic (95% O_2) and severely hypoxic (under 1% 0_2) conditions. Control conditions without medication were compared to conditions where one of the common AEDs Lamotrigine, Levetiracetam and Carbamazepine had been added to the artificial cerebrospinal fluid that surrounded the slice. None of the medications displayed a significant impact on either fictive sighs or fictive eupneic breathing activity. However, both Lamotrigine and Carbamazepine significantly reduced the frequency of or completely abolished fictive gasping during hypoxia. Furthermore, they delayed the resumption of eupneic breathing during recovery

from hypoxia under normoxic conditions. Levetiracetam, in contrast, did not show such an effect. As a conclusion, AEDs which alter sodium currents might impair the preBötC-mediated central response to hypoxia. This could lead to increased vulnerability for SUDEP in patients who continue to suffer from seizure-induced apneas despite medication with AEDs.

Disclosures: H. Koch: None. J. Brandes: None. L. Rüschstroer: None. H. Lerche: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.14/VV17

Topic: E.08. Respiratory Regulation

Support: NIH Grant NS72211

FCT Grant SFRH/ BD/51268/2010

Title: PreBotzinger Complex SST^+ neurons modulate breathing rhythm and pattern

Authors: *R. P. DE SOUSA ABREU[1,2], J. L. FELDMAN[1];
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Abstract: Within the respiratory rhythm generator, the preBötzinger Complex (preBötC), a specific subset of glutamatergic neurons that expresses the inhibitory peptide somatostatin (SST) is critical for breathing. In rats, these neurons constitute approximately 10% of the preBötC and project extensively to a broad range of respiratory-related regions (e.g., Bötzinger Complex and RTN/pFRG). We investigated the modulatory role of these neurons on breathing, in freely moving mice.

We used optogenetics to acutely manipulate the activity of preBötC SST^+ neurons and investigate their impact on preBötC rhythmic activity (preI and inspiratory), respiratory-modulated motor output (e.g., genioglossus and abdominal muscle activity) and, ultimately, breathing, in both anesthetized and behaving mice. To elucidate the mechanisms underlying modulation, we examined phase response curves (PRCs), in some cases during distinct external challenges (e.g., anesthetic depth, hypoxia, and hypercapnia). In freely moving mice, a chemogenetic approach was also implemented to investigate the impact of preBötC SST^+ neurons on breathing at a different timescale, i.e., when these neurons are activated over a period of hours.

In freely moving mice, we found that preBötC SST^+ neurons can rapidly and transiently modulate breathing shape and frequency in normoxia, hypoxia and hypercapnia. In anesthetized

mice, PRCs of preBötC neuronal activity revealed that preBötC SST^+ neurons do not appear to be rhythmogenic but rather powerful respiratory modulators. Further investigation of PRCs revealed differences across the respiratory cycle (e.g., Inspiration vs. Expiration) and during distinct respiratory network states of excitability. Moreover, it appears that the mechanisms underlying modulation are nonlinear, perhaps threshold-based.
We propose that the changes in breathing, which include the shape (e.g., peak airflow amplitude) and timing (e.g., expiratory duration; T_E), resulted from phase- and excitability-dependent modulation of rhythmogenic processes. For example, activation of pattern-generating mechanisms and consequent recruitment of inspiratory, but not expiratory, muscle activity, could explain the generation of sigh-like events by photostimulation. In contrast, effects on T_E may result of activation of sub- or supra-threshold (below or above bursting threshold) mechanisms that determine rhythm (increase T_E) or pattern (decrease T_E) generation, respectively.
Overall, our study suggests that preBötC SST^+ neurons play an essential role in rapid respiratory responses, a unique property necessary for a robust yet labile breathing behavior.

Disclosures: R.P. De Sousa Abreu: None. J.L. Feldman: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.15/VV18

Topic: E.08. Respiratory Regulation

Support: NSERC Discovery Grant (end 2020)

NSERC PGS-D (end 2017)

Title: Unbuckling the Buccal oscillator in the American Bullfrog

Authors: *M. I. BAGHDADWALA, R. J. A. WILSON;
Physiol. and Pharmacol., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: The American Bullfrog is a remarkable model organism to study the neural circuits underlying respiratory rhythm generation. It exhibits a breathing pattern that can be divided into four distinct ventilatory phases: lung-priming, lung-powerstroke, buccal-dilation, and buccal-constriction. We recently established that lung-priming and lung-powerstroke are generated by two separate burst generating substrates in the brainstem—the Priming Area and Powerstroke Area—located in rhombomere 4-5. The neural origin of the two phases of buccal, on the other hand, remains elusive. Our previous experiments suggest that a discrete, bilaterally iterated,

Buccal Area located in rhombomere 7-8 is responsible, at least in part, for generating the biphasic buccal rhythm. However, whether any additional burst generating substrates are involved is yet unknown. To further investigate the generation of biphasic buccal rhythm, we developed an isolated 'buccal-segment' preparation guided by a high resolution extracellular survey of unit activity in the buccal-generating region. **Extracellular survey:** We high-frequency stimulated the vagus (cranial nerve 10) and hypoglossal (CN12) nerve roots bilaterally under High Mg^{2+} saline to excitotoxically kill their motor nuclei. This was done to eliminate any motor neuron activity in close proximity to the Buccal Area. Following this, we extracellularly explored this region to map out patterns of unit activity. Our results showed that multiple separate loci had unit activity correlated to buccal bursts. This suggests that there might be several regions around the previously identified Buccal Area involved in the generation of buccal bursts. **Isolated 'buccal-segment' preparation:** Guided by the survey, we developed a reduced 'buccal-segment' preparation consisting only of the vagus and hypoglossal motor nuclei and nerve roots which, when exposed to 100nM AMPA, produced buccal bursts on both nerves. We further transected the brainstem at various levels and discovered that a central transection between vagus and hypoglossal nerve roots could sometimes produce two independently rhythmic slices suggesting that the buccal rhythm might be generated by multiple burst-generating substrates. **Significance:** We have previously suggested that the Buccal Area in frogs is an evolutionary homolog of the mammalian preBötzinger complex. Both are located in rhombomere 7, are resilient oscillators, staunchly insensitive to CO_2, and co-opted for sniffing. Our discovery that the buccal rhythm might be a multi-substrate phenomenon provides a novel insight into the evolution of breathing.

Disclosures: M.I. Baghdadwala: None. R.J.A. Wilson: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.16/VV19

Topic: E.08. Respiratory Regulation

Support: NIH/NCCAM R01 AT008632

Title: Kolliker Fuse orchestrates timing of abdominal nerve bursting

Authors: *W. H. BARNETT[1], A. P. ABDALA[2], J. F. R. PATON[2], D. B. ZOCCAL[3], Y. I. MOLKOV[1];
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Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom; [3]Dept. of Physiol. and Pathology, São Paulo State University, São Paulo, Araraquara, Brazil

Abstract: A hallmark of the respiratory reflex response to hypercapnia is the emergence of active expiratory pattern in the abdominal motor output (AbN). This pattern consists of late-expiratory (late-E) bursts attributed to the recruitment of expiratory neurons in the parafacial respiratory group (pFRG) putatively driven by chemosensitive cells in the retrotrapezoid nucleus (RTN). Evidence suggests participation of other cellular groups in control of AbN late-E activity generation. Late-E activity is abolished by systemic application of riluzole, indicating potential involvement of the persistent sodium current. However, there is no direct experimental evidence for the presence of persistent sodium channels in pFRG late-E neurons. In this scenario, we suggest that riluzole dependence of late-E activity is mediated by excitation from pre-inspiratory/inspiratory (pre-I/I) neurons of pre-Bötzinger complex (pre-BötC), which are known to contain persistent sodium channels. It has been previously suggested that the pFRG late-E population is suppressed by inhibition from post-inspiratory (post-I) neurons of the Bötzinger complex under eupneic conditions. Therefore, areas that control post-I activity, such as the Kölliker-Fuse (KF) nucleus, may be involved in the control of hypercapnia-induced late-E activity. Here, we explore the role of the KF nucleus and the pre-BötC pre-I/I neurons in modulating late-E respiratory activity. Based on model simulations, we propose that pre-I/I neurons in the preBötC are stimulated by RTN chemosensitive neurons during hypercapnia and which then drive late-E activity in pFRG neurons. Assuming that the KF area provides excitatory drive to BötC post-I inhibitory neurons, our model predicted that during hypercapnia: 1) KF inhibition lower the recruitment threshold of late-E activity; 2) KF excitation prevents emergence of late-E activity. These modeling predictions were tested experimentally in the *in situ arterially perfused* rat. We found that: i) inhibition of KF with isoguvacine (10mM) advanced by 1s the onset of late-E bursts in AbN after exposure to hypercapnia (8% CO2); and ii) disinhibition of KF with gabazine (100μM) greatly attenuated AbN late-E activity. The model suggests that the pre-BötC pre-I/I neurons are an important source of excitatory synaptic inputs to the pFRG expiratory neurons. Moreover, KF-driven inhibitory inputs to the pFRG, possibly through the post-I BötC neurons, may determine the presence and/or onset timing of AbN late-E bursts during hypercapnia.

Disclosures: W.H. Barnett: None. A.P. Abdala: None. J.F.R. Paton: None. D.B. Zoccal: None. Y.I. Molkov: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.17/VV20

Topic: E.08. Respiratory Regulation

Support: AFM

Title: Acetylcholinesterase and respiration: where this essential enzyme is required?

Authors: A. NERVO[1], C. REYMOND[1], F. NACHON[1], G. CALAS[1], *E. KREJCI[2];
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Abstract: Acute intoxication by pesticides (carbamates or organophosphates (OP)) or nerve agents affects several vital functions until death by asphyxia. The key target of these reactive molecules are cholinesterases, mainly acetylcholinesterase (AChE), whereas butyrylcholinesterase (BChE) appears more as a scavenger for these compounds. Both enzymes hydrolyze acetylcholine (ACh) and the well-known consequences of the inhibition of cholinesterases result presumably from the consecutive excess of ACh. However, it remains unclear where ACh is toxic. Indeed, deficit of AChE in the brain, when its anchoring protein PRiMA is absent, leads to a huge excess of ACh measured by microdialysis in the brain. PRiMA KO mice adapt well to this excess by the decrease of muscarinic receptors but remain as sensitive as WT mice to AChE inhibitors. Deficit of AChE in the skeletal muscle of AChE1iRR mice leads to muscle weakness and AChE1iRR mice are highly sensitive to OP intoxication, suggesting that AChE in the muscle may also serve as scavenger of OP. To explore where and how ACh could be toxic we have recorded the modifications of body movements and air exchanges of mice in two chambers plethysmography apparatus during the respiration. We used KO or Ki mice in which AChE is not properly localized, or in which nicotinic ACh receptors are absent. For each genotype, the nasal and thoracic flow parameters were recorded before and after intoxication with different inhibitors of cholinesterases such as paraoxon (model of OP pesticide), physostigmine or neostigmine (two carbamates that cross or not the blood brain barrier). We will present how the double chambers plethysmography helps to identify different alterations of respiration. All the inhibitors used block dramatically the respiration, even when the inhibitor does not cross the blood brain barrier. All the mice lacking of AChE in brain or in skeletal muscle are affected by these inhibitors. We will discuss how these results challenge the canonical view that AChE acts essentially to terminate the synaptic transmission at cholinergic synapses.

Disclosures: A. Nervo: None. C. Reymond: None. F. Nachon: None. G. calas: None. E. Krejci: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.18/VV21

Topic: E.08. Respiratory Regulation

Support: NIH R01-NS086088

Title: Parametrization of a closed-loop adaptive controller for respiratory pacing in a rodent model

Authors: *R. SIU[1], B. HILLEN[1], A. THOTA[1], S. RENAUD[2], J. ABBAS[3], R. JUNG[1]; [1]Florida Intl. Univ., Miami, FL; [2]IMS Lab., Univ. de Bordeaux, Bordeaux, France; [3]Arizona State Univ., Tempe, AZ

Abstract: Spinal cord injury at the cervical level can cause damage to the descending respiratory pathways, which can lead to a significant reduction in ventilatory capabilities. Individuals with impaired ventilatory capacity are often dependent on ventilators to achieve proper respiration. Respiratory pacing via electrical stimulation of the phrenic nerve or of the diaphragm has been shown to enhance quality of life compared to mechanical ventilation, by improving speech, preventing social stigma, and improving mobility. However, commercially-available respiratory pacing devices require initial manual specification of stimulation parameters and frequent adjustment to achieve and maintain suitable ventilation and ventilatory efficiency over long periods of time. We have developed a closed-loop neuromorphic controller capable of meeting the ventilatory demands of the user despite changes in muscle and electrode properties, as well as other extrinsic factors. Optimization of key parameters can increase stability and efficiency of the controller when considering its closed-loop implementation. Therefore, we conducted simulations based off rat experimental data to determine the range of relevant parameters over which the controller could achieve and maintain a root-mean-squared error (RMSE) of less than 5% within a period of 50 breathing cycles.

The controller's amplitude modulation algorithm is composed of a neural network in which an error signal at specific time points in the breathing cycle modifies connection weights for neurons that were active in the recent past. Based on prior work with this algorithm, it is expected that controller performance would be most affected by learning rate, number of neurons active at any time-point, and the length of the window used to average past activation values in the update algorithm. As a first step, the controller was tasked with matching a stimulation profile obtained from rat experimental data in which the controller was able to match a desired volume profile. The software-based controller was developed in LabVIEW. Learning rate was assessed for values of 0.0001 to 0.004, number of active neurons from 1 to 19, and past activation window length from 2 to 20 time steps. A limit of 50 cycles was allowed for

adaptation time. Simulations showed that the optimal parameters were a learning rate of 0.002 – 0.004 with 3 – 9 active neurons and 2 – 4 time steps for the past activation window length. These results were then validated in acute animal studies (n=4) in which 6 active neurons and a window of 4 past activations showed the best performance by maintaining RMSE values between 5% and 10% over periods of 100 breathing cycles or more.

Disclosures: R. Siu: None. B. Hillen: None. A. Thota: None. S. Renaud: None. J. Abbas: None. R. Jung: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.19/VV22

Topic: E.08. Respiratory Regulation

Support: NIHHD071302

Title: Developmental nicotine exposure alters the frequency of miniature inhibitory post-synaptic currents in hypoglossal motoneurons.

Authors: *L. B. WOLLMAN[1], R. B. LEVINE[2], R. F. FREGOSI[3];
[1]Physiol., The Univ. of Arizona, Tucson, AZ; [2]Neurosci., [3]Physiol., Univ. of Arizona, Tucson, AZ

Abstract: Prenatal nicotine exposure with continued exposure through breast milk over the first week of life (developmental nicotine exposure, DNE) is known to cause complex changes in hypoglossal motoneurons (XII MNs) including altered responses to inhibitory neurotransmitters and increased density of $GABA_A$ receptors on these neurons. Here we test the hypothesis that DNE alters the amplitude and frequency of spontaneous, action potential mediated, and miniature GABA inhibitory post-synaptic currents (IPSCs) recorded from XII MNs in neonatal rats. We did whole cell patch clamp recordings from XII MNs in medullary slices (700 micron thick) from DNE pups and control pups. Cells were voltage clamped at -55mV and spontaneous GABA IPSCs (sIPSCs), including both action potential mediated events and miniature events, were recorded in the presence of the glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and the glycine receptor antagonist Strychnine. Miniature GABA IPSCs (mIPSCs), representative of random quantal release of neurotransmitter, were recorded in the presence of CNQX, Strychnine, and the voltage-gated sodium channel antagonist Tetrodotoxin (TTX). Our results are as follows: 1) DNE does not alter the amplitude (Control {n=4} 21.4±2.7 pA, DNE {n=4} 22±2.1 pA) or frequency (Control {n=4} 366.4±107.6 counts/min, DNE {n=4}

311.5±82.8 counts/min) of GABA sIPSCs. 2) DNE does not alter the amplitude (Control {n=6} 14.6±1.6 pA, DNE {n=6} 15.3±1.4 pA) of GABA mIPSCs. 3) DNE did increase the frequency of GABA mIPSCs (Control {n=6} 26.5±9.1 counts/min, DNE {n=6} 59.2±21.3 counts/min). These results show that DNE alters the random, quantal release of GABA onto XII MNs by a currently unknown mechanism.

Disclosures: L.B. Wollman: None. R.B. Levine: None. R.F. Fregosi: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.20/VV23

Topic: E.08. Respiratory Regulation

Support: NIH Grant HD 071302

Title: Role of potassium conductances in shaping excitability in hypoglossal motoneurons in developmental nicotine exposure model

Authors: M. CHOLANIAN[1], R. B. LEVINE[1], *R. F. FREGOSI[2];
[1]Univ. of Arizona, Tucson, AZ; [2]Univ. Arizona, Tucson, AZ

Abstract: Developmental nicotine exposure (DNE) in rodents is a widely accepted model for studying neurotoxic effects of nicotine exposure *in utero*. Chronic nicotine exposure causes widespread desensitization of nicotinic acetylcholine receptors (nAChRs) in multiple brain regions, including brainstem neurons involved in the control of breathing. Our previous studies showed that DNE decreased excitatory synaptic input in hypoglossal motoneurons (XIIMNs) of neonatal rats and altered their excitability. Here we hypothesize that one of the major underlying mechanisms behind DNE-induced changes in excitability involves alterations in multiple potassium conductances. We have used whole-cell patch-clamp electrophysiology in a medullary slice prepared from neonatal rats to study the effect of DNE on K^+ conductances. We recorded from rhythmically active XIIMNs in an external solution containing 9mM K+ and non-rhythmically active XIIMNs in separate slices using a 3mM K+ external solution. Interestingly, DNE caused an increase in both early and sustained K^+ currents, as compared to XIIMNs from slices prepared from control animals. This effect was only observed, however, in the rhythmically-active XIIMNs. The K+ currents were not affected significantly by DNE in the non-rhythmically active XIIMNs. These results suggest that DNE affects distinct K^+ conductances in rhythmically-active XIIMNs.

Disclosures: M. Cholanian: None. R.B. Levine: None. R.F. Fregosi: None.

Poster

249. Neural Control of Respiration I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 249.21/VV24

Topic: E.08. Respiratory Regulation

Support: UCLA Children's Discovery and Innovation Institute's Seed Grant award

Title: Neuromodulation of limb proprioceptive afferents decreases apnea and stabilizes blood pressure (BP) in premature neonates

Authors: *K. KESAVAN[1], D. M. CORDERO[2], R. M. HARPER[3];
[1]Pediatrics, Univ. of California, Los Angeles, CA; [2]Pediatrics - Neonatology, [3]Neurobio., UCLA, Los Angeles, CA

Abstract: Background: Apnea of Prematurity (AOP) and intermittent hypoxia are common, affecting the majority of premature infants. Repeated episodes of hypoxemia during apneic episodes causes marked increases in sympathetic nerve activity, leading to substantial changes in BP. Adult patients with recurrent apneas and chronic IH exhibit increased sympathetic nerve activity and elevated plasma catecholamine levels. To date, the effects of on-going acute effects of such IH episodes on BP have not been systematically studied in premature neonates. It is important to maintain BP within the limits of cerebral autoregulation for optimal cerebral protection. In a pilot study, we found that neuromodulation of limb proprioceptive afferents using a vibration device was associated with fewer breathing pauses, IH episodes, and bradycardic episodes. Therefore, we hypothesized that, with proprioceptive stimulation decreasing apneas and improving oxygenation, we will reduce the associated BP changes.
Objective: The objective is to support breathing and maintain BP in premature infants by using a non-invasive vibratory device placed over limb proprioceptor fibers, an intervention using the principle that limb movements trigger reflexive facilitation of breathing.
Methods: Premature infants (23 -34 wks gestational age), with clinical evidence of AOP episodes were enrolled 1 week after birth. Small vibration devices were placed on one hand and one foot and activated in a 6 hour ON/OFF sequence for a total of 24 hours. Heart rate, respiratory rate, oxygen saturation (SpO_2) and BP, inferred from pulse transit time and calibrated by cuff measures, were continuously collected.
Results: 1. Fewer breathing pauses occurred during vibration periods, vs baseline ($p<0.05$).
2. Significantly fewer SpO_2 declines occurred with vibration ($p<0.05$), vs control periods.
3. Significantly fewer bradycardic events occurred during vibration periods, vs no-vibration

periods ($p<0.05$).
4. Acute falls in mean BP after apnea occurring during control periods were substantially reduced during vibration periods.'
Conclusions: Proprioceptive afferent activation reduced apnea and O_2 desaturation epochs, an outcome that also diminishes IH episodes and accompanying BP changes. Large BP changes pose a risk for impaired perfusion to brain structures. This low-cost neuromodulatory procedure using vibration to proprioceptive nerves has the potential to provide a non-invasive intervention to stabilize BP in premature neonates, thereby improving neurological outcomes.

Disclosures: **K. Kesavan:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **D.M. Cordero:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **R.M. Harper:** A. Employment/Salary (full or part-time): University of California, Los Angeles.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.01/VV25

Topic: E.10. Motor Neurons and Muscle

Title: Identification of cortical drive to arm muscles during arm cycling by analysis of power spectrum

Authors: *T. C. RICHARDS[1], S. CHAKRABARTY, Sr[1], K. POWER[2];
[1]Univ. of Leeds, Leeds, United Kingdom; [2]Sch. of Human Kinetics and Recreation, Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Cortico-muscular and intermuscular coherence studies have sought to relate activity in the sensorimotor cortex to activity in the muscle, or to identify muscles sharing common cortico-motoneuronal drive. Coherence is most commonly discussed in the context of three distinct bands: alpha (α) at 8 - 12 Hz, beta (β) at 15 - 30 Hz, and gamma (γ) at 30 - 100 Hz. Cortico-muscular coherence in the β-band is thought to reflect coupling of cortical oscillations to the spinal motoneuron pool during sustained contractions. Less commonly, coherence has been observed in the α-band, also during sustained contractions, and in the γ-band during dynamic movements. Cortico-muscular coherence within these bands is often assumed to reflect efferent activity of direct projections from the motor cortex to the spinal motoneurons. It has recently been demonstrated that TMS (transcranial magnetic stimulation) evoked responses increased in biceps brachii during arm cycling compared to an intensity-matched tonic contraction (Forman et al., 2015). The increased amplitude of the evoked response was attributed to increased cortical

neuronal excitability. By generating power spectrum of the EMG response, we will identify TMS induced changes in the frequency components of the response in this dataset (n = 10). In this context TMS evoked muscle activity reflects activity in any single and or combination of frequency bands previously reported in coherence studies. Forman, D. et al. 2015. Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. *Journal of Neurophysiology* [online]. (June 2014),pp.1142-1151.

Disclosures: T.C. Richards: None. S. Chakrabarty: None. K. Power: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.02/VV26

Topic: E.10. Motor Neurons and Muscle

Title: The effects of waveform and current direction on test-retest reliability of transcranial magnetic stimulation

Authors: *P. DAVILA PEREZ[1], A. JANNATI[1], M. SHAFI[1], J. CUDEIRO[2], A. PASCUAL-LEONE[1];
[1]BIDMC - Harvard Med. Sch., Boston, MA; [2]Univ. of A Coruña, A Coruña, Spain

Abstract: Objective: When using transcranial magnetic stimulation (TMS), different waveforms (monophasic vs. biphasic) and induced current directions in the primary motor cortex (posterior-to-anterior [PA] vs. anterior-to-posterior [AP]) may stimulate different cortical neural components. We assessed the effects of these factors on the test-retest reliability of single- and paired-pulse neuronavigated TMS protocols.
Methods: Twenty-six healthy participants between the ages of 18 and 35 (12 males, 22 right-handed) completed two sessions (intersession interval range 1-70 days; median = 10.5 days), assessing resting motor threshold (RMT), amplitude of motor evoked potentials (MEPs) (averaged over 10 pulses), contralateral cortical silent period (cSP), short-interval intra-cortical inhibition (SICI), long-interval intra-cortical inhibition (LICI), and intracortical facilitation (ICF). Estimates obtained for LICI, SICI and ICF were calculated as a percentage of baseline MEP amplitude, averaged over 40 pulses. Ten, nine, and seven subjects underwent two sessions of PA, AP, and biphasic stimulation, respectively. Neuronavigated TMS was performed with a MagPro X100 device and a Cool-B65 figure-of-eight coil (outer diameter 75mm), and Brainsight TMS neuronavigation system using a brain MRI template. MEPs were measured with a

PowerLab 4/25T data acquisition device. Analyses included individual and average absolute intraclass correlation coefficients (ICCs) using a two-way mixed-effects model.

Results:

		Individual ICC			**Average ICC**	
	Monophasic PA	Monophasic AP	Biphasic	Monophasic PA	Monophasic AP	Biphasic
RMT	0.67	0.98	0.90	0.81	0.99	0.95
Baseline MEPs	0.50	0.44	-0.16	**0.66**	0.61	-0.37
cSP	0.73	0.53	0.63	0.84	0.69	0.77
SICI	0.53	0.36	0.48	0.69	0.52	0.65
LICI	0.52	**0.93**	0.68	0.68	0.96	0.81
ICF	-0.13	0.06	**0.77**	-0.29	0.11	0.87
Note: Bold ICC values were significantly higher compared with a different waveform or current direction. The alpha level was 0.05.						

The average ICC for baseline MEP with PA pulse (0.66) was significantly higher than with biphasic pulse (-0.37), p=0.026. The individual ICC for LICI with AP pulse (0.93) was significantly higher than with PA pulse (0.52), p=0.04. The individual ICC for ICF with biphasic pulse (0.77) was significantly higher than with PA pulse (-0.13), p=0.006.

Conclusions: These results demonstrate the significant effect of waveform and current direction on test-retest reliability of TMS measures, thereby emphasizing the importance of choosing the appropriate parameters for a given single- or paired-pulse TMS protocol. Furthermore, these results allow for deeper understanding of the underlying neurophysiological mechanisms of TMS measures.

Disclosures: **P. Davila Perez:** None. **A. Jannati:** None. **M. Shafi:** None. **J. Cudeiro:** None. **A. Pascual-Leone:** F. Consulting Fees (e.g., advisory boards); Neosync- Member of Scientific Advisory Board, Company developing an EEG-guided transcranial current stimulation system, Company developing an improved method for transcranial current stimulation, Company developing a system for treatment of cognitive decline in Alzheimer's disease combining cognitive training and transcranial magnetic stimulation, Company in the transcranial magnetic

stimulation field developing improved neuronavigated TMS systems, Company that manufacturers and commercializes transcranial magnetic stimulation equipment, Company that manufacturers and commercializes a robot for targeting of transcranial magnetic stimulation.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.03/WW1

Topic: E.10. Motor Neurons and Muscle

Title: Probing causal relevance of oscillatory power and phase for corticospinal excitability in humans: a new experimental tacs-tms paradigm

Authors: *L. SCHILBERG, T. ENGELEN, T. DE GRAAF, T. SCHUHMANN, A. SACK; Maastricht Univ., Maastricht, Netherlands

Abstract: TMS measures of motor cortex excitability (Motor Evoked Potentials; MEPs, induced by single TMS pulses over primary motor cortex) are widely applied to study both brain function and plasticity in healthy and patient populations. Unfortunately these measures are subject to substantial and largely unexplained within- and between-subject variability, which affects reliability of MEPs as a measure of cortical excitability in research and clinical settings. Two candidate causes of trial-by-trial MEP fluctuations are the power and phase of cortical oscillations at the time of stimulation. Yet to test their functional relevance is challenging. It would require experimental control over both the power and phase of local oscillations in relation to TMS pulse timing. Transcranial alternating current stimulation (tACS) allows periodic electrical stimulation of human cortex, effectively enabling control over oscillations. TACS over M1 at 20Hz, a dominant frequency at sensorimotor areas, has been shown to increase corticospinal excitability. Alpha frequency (~10Hz) reflects pulsatile cortical inhibition and has been shown to mediate the inhibitory effects of patterned TMS, depending on the temporal relation between TMS pulses and oscillatory phase. To disentangle and evaluate the effects of oscillatory power and phase in cortical excitability, we applied a novel method in which TMS was phase-locked to tACS. We applied single TMS pulses at 8 equidistant phases of a signal entraining motor cortex oscillations (electrodes at M1 and Pz, 1mA current peak-to-peak, 36.5 minutes stimulation), at either alpha (10Hz), beta (20Hz), or sham tACS frequency. TMS intensity was 120% resting motor threshold, and single pulses were on average 6.75 seconds apart. TMS-induced MEPs were recorded with electromyography (EMG) from the targeted right first dorsal interosseous (FDI) muscle. A causal role of phase should lead to an oscillatory pattern of MEPs across the 8 TMS-tACS phase bins, while a causal role of power is tested by

comparing mean MEP from forty TMS-only trials before and after the tACS manipulation. By applying phase-locked tACS and TMS at the same location, a direct assessment of causal relevance of both power and phase of cortical oscillations can be made, which in the future can lead to a more reliable measure of motor cortex excitability.

Disclosures: L. Schilberg: None. T. Engelen: None. T. de Graaf: None. T. Schuhmann: None. A. Sack: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.04/WW2

Topic: E.10. Motor Neurons and Muscle

Support: NIH P20GM109098

NIH CoBRE P20GM109098

Title: Corticospinal excitability during normal gait at varying speeds and inter-limb velocities: a transcranial magnetic stimulation study.

Authors: *L. KOGAN, V. GRITSENKO, S. YAKOVENKO;
West Virginia Univ., Morgantown, WV

Abstract: Periodic movements are generally produced by oscillatory activity within dedicated neural elements, termed central pattern generators (CPGs). Our previous modeling work put forth the hypothesis that CPGs involved in gait in humans are driven by signals corresponding to limb velocity, which are high-level representations of desired whole-limb dynamics. Additionally, humans are sensitive to changes in inter-limb velocity during normal gait, and can sense a change in heading without access to visual information. This suggests that gait and inter-limb velocity may be represented within the visuomotor pathways, including the primary motor cortex (M1). The purpose of this work was to test this hypothesis in healthy human volunteers walking on a split-belt treadmill. Subjects walked at various speeds in tied-belt (identical left and right belt speeds) and split-belt regimes (with an inter-limb velocity differential). The acceleration of the treadmill was constant between conditions to allow the subjects to smoothly transition between target velocities. During the experiment, inter-limb velocity was varied sequentially and in randomized order. Eight surface EMG signals were recorded from representative muscles of each leg to allow for comparison between stimulated and contralateral limb response. Corticospinal excitability was measured using single-pulse transcranial magnetic

stimulation of M1 using a threshold value determined for each subject at rest. Integrated motor evoked potential (MEP) amplitudes were compared between baseline values collected during walking in different locomotor phases and under each condition. These normalized values representing corticospinal excitability were then compared across gait speeds and inter-limb velocities, and results were summarized across all subjects. We found that corticospinal excitability was modulated by gait velocity.

Disclosures: L. Kogan: None. V. Gritsenko: None. S. Yakovenko: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.05/WW3

Topic: E.10. Motor Neurons and Muscle

Support: NIH CoBRE P20GM109098

Title: Sequence of forelimb muscle activations during symmetric and asymmetric locomotion in rats

Authors: *E. M. SALIDO, K. TUNTEVSKI, S. YAKOVENKO;
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Abstract: Complex movements like reaching to a lever or locomotion are generated by a sequence of muscle actions. The leading theory is that CNS solves the problem of multiple possible control strategies using a subset of actions, termed motor primitives or synergies. The organization of neural and muscle motor primitives at different levels of CNS and how they subserve complex movements remain open fundamental questions. Previously, we have documented the activation sequence of forelimb muscles in cats (Yakovenko et al., 2011). While this animal model has been instrumental in understanding sensorimotor control, new genetic techniques and the development of multielectrode arrays for cortical recording and stimulation encourage transferring behavioral methodology to rodent models. To this purpose, we have developed the precise foot placement locomotor task that allows assessing skilled locomotor movements in rats (Tuntevski et al., 2016). We expected to find a similar sequence of muscle activations in rats walking on pegs as in cats reaching to a lever or stepping over obstacles (Yakovenko & Drew, 2016). Rats were trained to walk on peg walkway configured to produce either symmetric or asymmetric locomotor tasks where animals have to overstep with their left or right limbs. After brief training, selected forelimb muscles were bilaterally implanted with intramuscular electromyography (EMG), Teflon-insulated stainless steel wire pairs. After a week

of recovery, we recorded synchronized EMG and ground reaction forces (GRF) measured with the array of force sensors embedded into walkway pegs. The corresponding signals were digitized and filtered offline. Using supervised automatic threshold detection method (Yakovenko et al., 2005), we have marked locomotor events corresponding to stance and swing onsets for both forelimbs during consistent stepping behavior. The kinematic onset of swing phase was identified as the onset of deflection in GRF. The period between two successive onsets or offsets was defined as the cycle duration. EMG profiles normalized to the cycle duration were analyzed in all locomotor conditions. Average activity phase was determined for each muscle using inter-trial variability to mark onset and offset of EMG bursts. This data was used in the phase-space analysis of muscle activations. The sequence of activation was similar to that observed in cats during locomotion with and without obstacles. Moreover, the phase of bursts and their duration were modulated in the asymmetric tasks. These findings support the hypothesis that the profiles of muscle activations are qualitatively similar across quadrupeds during precise locomotor tasks.

Disclosures: E.M. Salido: None. K. Tuntevski: None. S. Yakovenko: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.06/WW4

Topic: E.10. Motor Neurons and Muscle

Support: ERC under the Advanced Grant DEMOVE #267888

EU's Horizon 2020 programme Marie Curie under grant agreement No #702491

Title: Linear vs nonlinear control of populations of motor neurons during voluntary contractions

Authors: *F. NEGRO[1], A. DEL VECCHIO[2], J. L. DIDERIKSEN[3], F. FELICI[2], D. FARINA[4]; [1]Inst. of Neurorehabilitation Systems, Univ. Med. Ctr. Göttingen, Goettingen, Germany; [2]Dpt. Human Movement Sci., Univ. degli Studi di Roma Foro Italico, Rome, Italy; [3]Dept. of Hlth. Sci. and Technol., Aalborg Univ., Aalborg, Denmark; [4]Inst. of Neurorehabilitation Systems, Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract: During voluntary contractions, motor neurons are controlled by the synaptic input generated by spinal and supraspinal centers. Measuring the correlation between populations of motor neuron spike trains *in vivo*, it has been recently demonstrated that this input is largely common across motor neuron pools [1]. Because of commonality, populations of motor neurons

substantially attenuate uncorrelated synaptic input (synaptic noise) by the averaging process performed in the generation of the neural drive to muscles [2]. Therefore, groups of motor neurons act as ideal amplifiers tuned on the common components of the synaptic input they receive and the neural drive to muscles almost perfectly reflects these components. Distributing correlated synaptic input to populations of motor neurons solves in a simple way the problems of non-linearity and noise at the individual neural level. Furthermore, due to the filter characteristics of muscle contraction, the "effective" neural drive resides in the low-frequencies (0-8 Hz). This view is partly in contrast with the possibility that high frequency neural oscillations may modulate force output [3]. In this study, we show that, although high frequency oscillations can be demodulated in low-frequency components that contribute to the effective neural drive to muscles, this mechanism cannot be use for accurate force control. First, a theoretical analysis based on a simplified integrate and fire model of motor neurons showed that input components modulated by high-frequency carriers are distorted when demodulated in the low-frequency band. Further, a numerical model comprising 445 motor neurons [4] that received both low-frequency input and the same input modulated by a high frequency carrier (20 Hz) confirmed the theoretical predictions. The direct transmission of low frequency components resulted more accurate and effective than that of the demodulated components. Finally, experimentally we found no association between the amplitude of neural drive oscillations in the delta and beta bands for motor units in the tibialis anterior muscle. In conclusion, our results points out that the voluntary control of force can unlikely be achieved by non-linear transmission of modulated high-frequency carriers.
[1] Negro F et al. (2016) J Physiol, In Press[2] Farina D & Negro F (2015). Exerc Sport Sci Rev, 43.1:23-33[3] Watanabe RN & Kohn AK J. Neurosc 35.40 (2015): 13687-13697. [4] Negro F & Farina D Plos One, 7(9), 2012. [5] Negro F et al. (2016) J Neural Eng. 026027.

Disclosures: F. Negro: None. A. Del Vecchio: None. J.L. Dideriksen: None. F. Felici: None. D. Farina: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.07/WW5

Topic: E.10. Motor Neurons and Muscle

Support: NIH/NICHD Grant 5K12HD001097-17

NIH/NINDS Center Core Grant P30-NS045758

Title: Sarcopenia and connectivity of the aging motor unit

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Abstract: The majority of people over the age of 80 are affected by sarcopenia, or loss of muscle mass and strength, resulting in impaired physical function and increased risk of mortality. Interestingly, after the age of 80 humans may lose up to 50% of their motor neurons. Sarcopenic patients' muscle biopsies show features of denervation and incomplete reinnervation. These findings implicate motor neuron loss as an important factor in the development of sarcopenia. This study was designed to determine the timing of motor unit loss in aging C57BL/6J male mice and to assess the interaction between motor unit function, grip strength and muscle mass. Mice were analyzed longitudinally between the ages of 10-25 months with electrophysiological measures of motor unit function including compound muscle action potential amplitude (CMAP) and motor unit number estimation of the triceps surae muscles, grip strength, and magnetic resonance imaging of triceps surae volume (MRI). CMAP amplitudes at 10 months(50.7±12.4mV; mean±standard deviation), 13 months (43.6±11.2mV), 15 months (47.5±6.7mV), and 17 months (45.3±5.7mV) were unchanged, but beginning at 20 months CMAP amplitudes are reduced (36.1±6.1mV) ($p<0.05$). Similarly, motor unit number estimation is stable at 10 months (341±93), 13m(302±98), 15 months(314±51) and 17 months (271±51), but beginning at 20 months, motor unit number estimation shows reduction (225±77) ($p<0.05$). Forelimb grip was reduced at 24 months (95±15g, $p<0.05$) compared with baseline measures at 15 months (124±21). However, all limb grip did not show significant reduction. Compared to baseline MRI volume at 17 months (21.05±1.11mm^3), measures at 19 months (19.46±1.85mm^3) were unchanged, but reduced at 25 months (18.97±1.02 mm^3, $p<0.05$). Histology of motor neurons, muscle, and axons is in progress to explore the linkage between functional and cellular loss. Our data demonstrate that motor unit loss occurs prior to deficits in grip strength, suggesting that motor unit loss may be an important and early determinant of aging-related sarcopenia. Deeper understanding of the neurological mechanisms of sarcopenia will enable development of effective treatment for this prevalent condition in aging demographics.

Disclosures: K. Sheth: None. **V.L. McGovern:** None. **K.M. Corlett:** None. **A. Bratasz:** None. **A. Burghes:** None. **W. Arnold:** 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; NICHD/NIH.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.08/WW6

Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant R43NS093651

NIH Grant R44NS077526

Delsys, Inc

Title: Grouping and averaging motor unit data obscures the adaptations of motor unit firing rates during muscle fatigue

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Abstract: Muscle fatigue is accompanied by adaptations in the firing rate and recruitment behavior of motor units. Both our own [1] and independent studies [2] have reported that, as a muscle fatigues during repeated or sustained sub-maximal voluntary contractions, active motor units increase their firing rates and new motor units are recruited. Yet these adaptations remain disputed by some [3] who report the opposite changes in firing behavior: namely that motor unit firing rates decrease with fatigue. Such differing results are typically reported in studies that group motor unit data across different subjects, contractions, and force levels. Although this practice has been suggested to obscure the firing behavior of individual motor units [4], the consequences of grouping motor unit data during fatigue have never been empirically investigated. Therefore we set out to evaluate the influence of different practices of analyzing motor unit data on the observed firing behavior during a fatigue protocol of repeated voluntary isometric contractions of the Vastus Lateralis muscle. When we assessed the behavior of individual motor units in individual subjects, we found that motor unit firing rates progressively increase during fatigue. But when the same motor unit data was analyzed using three different practices commonly applied in fatigue studies, we found that: 1) grouping and averaging motor unit firing rates within a contraction falsely indicates that the firing rates decrease with fatigue; 2) grouping motor unit data across subjects obscures specific adaptations in firing behavior unique to each subject; and 3) analyzing motor unit firing rates as a function of recruitment threshold falsely indicates that firing rates decrease due to the fatigue-induced decrease in recruitment threshold [5]. To mitigate this error we analyze the firing rates of individual motor units as a function of action potential amplitude. Each of these practices proved to obscure the actual increase in the firing rate among individual motor units as the muscle fatigued. We have previously explained the increase in firing rates as the result of increasing excitation to the

motoneuron pool to compensate for the diminishing force twitch of the muscle [1]. Contrary to opposing explanations, ours is supported by empirical observations of the actual firing behavior of individual motor units. References: [1] **Adam and De Luca.** *J Appl Physiol*, 2005. [2] **Mettler and Griffin.** *Exp Brain Res*, 2016. [3] **Enoka et al**. *J Neurophysiol*, 1989. [4] **De Luca and Contessa**. *J Neurophysiol*, 2012. [5] **Adam and De Luca.** *J Neurophysiol*, 2003.

Disclosures: J.C. Kline: None. **C.J. De Luca:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Delsys, inc. **P. Contessa:** None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.09/WW7

Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant R43NS093651

NIH Grant R44NS077526

Delsys Inc.

Title: Fatigue is characterized by a compensatory interaction between motor unit firing behavior and muscle force

Authors: *P. CONTESSA[1], C. DE LUCA[2,1], J. KLINE[1];
[1]Delsys Inc, Natick, MA; [2]Boston Univ., Boston, MA

Abstract: Previous studies have reported various adaptations in motor unit firing behavior during muscle fatigue, including a decrease [1] and an increase [2] in motor unit firing rates. The inconsistency among previous studies is derived primarily from the limited number (typically 1-5 per contraction) of motor units available for analysis using intramuscular electromyographic (EMG) techniques, and from the practice of grouping motor unit data from different subjects and contractions.
To establish a more clear understanding of the adaptations of motor unit firing rates during fatigue, we investigated the firing behavior of motor units in individual subjects and contractions during a fatigue protocol in the Vastus Lateralis muscle. Five healthy young (24-33 years old) subjects performed voluntary isometric contractions sustained for 60 s at a target level of 30% maximal voluntary contraction force and repeated to the endurance limit. Surface EMG decomposition technology [3] provided the firing behavior of 1,890 motor units. From these data

we found that to perform the target contractions as the muscle fatigued: 1) motor unit firing rates increased; 2) new motor units were recruited; and 3) motor unit recruitment thresholds decreased. The degree of the adaptations was subject-specific, but the behavior was consistent in all subjects. Importantly, in spite of these adaptations, the fundamental motor unit control scheme did not change. Earlier-recruited motor units displayed higher-firing rates than later-recruited ones in all contractions, indicating the Onion-Skin property of motor unit firings [4] was maintained during fatigue. The direct relationship between motor unit recruitment thresholds and action potential amplitude, known as the Size Principle [5], was also preserved throughout the fatigue protocol.

We compared our empirical findings with those obtained from simulation of the fatigue protocol [6]. The agreement between our empirical and simulated data indicated that the fatigue-induced adaptations in motor unit firing behavior can be explained by adjusting the operating point of the excitation to the motoneuron pool to compensate for the decrease in muscle force-twitch that is reported to occur during fatigue [7]. Yet, throughout these complementary adaptations, the control properties that govern motor unit firing behavior remained invariant with muscle fatigue.

References:

[1] **Kelly et al.** *J Neurophysiol* 2013
[2] **deRuiter et al.** *Eur J Appl Physiol* 2005
[3] **Nawab et al.** *Clin Neurophysiol* 2010
[4] **De Luca and Erim.** *Trens Neurosci* 1994
[5] **Henneman.** *Science* 1957
[6] **Contessa and De Luca.** *J Neurophysiol* 2013
[7] **Adam and De Luca.** *J Appl Physiol* 2005

Disclosures: **P. Contessa:** None. **C. De Luca:** Other; Delsys Inc.. **J. Kline:** None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.10/WW8

Topic: E.10. Motor Neurons and Muscle

Support: NSERC RGPIN-2014-06076

CIHR 0040678

Title: Motor point stimulation as a tool to modify spinal neural circuits

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Abstract: The motor point is the most sensitive location to generate contractions as it contains a high density of terminal motor axon branches when delivering transcunateous electrical stimulation over the muscle belly. It is known that motor point stimulation (MPS) induces a different recruitment curve from those generated by peripheral nerve stimulation (PNS). For example, when PNS is applied to the tibial nerve at the popliteal fossa, progressive increases in stimulation intensity results in the emergence and growth of the soleus H-reflex, concurrent with the M-wave, followed by H-reflex plateau and subsequent decline, while the M-wave amplitude increases unabated; Such reductions in H-reflex amplitude with increases in stimulation intensity are thought to be due to occlusion by back propagating (antidromic) action potentials along motor axons. In contrast, when MPS is applied to the ankle plantarflexors, progressive increases in stimulation intensity most commonly results in the emergence and growth of the M-wave, unaccompanied by the H-reflex. This implies that for a given stimulation intensity, MPS activates a substantially greater proportion of motor versus sensory axons compared to PNS. Such preferential motor axon activation during MPS may represent a unique modality to investigate and modify spinal neural circuitry via the antidromic volley. For example, our previous study showed that, when PNS was applied to the plantarflexors at various inter-stimulus intervals relative to MPS, the H-reflex was strongly inhibited with inter-stimulus intervals below 200 ms, suggesting that the MPS evoked antidromic volleys caused strong inhibition of the H-reflex through spinal circuits and not through direct antidromic occlusion. Here, we applied MPS to modify spinal circuitry via spike timing-dependent plasticity (STDP). Participants were seated on a chair with a torque sensor measuring ankle plantarflexion torque. PNS and MPS were repetitively applied 100 times at a specific inter-stimulation interval, where the antidromic firing of MPS was calculated to invade the motoneurone cell body 2-ms before Ia-afferent activation induced by PNS (estimated H-reflex inhibitory latency according to STDP). Comparing pre- and post-recruitment curves, the maximum amplitude of the H-reflex was reduced by 26%. On the contrary, when PNS or MPS alone was applied, there were no changes in the maximum amplitude of the H-reflex. These results suggest that paired associative stimulation involving PNS and MPS successfully modified spinal H-reflex circuitry. As such, we believe that MPS provides a unique modality to investigate and modify spinal neural circuitry.

Disclosures: T. Yamashita: None. A. Berguquist: None. T. Yoshida: None. K. Masani: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.11/WW9

Topic: E.10. Motor Neurons and Muscle

Title: Task dependent change in Rectus Femoris activity in normal participants

Authors: *P. SRIYA, T. RICHARDS, S. ASTILL, S. CHAKRABARTY;
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Abstract: The Rectus Femoris (RF) muscle is a bi-articular muscle that performs both leg flexion and extension. Given this, the RF, along with the vastus lateralis (VL) and medialis (VM) must exhibit a task dependent shift in their role during leg flexion and extension. Thus the aim of this study was to examine if RF, VL and VM act as agonists during a simple isometric task. Surface electromyography (sEMG) were recorded from Rectus femoris (RF), Vastus medialis (VM), Vastus lateralis (VL), and Semitendinosus (ST) simultaneously in 2 healthy young males while they performed isometric contractions of Quadriceps muscles at four different angles (0, 20, 60, 90 degrees). Activity in these muscles was compared using amplitude and waveform correlations, allowing quantification of the RF- VL, RF-VM and RF-ST interactions at the knee. Preliminary results suggest activity in RF increased with that in VL and VM at both 20 and 60 degrees but not at 0 or 90, suggesting it acts as an agonist at 20 and 60 but not at 0 and 90 degrees. However, ST and RF did exhibit antagonistic interactions at 0 and 20 degrees while being similar at 90 degrees. This might suggest that local interactions between RF and the other muscles are task dependent rather than being defined by their anatomical arrangement.

Disclosures: P. Sriya: None. T. Richards: None. S. Astill: None. S. Chakrabarty: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.12/WW10

Topic: E.10. Motor Neurons and Muscle

Support: NSERC

Title: Characterization of arm muscle activity during arm cycling at different workloads

Authors: *C. P. CHAYTOR[1], M. R. MONKS[1], D. A. FORMAN[1], J. M. BYRNE[1], A. LOUCKS-ATKINSON[1], K. E. POWER[1,2];
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Abstract: Arm cycling is commonly used in rehabilitation settings for individuals with upper- and/or lower-limb motor impairments. It is thought to induce neural plasticity that may lead to

increases in motor function in the affected limb(s). Arm cycling studies typically use absolute workloads for all participants whereas it is standard practice in studies using isometric contractions for participants to contract at relative intensities based on their isometric maximal voluntary contraction. This allows comparison between participants based on relative force outputs. The same does not occur during arm cycling studies. Thus, the objective of this study was to characterize arm muscle activity during arm cycling at different relative workloads. Participants (n=11) completed a 10-second maximal arm ergometry sprint to determine peak power output (PPO) followed by 11 randomized trials of 20-second arm cycling bouts ranging from 5-50% of PPO (5% increments) and a standard 25W workload. Electromyography (EMG) was recorded from the biceps brachii and triceps brachii bilaterally in 11 participants. Ln-ln regression models indicated a slight ns difference in the slopes for biceps brachii between flexion and extension, but not for triceps brachii. RM ANOVA models on ln-EMG found a positive highly significant main effect of workload ($p < .001$) up to 35% but did not differ between flexion and extension for either biceps brachii ($p = .143$) or triceps brachii ($p = .089$) above 35% of PPO. lnEMG was significantly larger in flexion than extension for biceps brachii ($p < .001$) but not triceps brachii ($p = .306$). These results demonstrate that there is a linear relationship between lnEMG and workload up to 35% of PPO in both biceps and triceps brachii. In addition, the slopes of the lnEMG/PPO relationship are not different between phases (i.e. flexion and extension) as intensity increases up to 35% in the biceps brachii and triceps brachii.

Disclosures: C.P. Chaytor: None. M.R. Monks: None. D.A. Forman: None. J.M. Byrne: None. A. Loucks-Atkinson: None. K.E. Power: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.13/WW11

Topic: E.10. Motor Neurons and Muscle

Support: Conacyt Grant LO591506

Conacyt Grant LM207849

Title: Neuronal circuits involved in the reflex activity of pubococcygeus muscle motoneurons of female rats

Authors: *O. LARA GARCIA[1], M. LARA GARCÍA[2,3], D. PÉREZ HERNÁNDEZ[2], M. MARTÍNEZ-GÓMEZ[3,4], E. CUEVAS[3], P. PACHECO[2,4];
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Abstract: Neuronal circuits which activity depends of hormonal actions participate in multifunctionality of pelvic striated muscles involved in micturition, defecation and copulatory behavior of female mammals. Thus, pubococcygeus muscle (Pcm) reflexively responds to perigenital tactile stimulation, particularly to pressure of the clitoris and during proestrous but diestrous conditions their motoneurons actively respond with intense after-discharges. Hence, in order to identify the neuronal components involved in its motoneuronal activity, we decided to use EMG multiunit activity recordings (EMA) during clitoral pressure or by electrical stimulation of the dorsal nerve of the clitoris (DNC). In i.p. urethane anesthetized adult female Wistar rats the EMA from both Pcms were bilaterally recorded with bipolar stainless steel electrode (0.1 mm diameter). The DNC stimulation was done through bipolar stainless steel electrodes. Results: Slow sweeps recordings showed that the EMA was compound of discharges with different amplitudes and frequencies. During clitoris stimulation a phasic "on", tonic "on", phasic "off" and tonic "off" (after discharge) were obtained. Occasionally, it was possible to record the axon and its corresponding innervated muscular fiber potentials which presented 1.2 msec of separation. Fast sweeps showed that discharges recorded after cesation of clitoris pressure were compound of groups of potentials with different discharge duration. Stimulation of the DNC evoked 8 and 10 msec latency potentials in the ipsi and contralateral Pcms respectively. Pulses at 10, 20 and 30 per sec produced synchronized and non-synchronized responses of both muscles. Discussion: Present results show that the Pcm motoneurons reflexively respond with a single or repetitive activity through interneuronal excitatory actions. The latency obtained by the DNC stimulation suggests that clitoris activates Pcm motoneurons through circuits that could be implicating the sensory neuron and 3-4 interneurons. It is likely the existence of two interneuronal polysynaptic circuits, one related with the phasic "on" and "off" responses and the other with the repetitive tonic "on" and "off" responses. Furthermore, the prolonged hyperactivity recorded could represent hormonal influence on this reflex. Conclusion: The EMA signals obtained represent multiple and concurrent motor unit activity that provides information concerning Pcm motoneuronal grouped activity present in: a) postural maintenance of the tail in the midline during copulatory behavior; b) intravaginal pressure increase during mating; and c) opening facilitation of the urethra during micturition.

Disclosures: O. Lara Garcia: None. M. Lara García: None. D. Pérez Hernández: None. M. Martínez-Gómez: None. E. Cuevas: None. P. Pacheco: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.14/WW12

Topic: E.10. Motor Neurons and Muscle

Title: Sex differences in the control of vastus medialis and vastus medialis oblique

Authors: Y.-L. PENG[1], M. S. TENAN[2], *L. GRIFFIN[1];
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Abstract: It is unresolved as to whether the vastus medialis (VM) and vastus medialis oblique (VMO) can be controlled independently, though they are known to have different pennation angles and possibly different functions. Weakness of the VMO relative to the vastus lateralis in women has been proposed to explain the high prevalence of knee pain in women compared to men. VMO training is commonly recommended in clinical practice, though it is unclear whether the VMO can be activated preferentially from the VM. The aim of this study is to determine if VM and VMO activation is varied between sexes and if control of the two muscles is different between rehabilitation exercises. Thirteen men and thirteen women performed isometric straight leg raises in two hip positions, neutral hip rotation (SLR-NR) and 30 degrees hip lateral rotation (SLR-LR). Participants performed isometric ramp contractions at a rate of 7.5% maximal voluntary contraction (MVC) per second up to 75% MVC. Bipolar intramuscular fine-wire electrodes were inserted into the VM and VMO and single motor unit action potentials were delineated. Recruitment threshold forces (RT) and initial motor unit firing rates (IFR) were measured. Linear mixed models and Tukey post hoc tests were used to assess significant differences. A total of 654 motor units were analyzed. There were significant main effects of Muscle ($p = 0.03$) and Task ($p < 0.01$) for RT. Holding all other factors constant, the VMO was recruited earlier than the VM (11.9 ± 1.2 vs. 14.8 ± 1.2 % MVC). Motor units were recruited earlier during SLR-NR compared to SLR-LR (11.5 ± 1.2 vs. 15.2 ± 1.2 % MVC). Significant main effects of Task ($p = 0.01$) and Sex ($p = 0.04$) were found for IFR. Motor units were recruited 0.5 Hz ± 0.2Hz faster ($p = 0.01$) during SLR-NR compared to SLR-LR. Women activated their motor units 1.2 Hz ± 0.6 Hz faster than men ($p < 0.05$). Thus, the VM and the VMO can be activated differentially and their motor unit recruitment properties are affected by sex and exercise type.

Disclosures: Y. Peng: None. M.S. Tenan: None. L. Griffin: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.15/WW13

Topic: E.10. Motor Neurons and Muscle

Title: Pattern of masseter muscle activity during chewing with dental implants

Authors: *A. GRIGORIADIS, M. TRULSSON;
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Abstract: Objectives
Jaw actions adapt to the changing properties of food that occur during a masticatory sequence. In the present study, we investigated how the time-varying activation profile of the masseter muscle changes during natural chewing and how it is affected by food hardness in subjects with implant-supported bridges.
Methods
We recorded surface electromyography of the masseter muscle together with the movement of the lower jaw in ten participants with implant-supported bridges in both jaws (mean age 67) and ten with natural dentition (mean age 71) when chewing gelatin-based model food of two different hardness. The muscle activity and the jaw kinematics were analyzed for the different phases of the chewing cycles in the beginning (three cycles) of the masticatory sequence.
Results
For the dentate group, the increase in the excitatory drive of the masseter muscle was biphasic during the jaw-closing phase showing an early and a late component. The transition between the two components occurred approximately at the time of tooth-food contact. The implant group, on the other hand, lacked to late component in the beginning of the masticatory sequence. Except for amplitude scaling, food hardness did not appreciably affect the muscle's activation profile for any of the groups.
Conclusions
When chewing food during natural conditions, subjects with implant-supported bridges lack the ability to generate a late excitatory component at food contact in the beginning of the masticatory sequence. We propose that the absence of tooth-food contact information from periodontal mechanoreceptors lead to this impairment. The temporal profile of muscle activity was virtually identical when chewing hard and soft food for both groups, implying that the principal effect of food hardness was due to scaling in the magnitude of activity.

Disclosures: A. Grigoriadis: None. M. Trulsson: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.16/WW14

Topic: E.10. Motor Neurons and Muscle

Support: University of Wisconsin-Milwaukee CHS Graduate Student Research Grant

Title: Differences in force steadiness and EMG amplitude during a dorsiflexion steadiness task in young and older adults

Authors: *J. J. PETERSON, K. G. KEENAN;
Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Background: Functional limitations, including walking, climbing stairs, and maintaining balance, become increasingly prevalent as adults age. Performance on force steadiness tasks has been related to functional limitations in older adults. However, the underlying neural mechanisms driving the change in performance with aging are unclear. Changes in electromyogram (EMG) amplitude may be related to performance, and although EMG amplitudes are frequently increased in older vs. young adults, few studies examine the relationship between force steadiness and increased EMG amplitude in older adults. This may be due to the inherent variability of using single bipolar EMG to estimate muscle activity. High-density surface EMG can increase the accuracy of muscle force estimation compared to single bipolar EMG. Therefore, the purpose of our study was to examine the differences in force steadiness and EMG amplitude between young and older adults during a 5% MVC dorsiflexion task, and to examine the relationship between force steadiness and EMG amplitude.
Methods: 18 young (26.5 ± 4.7 yrs) and 18 older (74.1 ± 7.4 yrs) adults performed a dorsiflexion task while seated on a bench with the left knee extended and ankle in a neutral position. Participants performed a series of MVCs followed by two 30 s trials at 5% MVC. Performance was quantified as the coefficient of variation of force. High-density surface EMG was collected from tibialis anterior (TA) using a 64-channel electrode (OTBioelettronica, Torino, IT). EMG recordings were visually inspected, rectified, normalized to MVC, and averaged across channels. Results reported as mean ± standard deviations.
Results: Force fluctuations increased for the older adults compared with the young adults (4.2 ± 2.0% and 2.2 ± 1.2%, respectively, $p = .008$). EMG amplitude increased for the older adults compared with the young adults (15.7 ± 5.5% and 9.5 ± 2.8%, respectively, $p < .001$). Moreover, there was a positive relationship for older adults between the coefficient of variation of force and EMG amplitude during the steadiness task ($R^2 = .36$; $p = .009$). The relationship was not significant for young adults ($p = .16$).
Conclusions: Over-activation of TA was related to worse performance on a dorsiflexion force

steadiness task in older adults. As previous studies using single bipolar EMG electrodes have failed to report this relationship, high-density surface EMG may be more accurately representing global muscle activity and its changes with advancing age. Future work should examine if the over-activity in TA in older adults is due to malfunction within the neuromuscular system or a compensatory mechanism to improve performance.

Disclosures: J.J. Peterson: None. K.G. Keenan: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.17/WW15

Topic: E.10. Motor Neurons and Muscle

Title: Precision and accuracy in human motor control

Authors: *A. KUMAR[1], Y. TANAKA[2], J. GRIGORIADIS[1], M. TRULSSON[1], P. SVENSSON[1];
[1]Karolinska Institutet, Huddinge, Sweden; [2]Dept. of Prosthodontics, Gerodontology and Oral Rehabil., Osaka Univ. Grad. Sch. of Dent., Osaka, Japan

Abstract: Background: Accuracy is measured by the degree of closeness of measurements of a quantity to that quantity's true value. While, precision is related to reproducibility and repeatability. We have compared the capacity to maintain an accurate and precise isometric contraction force with the teeth and fingers.
Aim: To investigate if targeting two different force levels with teeth and fingers affect the accuracy or precision of task performance and further investigate the optimization of target forces in terms of accuracy and precision due to repetition of the task.
Methods: Eighteen healthy volunteers (mean age 27 ± 4) participated in two experimental sessions. During each session, the participants were asked to target two different force levels (0.5 N and 5 N; randomized; displayed on a computer screen) by holding a force transducer with their anterior teeth or with their thumb and forefinger. During a single trial, the participant held the force transducer with a steady force (hold force) of either 0.5/5 N for about 3-5 s and subsequently increased the rapid ramp force to target a peak of 10 N (peak force). The accuracy (i.e., deviation from the target force) and the precision (i.e., coefficient of variation) were calculated and compared across the finger and teeth and across the different force levels. The participants performed 10 series of the task (with 10 trials in each series) with a total repetition of 400 trials during the entire experiment.
Results (preliminary): The deviation from the target (accuracy) for the hold force was

significantly higher when the participants targeted 0.5 N with teeth than when targeting 0.5 N with the finger ($P<0.001$) or 5 N with both the finger and the teeth ($P<0.001$). Further, the deviation from the target was significantly higher in all the series when targeting a hold force of 0.5 N than all the series when targeting 5 N ($P<0.001$). Moreover, the deviation from hold force (5 N) or the peak force (10 N) was significantly higher during the first series than the subsequent last five series ($P<0.001$). However, there were no significant interaction when the precision of task performance (assessed in terms of coefficient of variation) was analyzed for the teeth or finger with two different hold force levels and peak force.
Conclusion: Different force levels affect the accuracy but not the precision of task performance further the optimization of target forces is more obvious when targeting higher forces (5 N) than lower forces (0.5 N). Although the two words precision and accuracy can be synonymous in colloquial use, they could be deliberately distinguished in the context of the scientific method.

Disclosures: A. Kumar: None. Y. Tanaka: None. J. Grigoriadis: None. M. Trulsson: None. P. Svensson: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.18/WW16

Topic: E.10. Motor Neurons and Muscle

Support: NSERC

Title: Characterization of arm muscle activity during asynchronous and synchronous arm cycling using different forearm positions

Authors: *A. P. HYNES[1], C. P. CHAYTOR[1], L. R. ALCOCK[1], D. C. BUTTON[1,2], K. E. POWER[1,2];
[1]Sch. of Human Kinetics and Recreation, Mem. Univ. of Newfoundland, St John's, NL, Canada; [2]Fac. of Med., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: The purpose of this study was to characterize the muscle activation of the arm during arm cycling using different modes (i.e. asynchronous and synchronous) and forearm positions (i.e. pronated, neutral and supinated). Ten participants attended two sessions: (1) a familiarization session and (2) a testing session consisting of a warm-up followed by 12 randomized cycling trials (flexion/extension x ASY/SYN x pronated/neutral/supinated). The average rectified electromyography (EMG) of the biceps brachii (BB) and triceps brachii (TB) was recorded from the dominant arm throughout arm cycling and analyzed. Made relative to a

clock face with reference to the elbow joint, flexion was defined as from 3 to 9 o'clock as the hand crank moved towards the body and extension from 9 to 3 o'clock as the hand crank moved away from the body. Separate three-way ANOVAs were performed for BB and TB. Significant main effects for biceps brachii EMG were observed based on cycling phase (flexion > extension, p=0.001), forearm position (supinated > neutral > pronated, p=0.009) and cycling mode (synchronous > asynchronous, p=0.01). Within the flexion phase of arm cycling, BB EMG was higher as the forearm position moved from a pronated to a supinated position. There was no significant difference between the average rectified EMG in the neutral and supinated positions (p=0.536), however, both were significantly greater than the pronated position (supinated vs pronated, p=0.033; neutral vs pronated, p=0.005). BB EMG was significantly greater during synchronous compared to asynchronous arm cycling (p=0.01). There was no effect of forearm position or cycling mode on BB EMG during the elbow extension phase of arm cycling. TB EMG was not affected by phase, mode or forearm position. Though mechanisms associated with the observed changes in EMG were not assessed in the present study, the higher EMG of the BB as the forearm changes from a pronated to supinated position may reflect its biomechanical advantage as an elbow flexor as the forearm becomes supinated. Higher EMG during synchronous versus asynchronous arm cycling may reflect changes in motor control strategies associated with supraspinal and/or spinal factors.

Disclosures: A.P. Hynes: None. C.P. Chaytor: None. L.R. Alcock: None. D.C. Button: None. K.E. Power: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.19/WW17

Topic: E.10. Motor Neurons and Muscle

Title: Chronaxie of motor responses to S3 sacral nerve stimulation in sheep

Authors: D. DINSMOOR, M. CUTINELLA, J. AGRAN, *X. SU;
Medtronic, Minneapolis, MN

Abstract: Sacral neuromodulation (SNM; InterStim® therapy) at the third sacral foramen (S3) is an FDA-approved therapy for urinary urge incontinence, urgency-frequency and fecal incontinence. The recommended pulse width for SNM is 210 μs, but the strength-duration (SD) response developed from S3 neurostimulation has not been fully elucidated in the literature to-date. Using fully implanted, commercialized systems in a SNM sheep model, we evaluated electromyography (EMG) of the external anal sphincter (EAS) across different pulse widths.

Seven trials of six S3 nerve roots from four adult, female Polypay sheep were used for this study. Under propofol anesthesia (i.v., 6 mg/kg) in combination with 1-3% isoflurane, sheep were implanted with stimulation leads (Medtronic Model 3889) at S3 and two sensing electrodes (Medtronic Model 4351) at both the 3 and 9 o'clock positions of the EAS. The motor responses to SNM with different stimulation pulse widths were randomly examined using variable intensities from 0.1 V to 10 V. Stimulations triggered the EAS contractions. Response thresholds were obtained from both visual detection and EMG waveform analysis against the stimulus intensity. The strength-duration responses as ascertained both visually and from the EAS EMG were fitted with a monoexponential nonlinear regression; the resulting time constants (chronaxie) were of 74.35 ± 0.001 μs and 62.03 ± 0.001 μs, respectively. The rheobase values were 0.48 ± 0.29 V and 0.48 ± 0.12 V, respectively. The maximal values to minimal pulse width were 1.85 ± 0.39 V and 1.62 ± 0.22 V, respectively. These data suggest that a similar motor response may be evoked in the EAS at pulse widths much shorter (62 μs - 74 μs) than the 210 μs typically used with SNM. Shorter pulse widths translate directly to increased energy savings in the neurostimulator. Future studies are needed, however, to determine if the therapeutic efficacy of SNM in patients with incontinence at shorter pulse widths is equivalent to that at 210 μs.

Disclosures: **D. Dinsmoor:** A. Employment/Salary (full or part-time): Medtronic. **M. Cutinella:** A. Employment/Salary (full or part-time): Medtronic. **J. Agran:** A. Employment/Salary (full or part-time): Medtronic. **X. Su:** A. Employment/Salary (full or part-time): Medtronic.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.20/WW18

Topic: E.10. Motor Neurons and Muscle

Support: PRIN grant

COREA grant

Title: Emg regression as a measure of the contribution of central signals to force perception

Authors: ***S. TOMA**[1], F. LACQUANITI, 00173[2];
[1]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ. - Tempe Campus, Tempe, AZ;
[2]Systems Med., Univ. of Rome Tor Vergata, Roma, Italy

Abstract: It has been largely established that force estimation mainly relies on motor commands underlying force production, i.e., the so-called 'sense of effort'. Despite the large body of work investigating the relative contribution of descending and ascending signals in force perception, very few attempts have been made to link a measure of neural output (i.e., electromyography, EMG) to psychophysical performance. We propose that the strength of correlation between EMG activity amplitude and perceptual estimates can be interpreted as an estimate of the contribution of central signals to force perception. The current study was designed to quantify this correlation by recording EMG activity from eight arm muscles while participants performed a quasi-isometric force detection task. We used a method to quantify muscular activity ("*muscle-metric function"*) that could be directly compared to the description of participants' psychophysical judgment about the stimulus force. The muscle-metric curve was obtained by means of muscles averaged activity logistic regression. We found that muscle-metric absolute thresholds and the shape of the muscle-metric curves were closely related to those provided by the psychophysics. Moreover, the logistic regression of the distribution of just 3 of the 8 muscles considered was able to predict approximately 60% of the perceptual decisions total variance. Another important result is that the inter-subjects differences in participants perceptual absolute threshold revealed strong correlation with the thresholds extracted from the muscles-metric curve and participants' joint torques. Overall, our findings provide insights into the role played by descending motor commands in the performance of a force estimation task.

Disclosures: S. Toma: None. F. Lacquaniti: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.21/WW19

Topic: E.10. Motor Neurons and Muscle

Support: NIH Grant 5T32EB004314-15

Title: Static synergy patterns used to model time-varying functional task muscle activation of the hand.

Authors: *N. M. COLE[1,2], A. B. AJIBOYE[1,2];
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Abstract: A long standing theory of motor control is that the neuromotor system simplifies control of multi-degree-of-freedom (DOF) movements by recruiting coordinated patterns of

muscle activation (muscle synergies), which are combined to produce motor output. Previous studies have extracted synergy patterns from animal models and human subjects. It has been shown that muscle synergies can be extracted from a subset of hand postures and used to reconstruct a larger variety of hand shapes. The hypothesis of this study was that the multiple degrees of freedom in muscle activation in the human hand can be reduced to a set of static synergistic activations from a sample of electromyographic (EMG) data and those synergies can be scaled to produce a variety of time-varying functional output. In able-bodied persons, we recorded EMG from twelve intrinsic and extrinsic hand muscles. Each subject performed static hand postures and dynamic functional tasks with object manipulation. Synergies were extracted from average EMG recordings during static hand postures using a cross-validated NNMF decomposition algorithm. For each subject, the synergy model was used to reconstruct time-series EMG data. It has been concluded that a static synergy model extracted from hand postures can explain the temporal changes in muscle activation during functional tasks.

Disclosures: N.M. Cole: None. A.B. Ajiboye: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.22/WW20

Topic: E.10. Motor Neurons and Muscle

Support: Craig Neilsen Foundation Grant #128256

Title: Neural control of forelimb locomotor behaviors

Authors: A. ISLAM, T. PENG, ***P. SHAH**;
Stony Brook Univ., Stony Brook, NY

Abstract: Abundant evidence has established the existence of shared neural circuitry for different rhythmic hindlimb locomotor behaviors. However, whether common neural elements also control rhythmic forelimb locomotor movements remains largely unexplored. Understanding the neurophysiology of different functional forelimb motor behaviors will prove valuable in strategizing therapies for the recovery of hand function after neurotrauma. The primary goal of the present study is to determine the neuronal motor control of rhythmic forelimb movements - treadmill stepping (TM), overground walking (OG) and horizontal ladder walking (LD) - in adult rats. We hypothesized that neural mechanisms controlling rhythmic walking on the TM would be similar to that documented during OG and LD walking. Healthy adult rats were implanted with EMG electrodes in the deltoid (DEL), bicep, pronator, flexor

digitorum super-ficialis, and extensor digitorum (ED). Kinematic features of locomotor behaviors (TM, OG and LD) were captured using an advanced 3D Motion Analysis system while collecting EMG data. Burst characteristics (mean and peak amplitudes, burst duration), patterns and timing of muscle activity were analyzed from raw EMG signals. Cross-correlogram histograms examined synchronization of motor unit activity between TM and OG (low speed TM vs high speed, LSHS; low speed TM vs OG, LSOG; high speed TM vs OG, HSOG) for the DEL and ED. Bandpass EMG signals generated from one step cycle from each of the locomotor behaviors were used to construct correlogram histograms (total of ten individual steps). Our preliminary data show that the burst duration and amplitude between the three behaviors (TM, OG and LD) vary between muscles, but the order of muscle activation patterns is preserved such that there is a highly sequential pattern of proximal to distal muscle activation during TM, OG and LD. Additionally, center peaks appeared in the histograms for all correlation analyses. The width of the central synchronous peak in the cross-correlation histogram was broader in the DEL muscle than the ED between LSHS (average ± SD peak width = 31± 4.82ms vs 14.56± 2.59ms), LSOG (34.56 ± 3.97ms vs 29.33 ± 10.34 ms) and HSOG (33.22 ± 3.14ms vs 24 ± 5.91ms) conditions. These findings reveal that there is some degree of common neuronal drive to motoneurons during the TM and OG walking conditions. Moreover, there is also greater relative contribution of direct common inputs to the ED motoneurons during the two behaviors in comparison to the DEL. These data are novel and suggest that common neuronal drive regulates rhythmic forelimb locomotor behaviors in awake adult rats.

Disclosures: A. Islam: None. T. Peng: None. P. Shah: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.23/WW21

Topic: E.10. Motor Neurons and Muscle

Support: Swedish Dental Society

Karolinska Institutet

Title: Impaired oral motor control due to anesthesia during intraoral manipulation of food

Authors: *J. GRIGORIADIS, A. KUMAR, P. SVENSSON, K. SVENSSON, M. TRULSSON; Dept. of Dent. Med., Karolinska Institutet, Huddinge, Sweden

Abstract: Background: Mastication is a complex intermittent rhythmic act in which the tongue, facial and jaw muscles act in coordination with each other, to position the food morsel appropriately in between the teeth, break it down into smaller pieces and prepare it for swallowing. The fine-tuned coordination of tongue, facial and jaw muscles is achieved by the ability of the central nervous system to receive and integrate sensory information from various types of orofacial mechanoreceptors, including the periodontal mechanoreceptors (PMRs). Sensory information from PMRs surrounding the roots of natural teeth is important for optimizing the positioning of food, force levels and adjustment of force vectors during precision biting.
Aim: The aim of the experiment was to test the hypothesis; if decreased inputs from the PMRs, due to anesthesia, would perturb the oral fine motor control and the related jaw movements, during the intraoral manipulation of food morsels.
Material and Methods: Thirty healthy volunteers (16 female; mean age 27 (range 19-40)) with a natural dentition were equally divided into experimental and control groups. The participants in both the groups were asked to manipulate and split a spherical chocolate candy (diameter: 10mm, weight: 0.84 ± 0.01g) into two equal halves (ideal split) with the anterior teeth, thirty times each before and after an intervention. The intervention was made in the experimental group by anesthetizing the upper and lower front teeth. The control group continued the performance of the behavioral task without any intervention. During the experiment we evaluated the performance of the split and recorded the vertical jaw movement, electromyographic activity of masseter muscle and sound related to the cracking of the candy. We assessed the performance i.e., deviation from ideal split and the duration of jaw movements during the tasks.
Results: Preliminary results indicate that the participants in the experimental group showed a significant decrease in performance ($P<0.001$) while no difference was observed in the control group ($P=0.566$). However, there were no robust changes in the duration of jaw movements, due to anesthesia ($P>0,050$).
Conclusion: Sudden deprivation of sensory information from PMRs impairs oral fine motor control during intraoral manipulation of food, however, no significant changes in duration of jaw movements where observed. Our results maybe important because we have previously observed smaller duration of jaw movements in oral prosthetic rehabilitated patients in comparison to natural dentate individuals.

Disclosures: J. Grigoriadis: None. A. Kumar: None. P. Svensson: None. K. Svensson: None. M. Trulsson: None.

Poster

250. Motor Unit Recordings: EMG

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 250.24/WW22

Topic: E.05. Brain-Machine Interface

Support: DARPA-FDA IAA 224-10-6006

FDA Critical Path Initiative CPOSEL13

Title: Assessing movement variability with quantitative motion capture in able-bodied individuals performing the Jebsen Taylor Hand Function Test

Authors: L. WOZNICZKA[1,2], S. WANG[2,3], *K. KONTSON[2], E. CIVILLICO[2];
[1]Univ. of Marquette, Milwaukee, WI; [2]U.S. Food and Drug Admin., Silver Spring, MD; [3]Univ. of Maryland, College Park, MD

Abstract: The Jebsen Taylor Hand Function Test (JHFT), which is widely used in clinical settings to assess hand function, consists of seven standardized tasks based on activities of daily living. Although originally developed for individuals with neurological and musculoskeletal conditions, JHFT has also been utilized in the upper limb amputee population to evaluate rehabilitation progress and functional performance. Currently, the standard endpoint for each JHFT task is completion time. For upper limb prosthetic users, it is also important to assess *how* the subject performs each task: the completion time endpoint for JHFT is not sufficient to evaluate the potential compensatory movements (CMs) employed by subjects due to the loss of distal degrees-of-freedom in the arm. Through the use of motion capture technology, quantitative information on how the subject moves (i.e. movement quality) can be used to measure performance in a way that is potentially more clinically meaningful than completion time alone. To better understand the applicability of motion capture and variability in movement quality for each task in able-bodied individuals, five able-bodied subjects were recruited for a pilot study. Using a Vicon motion capture system with eight Bonita B10 infrared cameras, 27 markers on the upper body of each subject were tracked. Each subject performed two trials of the seven JHFT tasks with the dominant hand. Inter-subject variability for each task was determined by normalizing all subject trials with respect to completion time and calculating the maximum standard deviation for a particular task. The following angles were analyzed: wrist flexion, ulnar deviation, and internal rotation; elbow flexion; shoulder flexion, abduction, and internal rotation. Preliminary results indicate that shoulder flexion and abduction varied greatly between subjects during Task 1 (writing) and Task 5 (stacking checkers). There was high variability in shoulder abduction, but not shoulder flexion, while subjects performed Task 3 (picking up small objects) and Task 4 (simulated feeding). Wrist angles and elbow flexion were fairly consistent across all subjects for all of the JHFT tasks, with the exception of Task 2 (page turning). In future studies,

the utility of the motion capture in capturing compensatory movements will be assessed using able-bodied subjects performing these tasks with DOFs artificially reduced to more closely approximate the movements of an upper limb prosthesis user. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the DHHS.

Disclosures: L. Wozniczka: None. S. Wang: None. K. Kontson: None. E. Civillico: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.01/XX1

Topic: F.01. Neuroethology

Support: NIH Grant

Title: Principles of auditory processing in sensory and premotor structures of the songbird forebrain

Authors: *E. SOYMAN, D. S. VICARIO;
Rutgers Univ., Piscataway, NJ

Abstract: Sensory and motor brain structures are known to work in collaboration during speech perception, although their specific functions and the nature of the connectivity between them remain controversial. The present study exploits the special features of the songbird vocal communication system to explore the participation of auditory and vocal premotor structures in auditory processing of vocal signals. We simultaneously recorded the neural responses to auditory stimulation in both the higher-order auditory area NCM and premotor area HVC of the songbird brain in awake zebra finches (*Taeniopygia guttata*) using silicon probes to test multiple sites in each structure. Bird's own song (BOS) and various conspecific songs (CON) were presented in both blocked and shuffled sequences. The neural responses showed plasticity in the form of stimulus specific adaptation, although the dynamics were markedly different between the two structures. In NCM, the decrease in response with repetition of each stimulus was gradual and long-lasting. In contrast, HVC responses to CON stimuli decreased rapidly but transiently, as reflected by differential dynamics in the blocked versus shuffled stimulus presentation sequences. Responses to BOS in HVC decreased much more gradually than CON responses. Furthermore, the quality of neural representations and discrimination, as assessed by computing the mutual information between stimuli and neural activity, was higher in NCM than in HVC. Conversely, the internal functional connectivity, as analyzed by estimating the coherence

between the recording sites, was greater in HVC than in NCM. The cross-coherence between the two structures was limited to low frequencies (<18 Hz). An intriguing finding was that, in HVC, CON stimuli with high acoustic similarity to BOS elicited auditory responses that were more differentiated from BOS, compared to CON stimuli with low BOS-similarity. Our findings converge on the conclusion that auditory communication signals are processed according to very different, but complementary, principles in the two structures. NCM activity is governed by functionally heterogeneous neurons and/or neural subpopulations that reliably represent different parts of complex auditory stimuli at fine temporal resolutions; NCM thus is likely to play an important role in stimulus recognition and long-term memory formation. In contrast, HVC responses are globally synchronous and do not differentiate well between different stimuli; however, they show strong temporal and/or contextual sensitivity. These findings may inform study of the contributions of auditory and motor pathways to human speech processing.

Disclosures: E. Soyman: None. D.S. Vicario: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.02/XX2

Topic: F.01. Neuroethology

Support: NIDCD DC008854

Title: Effects of complex acoustic contexts on stimulus encoding in songbird auditory forebrain

Authors: *M. DONG, D. VICARIO;
Psychology, Psychology, Rutgers Univ., Piscataway, NJ

Abstract: An auditory stimulus can convey different information, depending on its timing and acoustic relationship to preceding stimuli. For example, oddball effects that depend on the relative frequency of two different stimuli have been demonstrated with simple sounds. The present study extends that approach to a novel paradigm that uses complex vocalizations from different acoustic categories (zebra finch and canary song syllables) as stimuli to investigate the effects of context-dependent encoding. We used multiple electrodes to record multi-unit neural activity in the auditory forebrain (Field L and area CLM) of awake male zebra finches in response to a sound presented in two different contexts. A zebra finch syllable (Target) was first presented in a sequence with 7 other zebra finch syllables as context; and then with 7 different canary syllables as context. All stimuli were played in a shuffled order with equal probability at an ISI of 1.2 secs.

The results show that the same Target syllable induced higher responses in the canary context than in the zebra finch context. This suggests that neural responses to a stimulus are "context"-dependent and is consistent with the stimulus-specific adaptation (SSA) hypothesis from oddball experiments. However, the current experiment differs from oddball experiments in two ways. First, most oddball experiments used pure tones as stimuli but we used complex stimuli. Second, oddball experiments using two stimuli show that a given stimulus elicits stronger neural responses as an oddball with low probability than as a standard with high probability. Our results show that neural responses differ depending on the type of context stimuli even though the probability of Target occurrence remains the same. This suggests an influence of the acoustic contrasts between syllables from different natural categories, independent of relative stimulus probability. Therefore, the current experiment expands our understanding of how the nervous system dynamically processes auditory information, which may be important for context-dependent tasks that depend on sequential information and use complex sounds, such as speech processing.

Disclosures: M. Dong: None. D. Vicario: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.03/XX3

Topic: F.01. Neuroethology

Support: NSERC 402417-2011

FRQ-NT PR-189949

Title: Mechanisms underlying the social enhancement of vocal learning in songbirds

Authors: *Y. CHEN, L. E. MATHESON, J. T. SAKATA;
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Abstract: Social processes profoundly influence speech and language acquisition. Despite the importance of social influences, little is known about how social interactions modulate vocal learning. Like humans, songbirds learn their vocalizations during development, and they provide an excellent opportunity to reveal mechanisms of social influences on vocal learning. Using yoked experimental designs, we analyzed the degree to which attention, multisensory stimulation, the structure of tutor vocalization, and reciprocal interactions mediated social influences on vocal learning. We demonstrated that social interactions with adult tutors for as

little as one day significantly enhanced vocal learning. Social influences on attention to song appeared central to the social enhancement of learning because socially tutored birds were more attentive to the tutor's songs than passively tutored birds and because variation in attentiveness and in the social modulation of attention significantly predicted variation in vocal learning. Attention to song was influenced both by the nature and amount of tutor song: pupils paid more attention to songs that tutors directed at them and to tutors that produced fewer songs. Tutors altered their song structure when directing songs at pupils in a manner that resembled how humans alter their vocalizations when speaking to infants, that was distinct from how tutors changed their songs when singing to females, and that could influence attention and learning. Furthermore, social interactions that rapidly enhanced learning increased the activity of noradrenergic and dopaminergic midbrain neurons. These data highlight striking parallels between humans and songbirds in the social modulation of vocal learning and suggest that social influences on attention and midbrain circuitry could represent shared mechanisms underlying the social modulation of vocal learning.

Disclosures: Y. Chen: None. L.E. Matheson: None. J.T. Sakata: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.04/XX4

Topic: F.01. Neuroethology

Support: NSERC 402417-2011

Title: Experimental evidence for innate biases in vocal sequencing learning

Authors: *L. JAMES, J. T. SAKATA;
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Abstract: Understanding how animals, including humans, learn and produce species-typical vocal sequences remains a fundamental question in neuroscience, ethology, and linguistics. Across languages, particular word and sound sequences are substantially more prevalent than others, and many have postulated that this results from innate sensory, motor, or cognitive biases (e.g., theories of universal grammar). However, there are no rigorous experimental tests of innate biases in vocal sequence learning across development. Zebra finches are a powerful animal model to experimentally assess innate predispositions underlying vocal sequence learning because they, like humans, learn their vocalizations during development. The songs of individual zebra finches consist of syllables arranged in a single stereotyped sequence (motif), and these

sequences vary extensively across individuals. In order to experimentally test the hypothesis that intrinsic biases guide vocal sequence learning, we tutored naïve juvenile zebra finches (i.e., raised in the absence of song; n=47), with randomized sequences of five species-typical syllables. All birds were tutored with the same five syllables and with all 120 possible five-syllable sequences in equal proportion. Because the stimuli do not provide any bias in sequencing, consistencies across tutored birds in the sequences produced would reflect intrinsic predispositions. Consistent with intrinsic biases in vocal learning, we found significant similarities in the sequencing of syllables across birds. For example, we observed significant biases in the location of individual syllables within a song motif (e.g., beginning, middle, or end). Additionally, we found directional biases between pairs of syllables wherein transitions from one syllable to the other were significantly more common than the reverse direction. These results provide strong experimental support for the hypothesis that sensory and/or motor systems are endowed with intrinsic biases that guide vocal sequence learning.

Disclosures: L. James: None. J.T. Sakata: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.05/XX5

Topic: F.01. Neuroethology

Support: NSF IOS 1453084

Title: Representation of calls in the activity of neurons in the songbird premotor nucleus hvc

Authors: *K. MURPHY, J. F. PRATHER;
Univ. of Wyoming, Laramie, WY

Abstract: Calls are vocalizations produced by both male and female songbirds. Although calls are shorter in duration than songs and have a less complex acoustic structure, portions of calls have been shown to be learned. Lesion and electrophysiological studies have indicated that nuclei in the song motor pathway (HVC and RA) are necessary for call vocalizations, however it remains unknown to what degree calls may be represented in activity of cells within the anterior forebrain pathway (HVC cells that project to Area X). This study investigated the role of HVC_{INT} and HVC_X neurons in both production and perception of call vocalizations. Individual HVC_X or HVC_{INT} neurons were recorded in awake and freely behaving adult male Bengalese finches (*Lonchura striata domestica*) using a miniature motorized microdrive. Neurons were identified through bipolar antidromic stimulation delivered to Area X. To determine the activity of cells

during call production and perception, calls were recorded through a microphone and played back through a speaker. A majority of HVC_X neurons were active in association with auditory playback of the bird's own call (BOC). This result indicates that the song learning pathway receives auditory activity associated with calls. These initial findings reveal that the song system is no longer involved in just song production and learning, but instead is a vocal communication system that contains representations of both songs and calls. In ongoing experiments, we are addressing the degree to which HVC_X neurons are selectively responsive to BOC versus other call stimuli such as heterospecific calls or conspecific female calls. In our recordings from HVC_{INT}, all cells that we sampled were active prior to call production. This could indicate that either HVC_{INT} neurons are active in driving the vocalization of calls (e.g. through precise regulation of HVC_{RA} neurons) or HVC_{INT} neurons are relaying a motor copy (e.g. corollary discharge) of the call from the HVC_{RA} neurons to a sensorimotor network. Together, these results indicate that call-related activity is present in HVC_X and HVC_{INT} neurons and support the role of the song system in representing the sounds used communication that include both songs and calls.

Disclosures: K. Murphy: None. J.F. Prather: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.06/XX6

Topic: F.01. Neuroethology

Support: NSF IOS 1453084

Title: Anatomical links between auditory perception and motor behavior in female mate choice

Authors: *J. L. DUNNING[1], S. MAZE[2], E. J. ATWOOD[2], K. MURPHY[2], J. F. PRATHER[2]; [1]Zoology and Physiol., [2]Univ. of Wyoming, Laramie, WY

Abstract: Females of many species use male courtship displays as a proxy of male fitness to inform decisions of mate choice. Female mate choice has been studied extensively in songbirds, in which females evaluate the quality of male songs for the purpose of choosing a mate. Female songbirds are superb in their abilities to discriminate amongst songs and will exhibit copulatory behaviors (i.e. copulation solicitation displays (CSDs) and calls) in response to songs played through a speaker, even when no male is physically present. It remains unknown, however, how perception of song quality influences expression of copulatory behaviors. Studies of female responses to song have implicated specific auditory cortical regions, such as the caudal

mesopallium (CM), in the expression of female mate preferences. Here we examined the projections from CM in female Bengalese finches (*Lonchura striata*) using an anterograde neural tracer. Our results demonstrate a novel projection from CM to the robust nucleus of the arcopallium (RA) as well as a region we suspect to be the ventral intermediate arcopallium (AIV). Ongoing experiments with dual tracers are examining the AIV projections more carefully. These arcopallial projections may enable CM to influence brain regions implicated in female courtship behaviors. In zebra finches, AIV projects to the ascending auditory stream, which in turn projects to the mediobasal hypothalamus (MBH), a region associated with female CSDs. In female Bengalese finches, RA projects to the dorsomedial nucleus of the intercollicular complex (DM), a site necessary for female call production. In addition, DM has recently been shown to project to the cloaca via the respiratory premotor nucleus retroambigualis (RAm) in canaries. Together, these data reveal putative pathways through which CM may influence both CSDs and calls in response to preferred song(s). To address the functionality of the projections emanating from CM in driving female courtship behaviors, we have begun using an adeno-associated virus (AAV) encoding the channelrhodopsin protein (ChR2) to selectively and reversibly manipulate CM neurons as female songbirds are engaged in evaluation of song quality. Our preliminary results demonstrate that CM displays robust expression of ChR2 and an increased firing rate in response to 420nm light. We are presently beginning tests of our expectation that manipulations of CM while females are listening to song will induce changes in female courtship behaviors via the projections of CM to the arcopallium.

Disclosures: J.L. Dunning: None. S. Maze: None. E.J. Atwood: None. K. Murphy: None. J.F. Prather: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.07/XX7

Topic: F.01. Neuroethology

Support: ImPACT program #16H01481

MEXT Kakenhi #26240019

Title: Mismatch response in auditory area of freely-moving songbird

Authors: *C. MORI, K. OKANOYA;
Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Mismatch negativity (MMN) is a component of the event related potentials (ERPs) that occurs in humans in response to unexpected and rare stimuli. The presentation of an oddball sequence of stimuli, infrequent sound (deviant) embedded in frequent regular sounds (standard), results in an evoked response. Several animals, including primates and rodents, show mismatch responses similar to that of humans. In this study, we investigated intracranial mismatch response from field L and the caudomedial nidopallium (NCM), in a songbird forebrain area, a brain structure considered to be analogous to parts of the primary auditory cortex in mammals. Songbirds communicate with each other by vocalization and develop a complex vocal pattern through vocal learning. Java sparrow, a species of songbird, is highly social and maintains communication with flock members using frequently repeated contact calls. It is crucial for Java sparrow to process repeated vocal signals and detect novel ones. We recorded local field potentials from Java sparrows by chronically implanted electrodes with wireless transmitter under freely moving condition. The auditory evoked potentials were recorded in passive listening. Pure tones (1-8 kHz) of 50 ms duration with 10 ms onset and offset ramps were presented at about 60-70 dB through a speaker placed near the perch. Three sound sequence designs (flip-flop sequence, many-standard sequence, and cascade sequence) were utilized to test whether the songbird auditory was capable of evoking human-like MMN.Significant negative and positive field potentials were observed between 50-250 ms after the onset of the deviant sound. We expect that songbird has a potential to be useful animal models that will contribute to the further understanding of neural mechanism of auditory sensory memory processing. (Work supported in part by ImPACT program and MEXT Kakenhi #16H01481 & #26240019 to KO).

Disclosures: C. Mori: None. K. Okanoya: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.08/XX8

Topic: F.01. Neuroethology

Support: University of Virginia

The Thomas F. and Kate Miller Jeffress Memorial Trust, Bank of America, N.A., Trustee

Title: Mechanism and function of physiologically heterogeneous cell types in caudolateral mesopallium

Authors: *A. N. CHEN[1,2], C. D. MELIZA[3];
[2]Neurosci. Grad. Program, [3]Psychology, [1]Univ. of Virginia, Charlottesville, VA

Abstract: The avian caudal mesopallium is one of the first regions in the auditory system responsible for selective, invariant representations of learned conspecific song. Using whole-cell electrophysiological recordings from caudolateral mesopallium (CLM) in zebra finches (*Taeniopygia guttata*), we have identified several putative classes of neurons by spike shape, frequency-current relationships, and firing patterns. A small proportion of cells had narrow spikes (NS; 9%) and little to no frequency adaptation, characteristics of inhibitory interneurons. The remaining putatively excitatory cells exhibited broad spikes and a range of phasic and tonic firing patterns that could be clustered into separate groups. Some neurons were regular-spiking (RS; 16%), with tonic, moderately adapting spiking responses to prolonged depolarizations. The predominant majority of neurons displayed a phasic firing pattern, responding only at the onset of depolarizing step currents. Within this group of cells we distinguished transient-spiking neurons (TS; 29%), which fired 3-5 action potentials at depolarization onset, followed by subthreshold oscillations, and onset-spiking neurons (OS; 46%), which fired only a single action potential at depolarization onset. Some neurons were filled with biocytin to reconstruct cell morphology; phasic neurons tended to have smaller, less elaborate dendritic trees. All four classes of neurons were present in adults and juveniles, but TS and OS neurons were more common in adults. Firing patterns are intrinsic, as they persisted in the presence of fast synaptic transmission blockers. Some TS cells became regular-spiking when treated with 4-AP but not other potassium-channel blockers, suggesting that a low-threshold outward current is responsible for phasic firing in these cells, as is also seen in vestibular ganglion cells and inferior colliculus in mammals. Based on linear and dynamical systems models fit from responses to complex, broadband current stimulation, phasic neurons are likely to function as coincidence detectors in processing complex auditory stimuli such as birdsong.

Disclosures: A.N. Chen: None. C.D. Meliza: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.09/XX9

Topic: F.01. Neuroethology

Support: Susan Todd Horton Class of 1910 Trust summer research fellowship

Hubel Neuroscience Summer Research Fellowship

Wellesley College Faculty Award

Wellesley College Early Career Funds

Seven College Conference of London Junior Year Abroad Award

European Research Council under the European Community's Seventh Framework Programme (ERC Grant AdG 268911 FP7/2007-2013)

Swiss National Science Foundation (grant 31003A_127024)

Title: Song exposure affects HVC ultrastructure in juvenile zebra finches

Authors: H. G. KHALED[1], Z. HUANG[3], R. H. R. HAHNLOSER[3], *S. M. H. GOBES[2]; [2]Neurosci., [1]Wellesley Col., Wellesley, MA; [3]ETH, Zurich, Switzerland

Abstract: Zebra finch song learning bears many similarities to human language learning, and thus the zebra finch is a useful model for understanding the biological processes involved in vocal skill acquisition. Song exposure in juvenile zebra finches triggers rapid spine enlargement and spine stabilization in HVC, an important brain area for adult song production. However, it is currently unknown whether in conjunction with learning-related spine changes there are also associated changes in synaptic structure and morphology. We paired juvenile male zebra finches with an adult bird (tutor) for various durations of time during the song-learning period. Birds were sacrificed at 59 days post-hatching and tissue from HVC was collected. Samples were processed for electron microscopy and imaged with a focus ion beam scanning electron microscope (FIB/SEM). Synapses in HVC were manually identified, and classified by subtype (symmetric or asymmetric) and shape (concave or convex). All identified synapses were segmented in 3D using ilastik, an interactive machine learning tool for image processing. Degrees of synapse curvature were quantified using EspINA, which enables measurement of synapse morphometric features. Comparisons of HVC synapse volumes and synapse curvatures were made among experimental groups that varied in their experience with tutor song. We found that asymmetric synapse volumes are significantly larger in bird groups exposed to song as compared to song isolates. Transient changes in synapse curvature emerged rapidly following tutor song exposure: the convexity difference between symmetric and asymmetric synapses significantly increased after one day of tutoring and decayed back to pre-tutoring levels within 3 weeks of first exposure. Overall, our results suggest that synapse morphology in the zebra finch HVC depends on tutor song exposure.

Disclosures: H.G. Khaled: None. Z. Huang: None. R.H.R. Hahnloser: None. S.M.H. Gobes: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.10/XX10

Topic: F.01. Neuroethology

Support: NSF IOS 0917918

Title: Motor and sensory coding in duetting wrens

Authors: E. S. FORTUNE[1], N. DAY[2], P. RIVERA[1], *M. J. COLEMAN[3];
[1]Dept. of Biol. Sci., New Jersey Inst. of Technol., Newark, NJ; [2]Dept. of Integrative Biol. and Physiol., Univ. California Los Angeles, Los Angeles, CA; [3]Keck Sci. Dept., Claremont McKenna, Pitzer and Scripps Colleges, Claremont, CA

Abstract: Male and female plain-tailed wrens (Pheugopedius euophrys) coordinate the production of syllables to generate a duet song that sounds as if a single bird is singing. We are interested in how these birds use both autogenous (self-generated) and heterogenous (other-generated) sensory feedback to coordinate motor patterns necessary for the production of duet songs. To do so, we examined patterns of action potentials in HVC, a site of sensorimotor integration for song control, both in awake and anesthetized birds. Chronic neurophysiological recordings were made using multiunit electrodes that were implanted in each bird. To compare activity between males and females during singing, we analyzed multiunit recordings. However, within a bird, we isolated single units to examine temporal patterns of activity to determine the roles of putative interneurons and projection neurons. We found that female activity was more tonic and male activity more phasic during duet production. The activity of putative interneurons appeared to encode both autogenous and heterogenous feedback, whereas the activity of putative projection neurons was correlated with the motor output of the animal. To examine the sensory responses in the birds, we subsequently anesthetized each bird with urethane and conducted playback experiments. We found that neurons in HVC of males and females exhibit similar response patterns to sensory playback of different song types. We predict that these common sensory codes contribute to the timing of the motor activity while the birds are singing their duet.

Disclosures: E.S. Fortune: None. N. Day: None. P. Rivera: None. M.J. Coleman: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.11/XX11

Topic: F.01. Neuroethology

Support: NSERC RGPIN 402186

Title: Early auditory experience does not affect c-Fos expression in catecholaminergic neurons in response to socially modulated song

Authors: *H. J. BARR[1], Y. CHEN[1], S. C. WOOLLEY[2];
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Abstract: Attribution of social salience to sensory information is a critical step in neural encoding of information and, across vertebrates, catecholaminergic systems appear to participate in assigning this salience. In songbirds, individuals often show strong biases for particular songs and catecholaminergic inputs into the auditory system have been postulated to shape neural responses to song. We have recently found that early exposure to song shapes auditory processing of song as well as behavioral preferences. For example, females raised in isolation of adult male song (isolate-reared) do not show species-typical preferences for courtship over non-courtship song, and it is uncertain whether they are able to discriminate between these two types of song. One possibility is that deficits in catecholaminergic responses to song contribute to this altered behavioral perception. To investigate this, we exposed both normally- and isolate-reared female zebra finches to either courtship or non-courtship songs and examined whether early life song exposure affected the activity of midbrain catecholaminergic neurons in response to contextually different song stimuli. Using immunocytochemistry, we quantified the degree of colocalization of cells expressing tyrosine hydroxylase (TH; the rate-limiting enzyme in the synthesis of catecholamines) and c-Fos (an immediate early gene and marker of neuronal activity) in three key catecholaminergic nuclei: the Locus Coeruleus (LoC), ventral tegmental area (VTA), and central gray (GCt). We found that while c-Fos expression in TH neurons was increased by song playback relative to silence, it was not significantly modulated by either early exposure to song or the type of song stimuli. These findings suggest that in normally-reared birds, differences in social salience of courtship and non-courtship song are not reflected in differences in c-Fos expression in midbrain catecholaminergic neurons. Furthermore, the finding that isolate-reared birds show similar levels of c-Fos expression in catecholamine neurons to normally-reared birds suggests that perhaps their initial appraisal of the general salience of song is comparable. Differences in song discrimination and preference could thus be modulated by more fine-tuned catecholaminergic activity at sites of auditory processing or by other neuromodulatory systems.

Disclosures: H.J. Barr: None. Y. Chen: None. S.C. Woolley: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.12/XX12

Topic: F.01. Neuroethology

Support: NIDCD DC013174

NIDCD DC008854

Title: Parental experience counts in a socially monogamous songbird: enhanced neural and behavioral responses to fledgling begging calls in male and female parents

Authors: *B. A. BELL, N. NARAYANAN, D. S. VICARIO;
Psychology, Rutger's Univ., Piscataway, NJ

Abstract: Animals invest heavily in pair bonds and offspring, and benefit if they are able to recognize their mates and family if they become separated. This study investigates how experience modulates the neural memory for auditory signals that can be used in individual recognition, with a focus on parent-offspring interactions in the zebra finch (ZF), a socially-monogamous species with bi-parental care. Male ZFs learn a single song early in life that is used as a social signal in adulthood. Each male's song contains unique variations that enable the song to be used as a recognition signal. In addition to songs, ZFs produce a 'long call' that is used in social situations, such as when birds are separated and want to locate one another. During the juvenile period (before long calls develop), ZFs produce another type of call, a short, high-frequency fledgling call (FC). FCs signal to parents that offspring need to be fed, and elicit a direct behavioral response. Thus, FCs are a behaviorally-relevant category of vocalization for ZF parents of both sexes, but may be meaningless to adult ZFs that have not yet mated and produced offspring (virgins). Although adult ZFs show behavioral and neuronal memories for the songs and long calls of familiar individuals, the neural processing of and behavioral responses to FCs have not been thoroughly examined.
Neural processing of these socially-relevant stimuli was assessed in the NCM and CMM regions of the avian auditory forebrain, in parents and virgin subjects. In addition, parental behaviors elicited by FCs were tested in a novel paradigm. Finally, the behavioral and neural data collected from parents were used to determine whether ZF parents can discriminate between the FCs of their own vs. unfamiliar fledglings. Results show that neural responses to FCs are stronger in parents of both sexes than in virgins. This may be due to differences in neural tuning that include

higher best frequencies in parents, perhaps reflecting a shift toward the high frequencies of FCs. Parents also show a neuronal memory for the calls of their own fledglings. In the behavioral paradigm, FC playback elicited parental behaviors more frequently in parents than in virgins. However, results also showed unexpected sex differences in the frequency of parental behaviors (nest-box entries, food-collected, etc.), as well as in the lateralization for parental-enhancement of neural responses to FCs. These sex differences support the hypothesis that maternal and paternal care develop through different neural mechanisms and establish the ZF as a valuable model for investigating how parental experience affects neural and behavioral processing in both female and male parents.

Disclosures: B.A. Bell: None. N. Narayanan: None. D.S. Vicario: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.13/XX13

Topic: F.01. Neuroethology

Support: NIH Grant DC013174

NIH Grant R15HD085102

Title: Statins affect the quality of auditory memories and performance on a behavioral discrimination paradigm in an avian model.

Authors: D. VALIVETI, S. SRINIVASAN, *D. S. VICARIO, M. L. PHAN;
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Abstract: The rise in childhood obesity has led to increased hypercholesterolemia (high cholesterol) and other risk factors in children normally associated with adult cardiovascular diseases. HMG-COA reductase inhibitors (statins) have been recommended for the management of high cholesterol in children. However, oral statins may affect cholesterol synthesis in the CNS, disrupting brain growth and maturation. We have developed the zebra finch (ZF) as a model system to examine potential cognitive and neural effects of statins in juveniles and adults by assessing song learning, auditory memory and performance on an auditory discrimination paradigm. Songbirds learn their vocalizations as juveniles through a process of vocal imitation similar to human speech learning. A songbird auditory region, the caudomedial nidopallium (NCM) is a specialized forebrain area that stores a memory of the tutor's song and subserves song discrimination.

Male ZFs received either oral simvastatin (40 mg/kg) or vehicle daily from ages 45±5 post-hatch days (phd) until the termination of the experiment (180 ± 5 phd). To test the hypothesis that statins impair auditory discrimination performance in adulthood, the birds were trained to peck for a food reward in response to a Go stimulus and to withhold responding from a NoGo stimulus. Vehicle treated birds reached criterion in ~2 weeks (M= 14.5 ± 1.9 days). In contrast, none of the statin treated birds learned the task well enough to reach the accuracy criterion (80% correct across two 100 trial sessions).
To test the hypothesis that chronic statin exposure impairs learning and memory, we then recorded simultaneously from multiple electrodes in NCM in the same birds in an awake restrained preparation. We compared multi-unit responses to novel and familiar songs (that had been presented 20h earlier). Response differences were quantified as Relative Response Strength (RRS) to provide a measure of long-lasting familiarity; the higher the RRS, the stronger the memory. RRS was significantly lower in statin-treated birds than in those that received vehicle alone ($p < 0.05$). Together, these results suggest that oral simvastatin treatment, given in therapeutic dosages chronically across development and into adulthood, produces deficits in learning and memory in adults.

Disclosures: D. Valiveti: None. S. Srinivasan: None. D.S. Vicario: None. M.L. Phan: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.14/XX14

Topic: F.01. Neuroethology

Support: NWO-Gravitation Programme, CID 024.001.003

Dynamics of Youth call Seed Money 2013, Utrecht University

Title: Zebra finches still recognize their tutor song after manipulation of temporal structure

Authors: *C. MOL[1], G. J. L. BECKERS[1], R. W. J. KAGER[2], J. J. BOLHUIS[1];
[1]Dept. of Psychology, [2]Dept. of Language, Literature and Communication, Utrecht Univ., Utrecht, Netherlands

Abstract: There are remarkable behavioral, neural and genetic parallels between birdsong and human speech and language. Song development in the zebra finch (*Taeniopygia guttata*) is a commonly used model for human speech development. Young male zebra finches learn their song by imitating the song of an adult bird (a 'tutor') during a sensitive period early in life,

comparable to speech acquisition in human infants. During vocal learning, zebra finches also form a memory of their tutor song, which they can still recognize when adult. However, it is not known which acoustic features of the tutor song are memorized during development, and are important for recognition in adult birds. Zebra finches typically sing a stereotyped motif consisting of a sequence of complex syllables separated by brief silent intervals. Within syllables, often several song elements can be distinguished based on rapid temporal or spectral changes. As a comparison, human infants use the temporal structure of speech, such as silent intervals, to parse continuous speech and to recognize linguistically relevant speech units. The aim of our study is to investigate whether the temporal sequencing of syllables, elements and silent intervals in a tutor song is important for song recognition in zebra finches. We assessed recognition of a tutor song using an auditory discrimination paradigm without reinforcement, in which adult zebra finches (raised by their parents) were exposed to playbacks of their tutor song and an unfamiliar song. Zebra finches typically respond differently towards tutor and unfamiliar song, which can be used as a behavioral measure for song recognition. In a previous study, we demonstrated that zebra finches were still able to recognize their tutor's song after systematically changing the temporal order of syllables within these songs. In the current study, we altered the temporal structure of silence intervals within a tutor song, such that: (1) all inter-syllable silence intervals were removed, (2) silence between song elements, within syllables was added, or (3) a combination of these two manipulations was applied. We found that adult zebra finches still recognized their tutor's song after all of the silence interval manipulations. Taken together, we conclude that zebra finches can recognize their tutor's song despite various and drastic manipulations of song temporal structure, and that therefore temporal integration of acoustic features over relatively brief song segments is sufficient for recognition.

Disclosures: C. Mol: None. G.J.L. Beckers: None. R.W.J. Kager: None. J.J. Bolhuis: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.15/XX15

Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant PHY-1058202

Tiberiu Tesileanu is also supported by the Swartz Foundation

Title: Matching tutors to students: efficient two-stage learning and birdsong

Authors: *T. TESILEANU[1], B. OLVECZKY[2], V. BALASUBRAMANIAN[3];
[1]Initiative for the Theoretical Sci., CUNY Grad. Ctr., New York, NY; [2]Ctr. for Brain Science, Dept. of Organismic and Evolutionary Biol., Harvard Univ., Cambridge, MA; [3]Dept. of Physics and Astronomy, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Existing models of birdsong learning assume that the outflow of the song-specialized basal ganglia (LMAN) plays the role of experimenter: it introduces vocal variability required for trial-and-error reinforcement learning. Recent data, however, suggests that LMAN activity also provides a corrective bias that drives short-term improvements in song that are later consolidated in the robust nucleus of the arcopallium (RA). This learning process is thought to occur through heterosynaptic plasticity, in which the strengths of HVC-RA synapses are modulated by activity at the LMAN-RA synapses. Here we develop a new model of such two-stage learning in which the teacher circuit (LMAN in the bird) provides both variability for experimentation and an instructive signal to the student circuit (RA in the bird). We demonstrate that for such learning to be efficient, the instructive signal should be adapted to the specific dynamics of the plasticity mechanism in the student. As an example, we show in our model that an adapted LMAN can effectively train RA to move from juvenile to adult patterns of firing, mimicking what is seen in songbirds. Specifically, using a stochastic gradient descent approach, we show that to guide RA effectively, the signal from LMAN should respond to an integral of the motor error on a timescale that is related to the parameters of the RA plasticity rule. We test this prediction in simulations using both a rate-based approximation and spiking neurons. Our results indicate that when LMAN uses an integration timescale that is significantly different from the one implied by our analysis, learning is slowed down or even abolished. Assuming that over evolutionary timescales circuits in the brain have adapted to learn more efficiently, our model predicts the structure of signals from LMAN. These predictions can be directly tested by recording activity in LMAN. We also demonstrate how the variability observed in the firing patterns of LMAN neurons can be used in a reinforcement learning paradigm to enable LMAN to build its teaching signal. Two-stage learning, in which one brain area learns information about the world and then, over longer timescales, transfers this knowledge to downstream circuits, has been observed in other parts of the brain. Our model can be applied generally to settings where a tutor circuit (such as LMAN in the bird) is used to teach a student circuit (such as RA) to produce a desired output under the drive of a conductor (such as HVC).

Disclosures: T. Tesileanu: None. B. Olveczky: None. V. Balasubramanian: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.16/XX16

Topic: D.05. Audition

Title: Mechanisms for category learning in songbirds

Authors: *T. SAINBURG[1], T. Q. GENTNER[2];
[1]Psychology, UCSD, LA Jolla, CA; [2]Psychology, UCSD, San Diego, CA

Abstract: My current project is to explore cognitive and physiological mechanisms which underlie acoustic category acquisition where explicit feedback only exists at the sequence level, or does not exist at all. Using an operant conditioning paradigm, combined with recent innovations in deep generative neural networks, we aim to isolate both unsupervised learning, and supervised learning where feedback exists only at the sequence level as exists in phoneme sequences in language. More specifically, we are training a VAE on Starling motifs to compress motif representations into a 2 Dimensional latent space. We are then drawing a circle in part of that latent space using samples points, arbitrarily dividing that circle in half, and calling half of those motifs 'A', and half of those motifs 'B'. Using this arbitrary division in the latent space of our network, we manipulate the information available to the bird to categorize motifs A vs B. We create three conditions:

Sequence Level Feedback (covert supervision): Classifying sequences (AB and BA vs AA and AB), the birds are provided feedback at the level of the sequence (left or right peck), where learning to divide A and B is necessary to correctly predict a reward. Specific instances of motifs (e.g. A31) are picked at random from an a-modal distribution (A24 is just as likely as A1)

Distributional (no supervision): While the bird is performing a cover task (classifying sequence loudness), a bird is experiences the same sequences as in I but with no feedback that provides information about phoneme category boundaries. Instead, the distribution of motifs (A and B) are gaussian, where motifs at the boundary between A and B have low probability (A24 is much more likely than A1)

Mixed Feedback: The bird performs the same task as in A, motifs are selected from a gaussian probability distribution function (A24 is much more likely than A1).

The purpose of this study is to create a controlled condition in which we can isolate the two leading theories in phonetic category learning: distributional learning where categories are learned using unsupervised clustering and covert reinforcement learning in which categories are learned using explicit feedback which occurs at the level of the sequence. Likely, phoneme categorization uses a combination of these two mechanisms, but a biological substrate for feedback learning in sequences has yet to be identified. This work would both demonstrate the plausibility of these mechanisms in sequence-component category learning, and enables further investigation into the computations which underlie feedback in sequence learning.

Disclosures: T. Sainburg: None. T.Q. Gentner: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.17/XX17

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

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (C) (15K06724) to Y.Y.-S.

Title: Early auditory experience modifies neuronal firing properties of neurons in zebra finch auditory cortex

Authors: *T. KUDO, Y. YAZAKI-SUGIYAMA;
OIST, Okinawa, Japan

Abstract: Juvenile, male zebra finches learn to sing based upon auditory experience with adult conspecifics during a limited time window called the critical period. They first hear and memorize tutor songs (normally their fathers') during a sensory learning period (<~50 days). Then they match their own vocalizations to memorized tutor songs using auditory feedback. We recently reported that early tutor song experience shapes neuronal auditory responsiveness in the caudomedial nidopallium (NCM), homologous to the mammalian higher auditory cortex (Yanagihara and Yazaki-Sugiyama, 2016). Here we examined development of neuronal properties with tutor song experience during the sensory learning period (between post-hatching day [PHD] 20, 40, and 60) in male and female NCM using whole-cell patch clamp *in vitro*. We found three types of neuron in NCM with distinctive spontaneous firing rates; silent, low frequency (< 7 Hz), and high frequency (> 9 Hz). High-frequency neurons were found only at PHD 20 and 40. The proportion of low-frequency neurons increased from PHD 20 to PHD 40, then decreased to the PHD 20 level by PHD 60 in both males and females. Some low- and high-frequency neurons showed tonic bursts during spontaneous firing (burst type neurons) in both sexes, but the percentage of burst neurons increased at PHD 40 only in males, returning to the PHD 20 level by PHD 60. When juvenile birds were isolated from their fathers at PHD 10, which extends the sensory learning period, high-frequency neurons could be found even at PHD 60. Also the proportion of low-frequency neurons and the ratio of burst-type neurons were higher at PHD 60, compared to normal juveniles, i.e. they are more like PHD 40 in normal juveniles. These findings suggest that early song experience modifies NCM neuronal properties, especially firing properties that might be necessary for song memory formation.

Disclosures: T. Kudo: None. Y. Yazaki-Sugiyama: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.18/XX18

Topic: D.05. Audition

Title: Relationship between song acoustic similarity and neural activity in female zebra finches

Authors: *R. TABATA, M. INDA, K. HOTTA, K. OKA;
Grad. Sch. of Sci. and Technology, Keio Un, Yokohama, Japan

Abstract: Zebra finches (*Taeniopygia guttata*) are songbirds, and they are known as an animal model of language learning. High order auditory areas in the zebra finch are analogous parts of the mammalian auditory cortex, so how acoustic information is encoded in these areas is crucial for understanding the neural bases of acoustic information processing. In particular, caudomedial nidopallium (NCM) in male zebra finch is related to the perceptual discrimination and memorization of birdsong. Previous study has reported the number of ZENK-expression cells in this area. ZENK, one of immediate-early genes, is commonly used as an index of neural activity. ZENK expression level was highest when they were exposed to conspecific song, in comparison with heterospecific song or pure tones (Mello *et al.*, 1992). In NCM of female zebra finch, song stimulus from her mate or unfamiliar directed song for courting induced the number of ZENK-expression cells (Woolley and Doupe, 2008). The number of ZENK-expression cells increased by unfamiliar directed song in comparison with mate's directed song. This result suggests that NCM detects whether the song is familiar or unfamiliar. However, how each single cell responses change temporally to each song is still unknown, because neural activities have been only estimated by the total number of ZENK expression cells previously. Here, we conducted electrophysiological experiments in NCM and NCL (NCM n=18, NCL n= 14) by using extracellular recording technique, and recorded temporal neural activities of single cell, induced by her pair's (familiar) or non-pair's (unfamiliar) directed songs. Pair-bonding is defined as occurrence of clumping behavior, and allopreening, following 8-13 days after male and female housing together. Spike sorting analysis was carried out by custom-developed MATLAB (MathWorks) code. Spike candidates were detected from recorded data and analyzed by principal component analysis, after detected waveforms were classified into several clusters by using k-means clustering. Neural activity is estimated as spike rate and spike precision; spike rates are detected during each song stimulus and spike precisions are average of inner products between trials during song stimulus. We detected that spike rates and spike precisions in NCM and NCL by pair's directed song are significantly higher than non-pair's directed song. Moreover, these neural activities are correlated with the similarity of several acoustic features between pair's and non-pair's directed songs. These results indicate that these neural indices

represent whether the song is familiar or unfamiliar and the song familiarity is caused by the acoustic similarity.

Disclosures: R. Tabata: None. M. Inda: None. K. Hotta: None. K. Oka: None.

Poster

251. Bird Song: Auditory Processing and Song Acquisition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 251.19/XX19

Topic: D.05. Audition

Title: Auditory neural activity in zebra finch forebrain depends on acoustic strucures of syllables

Authors: *M. INDA, R. TABATA, K. HOTTA, K. OKA;
Keio-Univ. Biophysics and Neuroinformatics Lab., Yokohama/Kanagawa, Japan

Abstract: Zebra Finch (*Taeniopygia guttata*) is a kind of songbirds. Male songs have stereotyped sequences of syllables which contain various acoustic characteristics. The male birds sing their own songs to court to females, and the females decide mating partners by their own preferences. Therefore, the auditory discrimination by difference of song structures is important ability for songbirds. The song discrimination system has been considered to be function of neurons in the avian forebrain, especially, higher-auditory regions. The caudal medial mesopallium (CMM) is a one of these regions, which is known to discriminate between conspecific and heterospecific songs. However, it doesn't discriminate between unfamiliar and familiar songs (Woolley and Doupe, 2008). This previous work only evaluated the neural responses to clearly different sound stimuli, thus we focused on song structures to clarify the details of song discrimination system. We mimicked syllable acoustic structures; 'Pitch' or 'Mean Frequency', by using pure tones with unchanging temporal characteristics to keep syllable sequences. In addition, we made syllable-shuffled song to disrupt syllable sequences. All motifs of these songs were divided into two parts 'first half' and 'second half' by syllable types, and these of natural song was exchanged for synthetic songs to investigate a factor of song discrimination. Furthermore, we prepared another synthetic song whose positions of 'first half' and 'second half' were exchanged. We recorded neural activities of CMM as spike sequences when these sounds were presented to female zebra finches. CMM neurons in females were more selective to the male songs comparing to tones mimicked 'Pitch', even though they were mixed in the same song. In contrast, these neurons did not show significantly different neural activities between the male songs and shuffled ones. These results indicate that these neurons discriminate between songs and the others by their acoustic structures. Meanwhile, these neurons were also significantly and selectively responded to 'second half' of songs. Syllables in 'second half'

induced higher mean frequencies and entropies than the ones of ‘‘first half’. We conclude that in CMM, there are some neurons that discriminate songs by not syllable sequence but these acoustic structures.

Disclosures: M. Inda: None. R. Tabata: None. K. Hotta: None. K. Oka: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.01/XX20

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH GRANT R01HD058638NICHD

CSULB BUILD NIH GRANT 8UL1GM118979-02; 8TL4GM118980-02; 8RL5GM118978-02

Arnold and Mabel Beckman Foundation Undergraduate Scholarship

Title: G protein-coupled estrogen receptor 1 (GPER) is found in the plasma membrane and cytoplasmic fractions of tissue from the arcuate nucleus of the hypothalamus

Authors: *M. FERI[1], J. PHAN[2], K. SINCHAK[3];
[1]Biol. Sci., California State Univ. Long Beach, Long Beach, CA; [2]Biol. Sci., [3]California State University, Long Beach, Long Beach, CA

Abstract: In the ovariectomized (OVX) rat primed with 2μg estradiol benzoate (EB), sexual receptivity (lordosis) can be facilitated within 30 minutes after the infusion of non-esterified 17β-estradiol (E2) into the arcuate nucleus of the hypothalamus (ARH). Our lab has shown that the E2 rapid facilitation of lordosis is mediated by G protein-coupled estrogen receptor (GPER). We have observed that GPER are coexpressed in ARH orphanin FQ (OFQ; aka nociceptin) neurons and induce the release of OFQ to facilitate lordosis (Long et al., 2014, Horm Behav 66:63). However, it is unclear whether the GPER signaling is initiated at the level of the plasma membrane or in the cytoplasm. Within the ARH we have observed that immunopositive GPER staining is located in the cytoplasm closely associated with the nucleus. Others have observed GPER in the plasma membrane of hippocampal tissue (Funakoshi, 2006). We tested the hypothesis that GPER are localized in the plasma membrane and the cytoplasm of the ARH. ARH block dissections were collected from OVX Long Evans rats treated with 2μg EB or oil. ARH plasma membrane and cytosolic fractions were produced using a Plasma Membrane Protein Extraction Kit (Abcam). GPER levels in plasma membrane and cytosolic fractions were

determined by western blot analysis. A band corresponding to the weight of GPER was observed for both plasma membrane and cytosolic fractions. Plasma membrane preparations lacked positive LIM kinase-staining indicating that the preparations were not contaminated with cytoplasmic fractions. Conversely, flotillin 1 was not observed in the western blots of cytoplasmic fractions free of plasma membrane contamination. These findings indicate that E2 may initiate signaling via ARH GPER at either the levels of the plasma membrane or intracellularly within the cytoplasm. Further, the SERMs tamoxifen and ICI 182,780 that antagonize classical estrogen receptors (α & β), activate GPER. Thus, understanding when and which GPER signaling pathways is mediating estrogenic signaling will be important for understanding estrogenic action throughout the cycle and better direct estrogenic therapies.

Disclosures: M. Feri: None. J. Phan: None. K. Sinchak: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.02/XX21

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01HD058638NICHD

Arnold and Mabel Beckman Foundation

Howell-CSUPERB Research Scholars Award

Title: Dopamine receptor D1 and D5 do not form physical complexes with Src kinase on the plasma membrane to mediate actions of progesterone signaling in the arcuate nucleus of hypothalamus

Authors: *J. PHAN[1], J. RODMAN[2], K. SINCHAK[2];
[1]Biol. Sci., [2]California State University, Long Beach, Long Beach, CA

Abstract: Classical progesterone receptor-B (PR-B) are necessary for progesterone facilitation of sexual receptivity (lordosis) and are upregulated by estradiol priming (EB). Infusion of progesterone in the arcuate nucleus of the hypothalamus (ARH) of estradiol-primed ovariectomized rats facilitates behavior within 30 minutes. These rapid actions of progesterone are mediated by extranuclear classical progesterone receptor which inhibit ARH β-endorphin neurons and induce lordosis. We have shown PR-B expressed in β-END neurons and present on plasma membrane from ARH neural tissue. Further, we have shown that this PR complexes and signal through Src to facilitates sexual receptivity. This PR-Src signaling are interdependent with

dopamine receptor (D1/D5) signaling--antagonizing one, blocks facilitation of lordosis by the other two. However, it was unclear which dopamine receptor, D1 or D5, associated with the PR-Src signaling. We have shown through immunohistochemistry that PR and D1 are colocalized and expressed in β-END neurons in the ARH. However, coimmunoprecipitation revealed D1 did not complex with either PR or Src on the plasma membrane. Thus, we tested the hypothesis that Src forms complexes with D5 in ARH plasma membrane. To test this, ovariectomized rats were treated with EB or EB + progesterone and plasma membrane and cytosolic fractions were extracted from ARH block dissections. Western blot analysis revealed Src and D5 were present in the ARH. However, coimmunoprecipitation experiments indicated that Src and D5 do not form complexes either. Our results suggest that rather than physically interacting with D1 or D5, Src may be regulated through the G protein subunit associated with the dopamine receptor to modulate the rapid actions of progesterone signaling through PR-Src complexes.

Disclosures: J. Phan: None. J. Rodman: None. K. Sinchak: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.03/XX22

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant-R01HD058638NICHHD

RISE Grant- R25GM071638

CSULB RISE- RO1HD058638

Title: Subpopulations of arcuate nucleus β-endorphin neurons express G protein-coupled estrogen receptor 1 and estrogen receptor-α

Authors: *T. CHUON[1], S. HUERTA[1], J. RODMAN[1], K. SINCHAK[2];
[2]Biol., [1]California State University, Long Beach, Long Beach, CA

Abstract: Sequential activation of estrogen receptor (ERα) and G protein-coupled estrogen receptor 1 (GPER; aka GPER30) over 48 hours induces sexual receptivity (lordosis) in ovariectomized rats (Long et al., 2014, Horm Behav 66:63). It is becoming more apparent that rapid extranuclear signaling and classical genomic signaling are important for synchronizing reproductive behavior and physiology. Estradiol, acting through ERα, initially inhibits lordosis via activation of β-endorphin (β-END) neurons in the arcuate nucleus of the hypothalamus (ARH). Meanwhile, estradiol is also upregulating systems, presumably via ERα as well, to

deactivate β-END neurons and facilitate lordosis. For example, in ARH β-END neurons, estradiol increases expression of opioid receptor-like (ORL)-1 along with its cognate ligand orphanin FQ (OFQ; nociceptin) in presynaptic neurons. Subsequently, estradiol acts rapidly via GPER to facilitate lordosis. In estradiol benzoate (EB, 2μg) primed ovariectomized (OVX) rats, ARH infusion of non-esterified 17β-estradiol (E2) 48 hours later will facilitate lordosis in 30 minutes. Our behavioral data and immunohistochemical colocalization of GPER in ARH OFQ neurons indicate this E2 acts via GPER directly in ARH OFQ neurons. Given that genomic actions of estradiol appear to be regulating ORL-1 expression in β-END and GPER's effects on regulating β-END activity are presynaptic, we hypothesized that ER-α will be expressed in β-END neurons, whereas GPER colocalization with β-END will be minimal. Double-label immunohistochemistry showed that β-END expresses both ERα and GPER. However, there seems to be a higher percentage of β-END immunopositive staining colocalized with ERα than GPER. These results are congruent with our previous findings: ERα has transcriptional effects in β-END neurons, and GPER is presynaptic to β-END in OFQ neurons.

Disclosures: T. Chuon: None. S. Huerta: None. J. Rodman: None. K. Sinchak: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.04/YY1

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01HD058638NICHD

CSULB RISE-NIH Grant RO1HD058638

CSULB RISE-NIH Grant GM07163

BUILD 8UL1GM118979-02; 8TL4GM118980-02; 8RL5GM118979-02

CSUPERB Howell Scholars Award

Title: Tamoxifen and ICI 182,780 activation of G protein-coupled estrogen receptor 1 (GPER) acts through orphanin FQ (nociception) neurons to rapidly facilitate sexual receptivity

Authors: *S. M. CHOKR, N. LONG, A. CHHAN, K. SINCHAK;
Biol. Sci., California State Univ. Long Beach, Long Beach, CA

Abstract: Estradiol can facilitate sexual receptivity (lordosis) by sequential activation of estrogen receptor-α (ERα) and G protein-coupled estrogen receptor 1 (GPER; aka GPER-1, GPER30; Long et al., 2014, Horm Behav 66:63). In the ovariectomized (OVX) rat, a priming dose of estradiol benzoate (2 μg; EB) initially activates β-endorphin (β-END) neurons in the arcuate nucleus of the hypothalamus (ARH) that project to the medial preoptic nucleus (MPN) to activate μ-opioid receptors (MOP), which inhibits lordosis. Subsequently, 47.5 hours after EB, infusion of non-esterified 17β-estradiol (E2) into the ARH reduces MPN MOP activation and facilitates lordosis within 30 minutes. This E2 acts through GPER and activates the orphanin FQ-opioid receptor-like-receptor-1 (OFQ-ORL-1) system to reduce the release of β-END into the MPN. Our lab has previously shown that GPER are expressed in 85.7% of ARH OFQ neurons. Thus, E2 appears to act directly through GPER in OFQ neurons to release OFQ and facilitate lordosis. We previously showed that the selective estrogen receptor modulators (SERMs), tamoxifen (TAM) and ICI 182,780 (ICI), facilitate lordosis and deactivate MPN MOP within 30 minutes through a GPER dependent pathway, similar to E2. Therefore, we hypothesized that infusion of TAM or ICI in the ARH of an EB-primed OVX rat will rapidly deactivate MPN MOP and facilitate lordosis via activation of the OFQ-ORL-1 system. As expected, infusion of either TAM or ICI into the ARH 47.5 hours after EB priming facilitated sexual receptivity within 30 minutes. Further, pretreatment with UFP-101, an ORL-1 selective antagonist, blocked TAM and ICI facilitation of sexual receptivity. These data indicate that, like E2, TAM and ICI rapidly induces ARH GPER signaling that induces the release of OFQ to facilitate lordosis by deactivation of β-END neurons. This GPER signaling is likely acting directly to induce ARH OFQ release which activates ORL-1 to inhibit β-END release. Understanding the regulation of GPER pathway activation by common anti-estrogen therapies TAM and ICI is important for better directing SERM therapies.

Disclosures: S.M. Chokr: None. N. Long: None. A. Chhan: None. K. Sinchak: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.05/YY2

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1256799

NIH Grant T32DA007234

Title: Measuring the tonic and phasic release of dopamine during sexual behavior in female hamsters using fixed potential amperometry

Authors: *B. T. HIMMLER[1], K. M. MOORE[1], L. E. BEEN[3], B. A. TEPLITZKY[2], R. L. MEISEL[1];
[1]Dept. of Neurosci., [2]Dept. of Biomed. Engin., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Psychology, Haverford Col., Haverford, PA

Abstract: In studying the neurobiology of sexual behavior, the majority of research has focused on the male experience, with little research focusing on the female experience. In contrast, reproductive success in most mammalian species, depends on a specific pattern of vaginal stimulation during mating, i.e., sexual selection. In many species, including hamsters, females behaviorally regulate the receipt of penile intromissions from the male. Past research has shown that sexual behavior in females is rewarding, a consequence of mating that is mediated by the release of dopamine in several brain areas, including the nucleus accumbens (NAc). However, the exact mechanisms involved with the receipt of intromissions and the release of dopamine is unknown. The objective of this study was to use Fixed Potential Amperometry in order to investigate the tonic increase of dopamine seen during sexual behavior in females, and also to investigate if there is a time-locked phasic pattern between intromissions during mating and with the release of dopamine in the NAc. In order to test this, adult female hamsters received bilateral ovariectomies and a carbon fiber electrode was sterotaxically lowered into their NAc. Females were then treated with estrogen and progesterone to induce sexual receptivity, and were tested with a stimulus male partner. Our preliminary results reveal a tonic increased release of dopamine in the NAc during mating bouts as well as a phasic time-locked release of dopamine with intromissions. These results have broader implications for how sexual experience may produce a vulnerability for drug addiction.

Disclosures: B.T. Himmler: None. K.M. Moore: None. L.E. Been: None. B.A. Teplitzky: None. R.L. Meisel: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.06/YY3

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1256799

T32DA007234

T32 GM008471

Title: Investigation of glutamate neurotransmission in the copulatory reward of sex behavior in female Syrian hamsters

Authors: *K. M. MOORE, R. L. MEISEL;
Dept. of Neurosci., Univ. of Minnesota Dept. of Neurosci., Minneapolis, MN

Abstract: The long-term focus of research in our lab has been to understand the neural underpinnings of sex behavior. Although dopaminergic signaling traditionally has been the focus of research in natural reward, glutamatergic neurotransmission, despite its involvement in synaptic plasticity in learned and motivated behaviors, has been largely ignored. The present study utilized enzymatic biosensing and immunohistochemistry to elucidate the effects of male copulatory behavior on glutamate release and activation in multiple regions of the female brain. Our enzymatic biosensor recordings suggest that the pattern of glutamatergic neurotransmission in different brain regions in the female hamster may be responsible for encoding distinct properties of the female's copulatory interaction with the male. Specifically, patterns of glutamate release in the prefrontal cortex (PFC) appear to encode overall bouts of male copulatory behavior, which we hypothesize may be responsible in signaling to downstream regions responsible for copulatory reward. In contrast, recordings in the nucleus accumbens (NAc) have revealed transient glutamate release in response to individual penile intromissions, which we hypothesize may signal reward so that future receptivity to this reproductively necessary behavior is reinforced. We next examined the underlying circuitry by which this activation takes place. We first determined potential sex-activated afferents to the NAc using stereotaxic injections of the monosynaptic retrograde tracer Choleratoxin subunit B and subsequent staining for cFos, a marker of neuronal activity. We confirmed that sex behavior increased activity in the NAc core as revealed through increased levels of cFos expression, and found that retrogradely labeled afferents in the PFC were also activated, corroborating the findings from our biosensor recordings. To further investigate this circuitry, we are currently using an inhibitory designer receptor exclusively activated by designer drug (DREADD) to selectively silence accumbal-PFC innervation during sex behavior, and will examine the impact of this manipulation on both cellular activation as well as reward-related behavioral conditioning to repeated sex experience.

Disclosures: K.M. Moore: None. R.L. Meisel: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.07/YY4

Topic: F.02. Behavioral Neuroendocrinology

Title: RU-486 enhances 8-OH-DPAT's inhibition of female rat sexual behavior

Authors: *R. S. HORNUNG[1], L. UPHOUSE[2];
[2]Biol., [1]Texas Woman's Univ., Denton, TX

Abstract: Gonadal hormones are critical for initiation of female rat sexual behavior. Estradiol is more important but progesterone allows expression of the entire repertoire of sexual activity. Neurotransmitters also influence the expression of sexual behavior and 5-HT_{1A} receptors have received special attention since 5-HT_{1A} receptor agonists inhibit the behavior. Both estradiol and progesterone reduce the effectiveness of the 5-HT_{1A} receptor agonist, 8-OH-DPAT [(±) -8-hydroxy 2-(di-*n*-propylamino) tetralin]. Mechanisms responsible for estrogen's reduction include a decline in 5-HT_{1A} receptor signaling but progesterone's role is not clear. Progesterone also reduces the effects of restraint on sexual behavior and the 5-HT_{1A} receptor has been implicated in this behavioral decline. Since the antiprogestin, RU-486 [(11β-(4-dimethylamino)phenyl-17β-hydroxy-17(1-propynyl)estra-4,9-dien-3-one)], attenuated the effect of progesterone, the intracellular progesterone receptor was implicated. By extension, the intracellular progesterone receptor may be involved in progesterone's reduction of sexual inhibitory effects of 5-HT_{1A} receptor agonists, but the effect of RU-486 has not been examined. The current project was designed to test this hypothesis. However, it has been reported that RU-486 blocks the serotonin transporter. To rule out the possibility that RU-486 was acting by such a mode, and independent of the intracellular progesterone receptor, RU-486 was administered before or after progesterone treatment. Ovariectomized Fischer rats were primed with 10 µg estradiol benzoate and 500 µg progesterone. RU-486 (5 mg/rat) was administered either 1 hr before progesterone (to block intracellular progesterone receptor-mediated events) or 4 hr after progesterone (when intracellular progesterone receptor-mediated events should have occurred). Prior to injection with 0-100 µg/kg 8-OH-DPAT, all females showed high lordosis frequency and lordosis quality. However, proceptivity was significantly reduced by RU-486 given before (Chi Square, $p \leq 0.05$), but not after, progesterone. RU-486 treatment before progesterone also enhanced the response to 8-OH-DPAT so that lordosis inhibition was evident at lower doses of the drug (ANOVA, $p \leq 0.05$). Comparable enhancement was not evident when RU-486 was given 4 hr after progesterone. Therefore, the intracellular progesterone receptor may be required for progesterone to reduce the sexual inhibitory effects of 5-HT_{1A} receptors.

Disclosures: R.S. Hornung: None. L. Uphouse: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.08/YY5

Topic: F.02. Behavioral Neuroendocrinology

Support: NIDDK award R01 HD081792

Title: Melanocortin 4 receptors expressed only on sim1 neurons are sufficient for male sexual behavior

Authors: *E. SEMPLE, J. HILL;
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Abstract: Sexual dysfunction affects 20-30% of men in the United States and world-wide. Unfortunately, sexual dysfunction is poorly understood, and existing treatments that target the periphery are often ineffective. Preclinical data has shown that central melanocortins, released by pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC), can lead to male erection and increased libido in rodents. When the melanocortin 4 receptor (MC4R) is knocked out globally, male mice show erectile dysfunction. The paraventricular nucleus of the hypothalamus (PVN), a known projection target of POMC neurons, expresses a high concentration of MC4 receptors. We hypothesize that the PVN is a key site of melanocortin-mediated regulation of male sexual behavior. To test this hypothesis, we compared the sexual behavior of tbMC4R/Sim1-cre mice to MC4R null mice and wild-type controls. The tbMC4R/Sim1-cre mouse model utilizes the cre-lox system to express MC4R only on Sim1-cre neurons, which are primarily found in the PVN. These mice were paired with a sexually experienced female and their behavior was filmed between 8pm and 2am. The behavioral videos were scored based on male sexual behaviors such as mounting, intromission, and ejaculation. Because MC4 receptors are also known to play a role in metabolism, a metabolic profile was obtained from these mice through the use of GTT, NMR, and weight gain. Expression of MC4R only on Sim1 neurons reversed the sexual deficits seen in MC4R null mice to those of controls, despite a similar metabolic profile to MC4R null mice. This study implicates MC4R on Sim1 neurons, likely in the PVN, in the central neurocircuitry underlying sexual behavior.

Disclosures: E. Semple: None. J. Hill: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.09/YY6

Topic: F.02. Behavioral Neuroendocrinology

Title: Prenatal exposure to diazepam or flunitrazepam produces sexual behavior modifications of male mice

Authors: *M. MARQUEZ-OROZCO[1], A. MARQUEZ-OROZCO[2], G. DE LA FUENTE-JUAREZ[2], L. A. I. HERNANDEZ-ALVAREZ[3];
[2]Embryology, [1]Univ. of Mexico (UNAM), Mexico, Mexico; [3]Educ. and Res., Hosp. Gen. Zona Norte de Puebla, Mexico, Mexico

Abstract: Studies performed in our laboratory have shown testicular alterations and dimorphic behavioral modifications in mature male mice exposed prenatally to diazepam (DZ), with facilitation patterns in females and copulatory alterations in males. Thus, the aim of this work was to assess sexual behavior of male mice exposed *in utero* to 1 mg/kg of DZ or to a single dose (2.5 mg/kg) of flunitrazepam (FLU) in an attempt to find a biological gradient. One group of CD-1 strain female mice (DZ) was sc. treated with DZ from 6^{th} to 17^{th} days of gestation, other group of dams (FLU) were exposed to a single dose of FLU on the 6^{th} day of gestation. The control groups (C) received saline solution. The spontaneous offspring male sexual activity to receptive females was tested in 3 sessions (1/week) on 6th month of age. Tested were performed during the dark stage of the photoperiod and video recorded under red light. Pre-copulating and copulating activities were evaluated. Assessment of copulatory stage included: mount (ML), intromission (IL) and ejaculation (EL) latencies, mount series with intravaginal penetration (MSWIP) or without it (MS), mount series with ejaculation (MSWE) and the number of interruptions of penile penetrations as falls, pauses or breaks were determined. DZ males exhibited a significant larger incidence of falls and breaks during MSWIP, nevertheless FLU males also showed a higher tendency to breaks. This may be explained by a motor coordination failure that depends of cerebral cortex, which in turn we have found to be affected by prenatal exposure to DZ in previous works. Prenatally treated males showed greater proportion of ejaculations, which may compensate the modifications of motor pattern. Data were statistically processed by frequency analysis test, Odds Ratio (OR) in 2x2 tables of Manttel-Haenzel's X^2 test, Cornfield confident limits, Student's *t* test and X^2 test for *K* independent groups with contingency C coefficient, using a software package with confidence level $\alpha = 0.95$. Results show a biological gradient to prenatal benzodiazepines (BDZ) exposure that may be correlated to modification of density of central receptors to BDZ during development and suggest a permanent modification in the GABAergic neurotransmission substrate mediated by CNS.

Disclosures: M. Marquez-Orozco: None. A. Marquez-Orozco: None. G. De la Fuente-Juarez: None. L.A.I. Hernandez-Alvarez: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.10/YY7

Topic: F.02. Behavioral Neuroendocrinology

Title: Diazepam administered prenatally induces sexual behavior alterations in adult and senile mice

Authors: *A. MARQUEZ-OROZCO[1], L. A. I. HERNANDEZ-ALVAREZ[2], G. DE LA FUENTE-JUAREZ[1], M. C. MARQUEZ-OROZCO[1];
[1]Univ. of Mexico (UNAM), Mexico 04510 DF, Mexico; [2]Hosp. Gen. Zona Norte de Puebla, Mexico, Mexico

Abstract: In former work, effects of prenatal exposure to diazepam (DZ) on sexual behavior of adult mice have been found. The aim of this work was to assess sexual behavior during reproductive span of CD-1 strain male mice exposed in utero to DZ. One group of female mice was treated with DZ (2.5 mg/kg/day; sc.) from 6th to 17th days of gestation and a control group received saline solution. The spontaneous offspring male sexual activity to receptive females was tested in 3 sessions (1/week) twice, on the 6th month of age and later on the 27th. Tests were performed during the dark stage of the photoperiod and video-recorded under red light. Pre-copulating and copulating activities were evaluated. Pre-copulating consisted in slow movements, interest, recognition and olfaction of females, self-licking and auto-exploration, olfaction of female genitals and rubbing. Assessment of copulatory stage: mount intromission and ejaculation latencies, mount series with intravaginal penetration or without it, mount series with ejaculation and number of interruptions of penile penetration were determinate. During copulating stage adult DZ-treated males showed greater incidence of interruptions of intravaginal penetration, while senile DZ-treated males had lower latencies of mount series and greater proportion of ejaculations (α=0.95).Both adult and senile DZ-treated males exhibited a significant larger incidence of falls and pauses during mount series with intromission. Data were statistically processed by frequency analysis test, Odds Ratio (OR) in 2x2 tables of Manttel-Haenzel's X^2 test, Cornfield confident limits, Student's *t* test and X^2 test for *K* independent groups with contingency C coefficient, using a software package with confidence level $\alpha = 0.95$. Results suggest a permanent modification in the GABAergic neurotransmission substrate mediated by CNS and show long-lasting effect of prenatal exposure of DZ on sexual behavior.

Disclosures: A. Marquez-Orozco: None. L.A.I. Hernandez-Alvarez: None. G. De la Fuente-Juarez: None. M.C. Marquez-Orozco: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.11/YY8

Topic: F.02. Behavioral Neuroendocrinology

Support: DA030517

T32GM008181

Title: The converging roles of progesterone receptor, dopamine receptor, and neuronal activation in the medial amygdala to mediate female sexual motivation

Authors: *S. A. RUDZINSKAS, S. JURADO, J. A. MONG;
Univ. of Maryland Baltimore, Baltimore, MD

Abstract: The neural mechanisms which mediate sexual motivation in women are not well understood. Previous work in our laboratory investigating mechanisms underlying methamphetamine (MA)-facilitated female sexual motivation have given us new insights into the neural circuitry and components contributing to sexual motivation in the normal female brain. Using cFos and lesion studies in a rodent model, we have demonstrated that the medial amygdala (MeA) is the crucial mediator of MA's ability to increase sexual motivation in females. These studies resulted in the novel finding that MA, in the absence of hormones, could increase progesterone receptor (PR) in the MeA. This is significant since dopamine and dopamine receptor 1 (D_1R) activation have been previously demonstrated to initiate PR signaling via ligand independent activation. Furthermore, a PR antagonist RU486 infused into the MeA after MA administration blocks MA's ability to increase sexual motivation. Taken together, this evidence led us to hypothesize that D_1R mediated PR upregulation in the MeA is responsible for increasing both sexual motivation and cFos activation of this nucleus. To test the behavioral aspect of this hypothesis, we designed a lentivirus containing an ubiquitin-PR-GFP-construct to ubiquitously overexpress PR. This virus was stereotaxically injected into the MeA two weeks prior to sex behavior testing, during which proceptive, or sexually motivated, behaviors and receptive events were recorded for 15min following exposure to a novel male. Our preliminary results suggest that PR upregulation in the MeA significantly increases overall number of proceptive behaviors in a hormonally-primed female rat. In fact, specific proceptive behaviors such as hops and darts also show significant increases, with no significant changes to non-

proceptive behaviors. To test whether PR upregulation may account for the increases in cFos in the MeA after MA, we are currently administering RU486 to rats 30min prior to progesterone on the final day of the MA-binge, and quantifying changes in cFos activation after RU486 using immunohistochemistry. Our future studies will aim to further link the role of PR, D_1R, and cFos neural activation play in the MeA to mediate sexual motivation.

Disclosures: S.A. Rudzinskas: None. S. Jurado: None. J.A. Mong: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.12/YY9

Topic: F.02. Behavioral Neuroendocrinology

Support: ANR-Neurofem

Title: Neural estrogen receptor alpha and beta signalling pathways in mice

Authors: *S. MHAOUTY-KODJA;
CNRS UMR 8246, INSERM U1130, UPMC, Paris, France

Abstract: Estrogens through their nuclear receptors play a key role in the regulation of neuroendocrine functions and behaviors related to reproduction in both males and females. In males, estradiol derived from neural aromatization of gonadal testosterone acts perinatally in the neural structures underlying these responses to induce their irreversible organization. It is also necessary during adulthood in their activation and maintenance. In females, ovarian estradiol liberated during the postnatal period is required in pubertal maturation and feminization of neural structures involved in the expression of female sexual behavior. As for males, estradiol is also important during adulthood in the regulation of neuroendocrine and behavioral responses related to reproduction. In order to precise the neural roles of estrogen receptor alpha (ERalpha) and beta (ERbeta) in these neuroendocrine and behavioral responses, we recently generated mouse lines lacking each of these receptors in the nervous system. This restricted mutagenesis does not interfere with their peripheral functions. We recently showed that neural ERbeta invalidation delays pubertal maturation in females, but does not interfere with the organization and activation of sexual behavior in both males and females (Naulé et al., 2015; 2016). We recently assessed the effects of ERbeta invalidation on aggressive behavior by using the resident-intruder test in males. We will also present the first results we obtained on the recently generated mouse model lacking neural ERalpha gene. This characterization included behavioral (mating, olfactory preference, aggressive behavior…) and neuroanatomical analyses in brain areas underlying

sexual and aggressive behaviors. The goal of this study is to get a precise view of the neural phenotypes induced by these gene invalidations. This will allow then a detailed molecular analysis of these signaling pathways in the relevant brain areas underlying neuroendocrine and behavioral responses related to reproduction in both males and females.

Disclosures: S. Mhaouty-Kodja: None.

Poster

252. Molecular and Pharmacological Mechanisms of Sexual Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 252.13/YY10

Topic: F.02. Behavioral Neuroendocrinology

Support: CIHR

Title: Disruption of conditioned sexual inhibition in male rats: alcohol and cue effects on nucleus accumbens GABA release.

Authors: *K. GERMÉ[1], J. ALVAREZ-BARKHAM[2], L. CUSACK[2], L. R. GOSSIP[2], H. S. POUPEL[2], H. RAJABI[2], J. G. PFAUS[2];
[1]Dept. of Biol. / Ctr. for Studies in Behavioral Neurobio., [2]Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Sexual inhibition can be induced in male rats when they are given access on alternate trials to a sexually nonreceptive female scented with a neutral odor (almond) and an unscented receptive female. On a final choice test with two receptive females, one scented and the other unscented, males show a conditioned avoidance of the scented female. An acute systemic treatment with a low dose of alcohol (0.5g/kg) before the final test disrupts this preference and those males show greater numbers of Fos-immunoreactive cells in the nucleus accumbens (NAc) when exposed to the odor alone. We have previously shown that males receiving this low dose of alcohol (0.5 g/kg) display an increase in both dopamine (DA) and serotonin (5-HT) concentrations in the NAc, however in alcohol-treated males only, the cue increased DA but not 5-HT. As GABAergic neurotransmission is a major target of ethanol effects, we examined here the effects of the same low dose of alcohol on extracellular concentrations of GABA in the NAc in the presence of an inhibitory cue. Sexually naïve male Long-Evans rats were conditioned for sexual inhibition to an odor (almond) using our established paradigm and injected with saline or alcohol (0.5g/kg) before the final choice test. Males were then implanted with microdialysis probes aimed at the NAc and given access to the odor under the influence of saline or alcohol. The alcohol alone had no effect on GABA concentrations. However, in the presence of the

inhibitory cue, there was an increase in GABA concentrations in NAc in alcohol-treated but not in the saline-treated rats. These data suggest that, as with DA transmission, enhanced transmission of GABA in the NAc is involved in sexual disinhibition in male rats.

Disclosures: K. Germé: None. J. Alvarez-Barkham: None. L. Cusack: None. L.R. Gossip: None. H.S. Poupel: None. H. Rajabi: None. J.G. Pfaus: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.01/YY11

Topic: F.04. Stress and the Brain

Support: NIH DA09082

Title: Cellular substrates for interactions between neuropeptide Y (NPY) and corticotropin-releasing factor (CRF) in the rat locus coeruleus

Authors: *M. J. WARNER, B. A. S. REYES, E. J. VAN BOCKSTAELE;
Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Neuropeptide Y (NPY) transmission in the brain mediates behavioral responses to stress, anxiety and stress resilience. This neuropeptide is abundant in the central nucleus of the amygdala (CeA), a limbic region that sends CRF projections to noradrenergic neurons of the locus-coeruleus (LC). The LC-norepinephrine (NE) system contains the largest cluster of noradrenergic neurons within the brain and provides the major source of NE throughout the neuraxis. From a functional perspective, the amygdalar-LC circuit is thought to mechanistically link limbic and arousal centers in response to stress. Several lines of evidence have proposed that NPY and CRF are strategically positioned to interact with each other. While previous independent studies have described the anatomical organization of NPY and CRF in the CeA and its influence on LC-NE neuronal activity (Enman et al., 2015; SFN abstract), the cellular substrates for interactions between NPY and CRF in the rat LC remain largely unknown. In the present study, we investigated anatomical substrates for interactions between NPY and CRF using immunofluorescence and immunoelectron microscopy in the male rat LC. Forty-micron thick tissue sections from the LC were collected and processed for immunocytochemical detection of NPY, NPY receptors (Y1r or Y2r) and CRF using commercially available antibodies that underwent rigorous specificity testing. Immunofluorescence microscopy showed that individual CRF-immunoreactive fibers within the LC exhibited labeling for NPY. Ultrastructural analysis using immunogold-silver labeling for CRF and immunoperoxidase labeling for NPY

confirmed that CRF and NPY co-exist in single axon terminals in the LC. Furthermore, immunogold-silver labeling of Y1 or Y2 and immunoperoxidase labeling for CRF revealed that CRF is presynaptically distributed in axon terminals that directly contact Y1-labeled dendrites while CRF and Y2r co-exist in the same axon terminals. On occasion, CRF-labeled axon terminals contacted Y2-labeled dendrites. Taken together, these findings indicate multiple sites of interaction between CRF and NPY in the LC.

Disclosures: M.J. Warner: None. B.A.S. Reyes: None. E.J. Van Bockstaele: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.02/YY12

Topic: F.04. Stress and the Brain

Support: MH 093981

DA 09082

Title: Activation of amygdalar corticotropin releasing factor and brainstem enkephalinergic afferents to the locus coeruleus in female rats following social stress

Authors: *B. A. REYES[1], H. GUAJARDO[2], E. C. DUFOURT[1], R. J. VALENTINO[2], E. J. VAN BOCKSTAELE[1];
[1]Pharmacol. & Physiol., Drexel Univ., Philadelphia, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Stress-related psychiatric diseases are nearly twice as prevalent in women compared to men. We demonstrated sex differences in signaling of the corticotropin-releasing factor (CRF) receptor in the locus coeruleus (LC) that can increase female vulnerability to stress-related psychopathology. Sex differences in afferent regulation of the LC could also affect stress vulnerability.We recently showed in male rats that social stress differentially engages CRF neurons in the central nucleus of the amygdala (CeA) and opioid-containing neurons in the nucleus paragigantocellularis (PGi) that project to the LC depending on coping strategy as determined by their latency to assume a defeat posture. Using the same model of social stress, the present study was designed to examine engagement of these neural circuits following social defeat in female rats. Female Sprague-Dawley rats were injected with the retrograde tracer, Fluorogold (FG) into the LC. Three days following the FG injection, rats were subjected to repeated (5 days) resident-intruder stress or control manipulation and perfused 90 minutes 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). Cell counts revealed that while c-fos expression in the PGi was comparable among all groups, significant increases in c-fos expression were observed in the CeA of rats that have a short latency to defeat (SL) rats ($P < 0.05$) when compared to control and long latency (LL) rats. Triple labeling of c-fos, FG and CRF revealed that 82% of c-fos and FG-immunoreactive neurons in the CeA of SL rats also expressed CRF, which was significantly higher ($P < 0.01$) when compared to control and LL rats. In the PGi, approximately 34% of c-Fos and FG-immunoreactive neurons of LL rats also expressed ENK,which was significantly higher ($P < 0.05$) when compared to SL rats. These preliminary results indicating that repeated social stress engages divergent circuitry to regulate the LC in female rats with different coping styles is consistent with previous findings in males and suggests that coping strategy rather than sex is an important determinant of this regulation.

Disclosures: B.A. Reyes: None. H. Guajardo: None. E.C. Dufourt: None. R.J. Valentino: None. E.J. Van Bockstaele: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.03/YY13

Topic: F.04. Stress and the Brain

Support: NIH P50 MH096889

NIH NS28912

NIH MH73136

Title: Simultaneous acute stresses impair memory enduringly via novel convergent actions of multiple stress hormones

Authors: *Y. CHEN[1], J. MOLET[1], J. C. LAUTERBORN[2], B. H. TRIEU[2], J. L. BOLTON[2], K. P. PATTERSON[2], C. M. GALL[2], G. LYNCH[2,3], T. Z. BARAM[1,2];
[1]Pediatrics, [2]Anatomy/Neurobiology, [3]Psychiatry and Human Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Terrorist attacks, natural disasters and battles confront us with simultaneous physical, emotional and social stresses. However, despite vast information about effects of individual acute or chronic stresses on the brain, it is unknown if multiple concurrent short stresses

influence crucial brain functions, if these problems persist, and the nature of the responsible mechanisms. Whereas an individual short stress did not affect memory, here we show that concurrent stresses lasting 1-2 hours provoked profound and enduring spatial memory deficits in mice. These stemmed from disrupted hippocampal synaptic plasticity due to loss of functional excitatory synapses. The effects of the concurrent stresses were recapitulated by combined actions of the canonical stress hormone corticosterone and the hippocampal peptide corticotropin-releasing hormone. Together, these hormones disrupted synaptic function and integrity via convergent mechanisms on dendritic spine scaffolding. Accordingly, blocking the actions of both hormones, but not of each, within the brain of stressed mice abrogated the memory problems. The inability of the mammalian brain to preserve memory when challenged with multiple simultaneous acute stresses might be adaptive.

Disclosures: Y. Chen: None. J. Molet: None. J.C. Lauterborn: None. B.H. Trieu: None. J.L. Bolton: None. K.P. Patterson: None. C.M. Gall: None. G. Lynch: None. T.Z. Baram: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.04/YY14

Topic: F.04. Stress and the Brain

Support: NIH Grant 1DP2OD007363

NIH Grant 1DP2OD008398

Title: Mood, stress and longevity: convergence on Ankyrin 3

Authors: *A. NICULESCU[1], S. RANGARAJU[3], D. LEVEY[2], K. NHO[2], H. LE-NICULESCU[1], A. SAYKIN[2], D. SALOMON[3], M. PETRASCHECK[3];
[1]Psychiatry, [2]Indiana Univ. Sch. of Med., Indianapolis, IN; [3]The Scripps Res. Inst., La Jolla, CA

Abstract: Antidepressants have been shown to improve longevity in C. elegans. It is plausible that orthologues of genes involved in mood regulation and stress response are involved in such an effect. We sought to understand the underlying biology. First, we analyzed the transcriptome from worms treated with the antidepressant mianserin, previously identified in a large-scale unbiased drug screen as promoting increased lifespan in worms. We identified the most robust treatment-related changes in gene expression, and identified the corresponding human orthologues. Our analysis uncovered a series of genes and biological pathways that may be at the interface between antidepressant effects and longevity, notably pathways involved in drug

metabolism/degradation (nicotine, melatonin). Second, we examined which of these genes overlap with genes which may be involved in depressive symptoms in an aging non-psychiatric human population (n=3,577), discovered using a genome-wide association study (GWAS) approach in a design with extremes of distribution of phenotype. Third, we used a convergent functional genomics (CFG) approach to prioritize these genes for relevance to mood disorders and stress. The top gene identified was ANK3. To validate our findings, we conducted genetic and gene expression studies, in C.elegans and in humans. Taken together, these studies uncover ANK3 and other genes in our dataset as biological links between mood, stress and longevity/aging, that may be biomarkers as well as targets for preventive or therapeutic interventions.

Disclosures: A. Niculescu: None. S. Rangaraju: None. D. Levey: None. K. Nho: None. H. Le-Niculescu: None. A. Saykin: None. D. Salomon: None. M. Petrascheck: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.05/ZZ1

Topic: H.01. Animal Cognition and Behavior

Support: The Hartwell Foundation

NIDA RO1 1R01DA029122-03(AMR)

Title: The role of $Ca_v1.2$ in mediating susceptibility to chronic stress in the prefrontal cortex

Authors: *C. C. BAVLEY, A. M. RAJADHYAKSHA;
Pediatric Neurol., Weill Cornell Med., New York, NY

Abstract: Chronic stress increases the risk for developing neuropsychiatric disorders, particularly in genetically vulnerable individuals. Single nucleotide polymorphisms (SNPs) in the gene *CACNA1C*, which codes for the $Ca_v1.2$ subunit of the L-type Ca2+ channel (LTCC), has been associated with several stress-related disorders, including bipolar disorder, schizophrenia, and major depressive disorder. The prefrontal cortex (PFC) is responsible for cognitive and emotional processing, and deficits in the functional connectivity of the PFC have been associated with most neuropsychiatric disorders. The PFC is particularly susceptible to the effects of chronic stress, displaying changes in gene expression, dendritic spine morphology and synaptic plasticity. Additionally, carriers of the risk-conferring SNPs in *CACNA1C* show alterations in PFC structure and function, suggesting a critical role of $Ca_v1.2$ in maintaining

functionality of this region. Here we demonstrate that chronic stress increases $Ca_v1.2$ protein expression in the PFC of mice, and that $Ca_v1.2$ heterozygous mice are resilient to the effects of chronic stress on anxiety-like behavior and working memory, demonstrated in the elevated plus maze and y-maze spontaneous alternation tests, respectively. Current experiments are examining the molecular mechanisms downstream of $Ca_v1.2$ within the prefrontal cortex that may mediate these stress-induced behavioral deficits. Understanding how $Ca_v1.2$ regulates susceptibility to chronic stress will help further our understanding of $Ca_v1.2$ signaling, as well as improve our understanding of the role of $Ca_v1.2$ in the development of stress-related neuropsychiatric disorders.

Disclosures: C.C. Bavley: None. A.M. Rajadhyaksha: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.06/ZZ2

Topic: F.04. Stress and the Brain

Support: NIH R01MH062044

Title: Effects of social housing on measures of stress in male and female Syrian hamsters

Authors: *A. P. ROSS, A. NORVELLE, D. C. CHOI, J. C. WALTON, H. E. ALBERS, K. L. HUHMAN;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Although Syrian hamsters are thought to be naturally solitary, research from our laboratory suggests that hamsters prefer social interaction and that this interaction can induce a conditioned place preference. We have also demonstrated, however, that social defeat stress stimulates stress hormone release and promotes hyperphagia and weight gain in male hamsters. In contrast, it has also been proposed that single housing of female hamsters leads to stress-induced anorexia. Thus, there are conflicting ideas about whether group housing or single housing is more stressful to hamsters. The purpose of this study was to compare a variety of stress endpoints in singly housed and group housed males and females. Male (n = 20) and female (n = 20) hamsters were housed singly or in groups of 5. After 4 weeks, the hamsters were euthanized by rapid decapitation and trunk blood was collected at lights on, a time that is at the nadir of the daily glucocorticoid rhythm. Fat pads, thymus and adrenal glands were extracted and weighed. Serum was collected, and cortisol was measured using a radioactive immunoassay. We found that group-housed females weighed more than any other group. In addition, both group-

housed females and males had significantly more fat than did singly housed animals. Interestingly, there was no effect of housing on basal cortisol concentration or adrenal gland weight. There was, however, an effect of housing on thymus gland weight, such that the thymus glands of group-housed animals was less than that of singly-housed hamsters, but this effect was only significant when thymus weight was normalized to body weight. Together, these data suggest that while different housing protocols can impact aspects of hamster physiology, neither group nor single housing is a strong stressor for Syrian hamsters. Supported by NIH R01MH062044 to KLH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: A.P. Ross: None. A. Norvelle: None. D.C. Choi: None. J.C. Walton: None. H.E. Albers: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.07/ZZ3

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH062044

Title: Acute and repeated exposure to social stress alters gut microbiota composition in Syrian hamsters.

Authors: *K. A. PARTRICK[1], B. CHASSAING[2], A. T. GEWIRTZ[2], K. L. HUHMAN[3]; [2]Biol., [1]Georgia State Univ., Atlanta, GA; [3]Neurosci., Georgia State Univ., Altanta, GA

Abstract: Recent data indicate gut microbiota can affect their host's brain and behavior. In humans, social stress is the primary form of stress experienced, and this exposure promotes a wide variety of gastrointestinal and neuropsychiatric illnesses. It is still not known if alterations in gut microbes are involved in these outcomes. Syrian hamsters are ideal subjects for social stress research because they are territorial and aggressive and rapidly form stable dominant/subordinate relationships. Importantly, their agonistic interactions are highly ritualized and thus rarely result in physical injury, making it possible to focus on psychological effects of social stress. The purpose of this study was to determine if exposure to social stress in hamsters alters gut microbiota and if gut microbiota composition can predict the outcome of an agonistic encounter. Hamsters were paired for 15 min on Day 1, leading to rapid formation of a dominance hierarchy. Animals were then paired twice a day on Days 2-5. Fecal samples were collected before and after the initial interaction on Day 1 and again on Day 5. Microbiota composition was

assessed via 16S mRNA Illumina sequencing on fecal samples. After the first interaction, a decrease in alpha diversity was observed in both dominant and subordinate animals, with this decrease being more pronounced after repeated interactions. Importantly, beta diversity analysis revealed distinct clustering, reflecting alterations of microbiota composition, between hamsters undergoing social interaction versus their controls. Linear discriminant analysis (LEfSe) identified specific microbiota members that drove those differences. Bacteria from the order Lactobacillales, linked to reduced inflammation and anxiolytic effects, were significantly reduced following social stress in both dominants and subordinates. Differences between these two groups were also seen including Bifidobacterium, linked to anxiolytic effects, and Clostidiacea, linked to gastrointestinal disease, which were significantly altered only in winners (dominants). Importantly, LEfSe analysis on samples collected before social interaction revealed some microbiota were powerful predictors of whether an animal achieved dominant or subordinate status. Together, these data suggest that agonistic social interactions impact gastrointestinal health in both winners and losers, and that the state of the microbial community before social stress may predict the outcome of dominant/subordinate relationships. Supported by NIH R01MH062044 to KLH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: K.A. Partrick: None. B. Chassaing: None. A.T. Gewirtz: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.08/ZZ4

Topic: F.04. Stress and the Brain

Support: NSF IOS-0923301

NIH MH062044

Title: Sex differences in the effects of arginine vasopressin (AVP) in the anterior hypothalamus (AH) on resilience to social stress in Syrian hamsters

Authors: *J. I. TERRANOVA[1,2], N. HARDCASTLE[1,2], K. L. HUHMAN[1,2], H. E. ALBERS[1,2]; [1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Ctr. for Behavioral Neurosci., Atlanta, GA

Abstract: Syrian hamsters readily establish dominance relationships that are formed and maintained by agonistic behaviors such as aggression and social communication. Hamsters that

lose agonistic encounters subsequently abandon territorial aggression and become more susceptible to subsequent social stress, whereas animals that have dominant experience become more resistant to social defeat stress. Dominant male hamsters have increased arginine vasopressin (AVP) 1a receptors, whereas subordinate males have decreased AVP cell bodies in the hypothalamus, a critical brain region that mediates aggressive behavior. Thus, the purpose of this project is to test the hypothesis that neural systems that increase aggressive behavior promote resistance to social stress, whereas neural systems that reduce aggressive behavior decrease resistance to social stress. Interestingly, AVP microinjection into the anterior hypothalamus (AH) *facilitates* aggression in male hamsters but *inhibits* aggression in female hamsters. Therefore, we also tested the hypothesis that there is a marked sex difference in how susceptibility to social stress is mediated. Hamsters were singly housed for two weeks and implanted with unilateral guide cannula aimed at the AH. The week following surgery, hamsters were handled daily and the estrous cycle was monitored in females. During the testing week, hamsters experienced a single 15 min defeat in the home cage of a sex-matched resident aggressor (RA). Females were defeated and tested during diestrus. Twenty-four hours later, hamsters were microinjected with saline vehicle or 0.9 μM AVP and placed in a neutral arena with an unfamiliar RA behind a mesh cage. Time spent avoiding the RA and time spent investigating the RA was quantified. There was a trending interaction of drug treatment and sex on avoidance duration ($p = 0.137$) and a significant interaction of drug treatment and sex for investigation duration ($p < 0.05$). Females who received AVP in the AH spent less time investigating the RA than control females (Controls: 201.37 ± 19.25 seconds; AVP: 108.59 ± 26.49 seconds) and more time avoiding the caged RA than control females (Controls: 55.76 ± 17.70 seconds; AVP: 148.03 ± 26.34 seconds). There was no effect of AVP treatment on male avoidance or investigation. These data support the hypothesis that neural systems that decrease aggressive behaviors increase susceptibility to social stress. Furthermore, there are sex differences in the contributions of these neural systems. (This work is supported by NSF IOS-0923301 and NIH MH062044. The content is solely the responsibility of the authors and does not necessarily represent the official view of the NSF or NIH.)

Disclosures: J.I. Terranova: None. N. Hardcastle: None. K.L. Huhman: None. H.E. Albers: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.09/ZZ5

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH062044

Title: An acute social defeat stressor during puberty increases susceptibility to conditioned defeat in adulthood

Authors: *A. M. ROSENHAUER, K. E. MCCANN, A. NORVELLE, K. L. HUHMAN; Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Syrian hamsters readily display territorial aggression and, if they lose an agonistic encounter, show striking reductions in aggressive behavior and increases in submissive behavior, a distinct behavioral change termed conditioned defeat. This model is particularly useful because the behavioral change occurs after a single defeat in both males and females. Additionally, this stressor is primarily psychological with no tissue damage or inflammation, and therefore, makes an ideal model for studying social stress. Although our lab has made significant progress delineating the neural circuit involved in conditioned defeat, all research to date has been in adults. However, social stress is also common during human adolescence, and stress experienced during puberty is a known risk factor for the development of stress-induced disorders in adulthood. In this project, we expanded the conditioned defeat model to include social defeat in early puberty. We found that hamsters exposed to a single, 15 min social defeat on P35 exhibited significantly increased social avoidance 24 hrs later when compared to no defeat controls, ($F(1,54) = 40.072$, $p < .001$). When these hamsters were tested for territorial aggression on P64, all animals attacked an intruder placed in their home cages. Pubertal stress and no defeat control animals were then subjected to a single 15 min social defeat in adulthood (P65) and were tested for social avoidance on P66 to determine if the pubertal social defeat increased vulnerability to social stress in adulthood. Indeed, hamsters that experienced pubertal social stress responded to the adult social defeat with increased social avoidance when compared with hamsters that were only defeated in adulthood or with no defeat controls ($F(2,81) = 8.214$, $p = .001$). Thus, a single social stressor in puberty increased susceptibility to acute social stress in adulthood. It is possible that the rapid changes occurring within the brain during puberty may constitute a stress-sensitive period resulting in this enhanced vulnerability. This pubertal social defeat and adult re-defeat protocol is a promising model with which to explore the long term effects of adolescent social stress and may shed light on how stress during development contributes to the increased risk of developing psychopathology in adulthood. We are currently using this behavioral protocol to examine the role of DNA methylation following adolescent stress to test if inhibiting DNA methylation blocks the increased stress responsivity later in life. Supported by NIH R01MH062044 to KLH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: A.M. Rosenhauer: None. K.E. McCann: None. A. Norvelle: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.10/ZZ6

Topic: F.04. Stress and the Brain

Support: NIH R01MH062044

Title: Prenatal exposure to social stress alters adult response to social stress in Syrian hamsters

Authors: *A. NORVELLE, G. M. F. JONES, A. M. ROSENHAUER, K. E. MCCANN, K. L. HUHMAN;
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Abstract: Maternal stress can have long lasting cognitive, emotional and physical effects on the offspring of stressed mothers. The majority of the relevant studies in this area have used relatively severe, non-social stressors to induce behavioral change in the offspring. Additionally, many studies have indicated that stress-induced alterations in maternal behavior is a critical mechanism underlying these behavioral changes. The purpose of this project was to assess the utility of our hamster social defeat model as an alternative with which to examine the effect of mild social stress of dams on the behavior of their offspring. Syrian hamsters are an ideal species with which to study the effects of social stress because both males and females are highly aggressive and will readily defend their home cage from an intruder. Yet when a hamster loses an agonistic encounter, it will abandon territoriality and will instead display social avoidance. In the current study, 6 of 12 females were defeated 3 times for 15 min in early pregnancy (prenatal days 2, 4 and 6) by larger, ovariectomized females. Following parturition, maternal behavior was scored for 7 days. Surprisingly, we observed no difference between groups in maternal behavior including licking and grooming or pup contact time. The pups of all 12 dams were weaned at P25 and were group housed until P60 whereupon they were singly housed. Half of the offspring of both groups of dams were defeated and all offspring were tested for avoidance behavior with a caged, non-threatening intruder. Defeated males of non-stressed dams avoided more than no defeat controls ($t(11)=2.5$, $p=0.03$). Male offspring of stressed dams did not show the same effect ($t(13)=1.53$, $p=0.15$). This change in stress responsivity cannot be attributed to differences in maternal behavior. There were no differences in the behavior of female offspring of stressed or non-stressed dams. These data suggest that males may be more susceptible to prenatal stress. This sex difference is consistent with the increased prevalence of specific neurodevelopmental disorders in males, including autism and schizophrenia. Supported by NIH R01MH062044 to KLH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: A. Norvelle: None. G.M.F. Jones: None. A.M. Rosenhauer: None. K.E. Mccann: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.11/ZZ7

Topic: F.04. Stress and the Brain

Support: NIH MH62044

Title: Gene expression and network analyses in the basolateral amygdala dominant and subordinate Syrian hamsters

Authors: *K. E. MCCANN, D. M. SINKIEWICZ, K. L. HUHMAN;
Neurosci. Institute, Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Social stress is the most common stressor experienced by humans and is a risk factor for developing a number of neuropsychiatric illnesses, including anxiety and mood disorders. Hamsters are ideal for modeling social stress because both males and females readily exhibit intraspecific aggression. After losing one agonistic encounter, however, both males and females abandon all territorial aggression and become highly submissive, a behavioral shift termed conditioned defeat. Our laboratory has made significant progress in delineating the neural circuitry and many of the neurochemical correlates of conditioned defeat and have demonstrated that the basolateral amygdala (BLA) is a critical component of this neural circuit. The purpose of this project was to sequence the BLA transcriptome of dominant and subordinate animals and to compare their gene expression to one another and to same-sex controls. We examined genes associated with learning and memory, mood and anxiety disorders, and social behavior. Several genes that had lower expression in animals that experienced an agonistic encounter have been linked to bipolar disorder (Akap5), general mood disorders (Aldh1a1), anxiety (Kif13a), and depression (Mgat5). Other genes linked to major depressive disorder (Gad2, Gria2), PTSD (Dicer1), and anxiety (Spock3) had higher expression in dominant and/or subordinate animals when compared with controls. Next, we used a weighted gene co-expression network analysis to determine the similarity in gene expression patterns of dominant, subordinate, and control samples and graphed the connectivity of the samples based on overall gene expression patterns. All samples from subordinates grouped closely together, suggesting overall gene expression patterns in the BLA were consistent in this group, regardless of sex. Samples from dominants and controls, however, were intermixed, and overall expression patterns in these groups were not distinct from one another, again independent of sex. This is not surprising given that the

behavioral phenotype of controls is aggressive, closely resembling that of dominants. We are now examining specific gene network patterns to further define the similarities and differences of gene expression in males and females of different social status. Supported by NIH R01MH062044 to KLH and Georgia State University Brains and Behavior Seed Grant. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: K.E. McCann: None. D.M. Sinkiewicz: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.12/ZZ8

Topic: F.04. Stress and the Brain

Support: NIH Grant MH062044

Title: Prelimbic and infralimbic cortical TrkB signaling differentially regulates conditioned defeat learning

Authors: *D. C. CHOI[1], K. A. PARTRICK[2], A. M. ROSENHAUER[2], K. L. HUHMAN[2]; [1]GSU Neurosci. Inst., Atlanta, GA; [2]GSU, Atlanta, GA

Abstract: Social defeat stress is well established to result in pervasive changes in social avoidance and behavioral adaptations that parallel the development and symptoms of depression and other psychiatric disorders. In order to identify targets for the treatment of these mental health disorders, our laboratory utilizes social defeat stress to elucidate the specific circuitry and mechanisms that regulate these long-lasting behavioral changes. The medial prefrontal cortex (mPFC) is known to regulate emotional learning and to modulate conditioned defeat learning. Previous work has also found BDNF in the prelimbic cortex (PL) and infralimbic cortex (IL) subregions of the mPFC are critical for consolidation of fear learning and extinction, respectively (Choi et al 2010, Peters et al., 2010). The purpose of this study was to test the hypthothesis that BDNF/TrkB signaling in the PL and IL differentially regulate conditioned defeat learning. Hamsters were implanted with cannulae targeting either the PL or IL and microinjected with BDNF (0.4ug/100 nl saline) or vehicle 30 min prior to a 15 min defeat stress by a resident aggressor. The following day, the defeated hamsters were tested for defeat-induced social avoidance with a confined resident aggressor in a novel testing arena. During this test, animals previously infused with BDNF in the PL demonstrated a robust attenuation in avoidance relative to vehicle -infused controls. In contrast, the animals infused with BDNF in the IL had greater

avoidance behavior relative to vehicle controls. These data suggest that TrkB signaling in the PL and IL have opposing roles in modulating conditioned defeat learning. With the specificity and solubility limitations of currently available TrkB receptor antagonists, we are now beginning to investigate the necessity of PL and IL TrkB signaling in social stress-induced behavioral change utilizing $TrkB^{F616A}$ mutant mice. These mice have a single point mutation within the TrkB receptor, resulting in its being highly sensitive to inhibition by small PP1-derivative molecules, in particular, 1-NM-PP1. Future experiments will also utilize site-specific inducible knockdown of BDNF gene expression in BDNF floxed mice to test whether PL and IL BDNF expression is critical for conditioned defeat learning. Supported by NIMH award R01MH062044 to KLH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosures: D.C. Choi: None. K.A. Partrick: None. A.M. Rosenhauer: None. K.L. Huhman: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.13/ZZ9

Topic: F.04. Stress and the Brain

Support: NIH NRSA F31AI106357

Title: Transcriptional regulation of corticotropin-releasing factor gene expression

Authors: *L. AGUINIGA[1], D. J. KLUMPP[2];
[2]Urology, [1]Northwestern Univ., Chicago, IL

Abstract: Corticotropin−releasing factor (CRF) has been well established as a key mediator of stress responses and voiding control, where increased CRF levels in Barrington's nucleus induce urinary retention and bladder dysfunction. Arachidonic acid (AA) metabolites have been shown to modulate CRF expression, however the transcriptional mediators of this modulation are unknown. We are investigating the role of PPARgamma and Ahr in AA−dependent CRF induction. We used MIRAGE software to identify candidate transcription factor binding sites in a 1kb region of the human CRF gene promoter. We identified a peroxisome proliferator−activated hormone response element (PPRE) and two Xenobiotic Responsive Element (XRE) sites as candidate mediators of AA−dependent CRF induction. Site−directed mutations of the PPRE and XRE sites were generated in a CRF−luciferase reporter plasmid. We evaluated expression of WT and the mutants in HEK 293T cells for their responses to AA. We

also transfected in transcription factors AhR and PPAR and evaluated AA−dependent CRF induction. Mutation of XRE1 resulted in significantly decreased basal CRF expression, while mutation of XRE2 or PPAR had modest effects. However upon AA induction, the PPRE mutant had increased CRF expression compared to WT, whereas XRE1 had decreased expression. The double mutation of XRE1 and XRE2 resulted in decreased responsiveness to AA. Co−transfection with PPAR had modest effects; however, AhR resulted in a significant increase in AA−dependent CRF expression. Over−expression of AhR and PPAR resulted in inhibition of AhR−dependent CRF induction. These results suggest AhR binding to the XRE sites modulates AA−dependent CRF gene expression. Continued studies using animal models will examine the role of such factors in modulating voiding in response to stress.

Disclosures: L. Aguiniga: None. D.J. Klumpp: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.14/ZZ10

Topic: F.04. Stress and the Brain

Support: grants-in-aid for Scientific Research (C) from Japan Society for the Promotion of Science (JSPS) No. 24500443

Title: The aberrant behavior and altered protein expression associated with neuropsychiatric disorders in crmp2 gene-deficient mice

Authors: *H. NAKAMURA[1,2], N. YAMASHITA[1], A. KIMURA[5], Y. KIMURA[5], H. HIRANO[5], H. KIYONARI[6], G. SHIOI[6,7], H. MAKIHARA[1], Y. KAWAMOTO[2], A. JITSUKI-TAKAHASHI[1], K. YONEZAKI[3], K. TAKASE[8], T. MIYAZAKI[3,4], F. NAKAMURA[1], F. TANAKA[2], Y. GOSHIMA[1];
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Abstract: Collapsin response mediator protein 2 (CRMP2) was originally identified as an intracellular mediator for the repulsive axon guidance molecule, Semaphorin3A. CRMP2 plays a key role in axon guidance, dendritic morphogenesis, and cell polarization. CRMP2 is implicated

in various neurological and psychiatric disorders. However, *in vivo* functions of CRMP2 remain unknown. We generated CRMP2 gene-deficient (*crmp2*$^{-/-}$) mice to examine their behavioral phenotypes. During 24-h home cage monitoring, the activity level during the dark phase of *crmp2*$^{-/-}$ mice was significantly higher than that of wild-type (*WT*) mice. Moreover, the time during the open arm of an elevated plus maze was longer for *crmp2*$^{-/-}$ mice than for *WT* mice. The duration of social interaction was shorter for *crmp2*$^{-/-}$ mice than for *WT* mice. *Crmp2*$^{-/-}$ mice also showed mild impaired contextual learning. Because the mechanisms that contribute to the response to methamphetamine may be relevant to a variety of psychiatric disorders, we examined the methamphetamine-induced behavioral change of *crmp2*$^{-/-}$ mice. Methamphetamine-induced hyperlocomotion was observed in both *WT* and *crmp2*$^{-/-}$ mice, but *crmp2*$^{-/-}$ mice showed drastic behavioral changes compared to *WT* mice. *Crmp2*$^{-/-}$ mice also showed altered expression of proteins involved in GABAergic synapse, glutamatergic synapse and neurotrophin signaling pathways. In addition, SNAP25, RAB18, FABP5, ARF5, and LDHA, which are related genes to schizophrenia and methamphetamine sensitization, are also altered in *crmp2*$^{-/-}$ mice. Our study implies that dysregulation of CRMP2 may be involved in pathophysiology of neuropsychiatric disorders.

Disclosures: H. Nakamura: None. N. Yamashita: None. A. Kimura: None. Y. Kimura: None. H. Hirano: None. H. Kiyonari: None. G. Shioi: None. H. Makihara: None. Y. Kawamoto: None. A. Jitsuki-Takahashi: None. K. Yonezaki: None. K. Takase: None. T. Miyazaki: None. F. Nakamura: None. F. Tanaka: None. Y. Goshima: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.15/ZZ11

Topic: F.04. Stress and the Brain

Support: 2014 CARIPLO Foundation BIomedical Research conducted by Young Researchers

MIUR (PRIN 2012 prot-2012A9T2S9)

Title: Acute ketamine reverses changes in glutamate release and related molecular mechanisms induced by chronic stress

Authors: *L. MUSAZZI[1], P. TORNESE[1], N. SALA[1], M. SEGUINI[1], M. MILANESE[2], T. BONIFACINO[2], D. BONINI[3], G. RACAGNI[1], A. BARBON[3], G. BONANNO[2], M. POPOLI[1]; [1]Univ. of Milano, Milano, Italy; [2]Univ. of Genova, Genova, Italy; [3]Univ. of Brescia, Brescia, Italy

Abstract: Increasing evidence has associated dysfunction of the glutamate system with the pathophysiology of neuropsychiatric disorders. Consistently, the NMDA receptor antagonist ketamine (KET) was demonstrated to induce rapid and sustained antidepressant effects.
Using a chronic mild stress (CMS) model of depression, we studied the effects of chronic stress and KET on glutamate release and related molecular mechanisms in the hippocampus (HPC) and prefrontal/frontal cortex (PFC/FC) of rats. Sucrose preference test was used to distinguish stress resilient (CMS-R) from vulnerable rats (CMS-V).
A significant increase of basal glutamate release from superfused synaptic terminals was measured in HPC and PFC/FC of CMS-V; acute KET restored glutamate release to control levels. Moreover, CMS significantly increased the levels of synaptic mineralocorticoid receptor levels, and decreased total and synaptic metabotropic glutamate receptor 2 expression, selectively in CMS-V. These changes were reversed by acute KET treatment. Finally, a significant reduction in dendritic trafficking of total BDNF and BDNF-6 splice variant transcripts was measured in CA1 and PFC/FC of both CMS-R and CMS-V, and in CA3 of CMS-V. KET partly reversed these changes.
These results suggest that chronic exposure to stress induces significant functional and molecular alterations, which are modulated by KET. Further investigation of the mechanisms underlying individual vulnerability to stress could help to clarify the neurobiological underpinnings of depression and fast-antidepressant effect.

Disclosures: L. Musazzi: None. P. Tornese: None. N. Sala: None. M. Seguini: None. M. Milanese: None. T. Bonifacino: None. D. Bonini: None. G. Racagni: None. A. Barbon: None. G. Bonanno: None. M. Popoli: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.16/ZZ12

Topic: F.04. Stress and the Brain

Support: National Institute of Mental Health Grant P50MH096890

Title: Engineered transcription factors targeted to the Fosb gene are sufficient to model the epigenetic and transcriptional phenomena that underlie stress-related behaviors

Authors: *P. J. HAMILTON[1], E. A. HELLER[4], D. D. BUREK[2], S. I. LOMBROSO[2], S. T. PIRPINIAS[2], M. FARIS[2], J. D. KOURIS[2], A. D. AVAKIAN[2], H. M. CATES[2], R. L. NEVE[5], E. J. NESTLER[3];

[1]Neurosci. Program, [3]Fishberg Dept. of Neurosci. and Friedman Brain Inst., [2]Mount Sinai Sch. of Med., New York, NY; [4]Univ. of Pennsylvania, Philadelphia, PA; [5]MIT, Boston, MA

Abstract: Multiple studies have implicated genome-wide epigenetic remodeling events in brain reward regions following stress exposure. However, only recently has it become possible to target a given type of epigenetic remodeling to a single gene of interest, in order to probe the causal relationship between such regulation and neuropsychiatric disease (Heller et al., *Nat Neurosci*, 2014). Here, histone post-translational modifications were targeted specifically to the *Fosb* gene locus using engineered zinc-finger proteins (ZFPs). *Fosb*-ZFPs were fused to either the transcriptional repressor, G9a, that promotes histone methylation or the transcriptional activator, p65, that promotes histone acetylation. These ZFPs were expressed selectively in D1 vs. D2 medium spiny neurons (MSNs) using Cre-dependent viral expression in the nucleus accumbens (NAc) of mice transgenic for Cre-recombinase in these MSN subtypes. We find that *Fosb*-targeted histone acetylation in D2-MSNs and *Fosb*-targeted histone repressive methylation in D1-MSNs promotes susceptibility to social stress, while *Fosb*-targeted histone methylation in D2-MSNs and *Fosb*-targeted histone acetylation in D1-MSNs promotes resilience to social stress. Thus, we observe that cell- and gene-specific targeting of histone modifications *in vivo* is sufficient to model natural transcriptional phenomena that underlie stress-related behaviors. In light of these findings, we are broadening our technical repertoire to include CRISPR/Cas9 technology, in an effort to model other transcriptional events that underlie stress-related behaviors. To this end, we designed guide RNAs to target nuclease dead Cas9 (dCas9) fused to novel effector domains to the *Fosb* gene locus. We observe that dCas9 fused to the transcriptional activator, VP64, or the transcriptional repressor, KRAB, and targeted to specific locations in the *Fosb* promoter is sufficient to regulate *FosB* and *deltaFosB* mRNA levels, in both cultured cells and the NAc. These results suggest the validity in engineering ZFPs and dCas9 as DNA-binding factors to induce transcriptional events that control stress responses.
Supported by NIMH

Disclosures: P.J. Hamilton: None. E.A. Heller: None. D.D. Burek: None. S.I. Lombroso: None. S.T. Pirpinias: None. M. Faris: None. J.D. Kouris: None. A.D. Avakian: None. H.M. Cates: None. R.L. Neve: None. E.J. Nestler: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.17/ZZ13

Topic: F.04. Stress and the Brain

Support: NWO ALW 823.02.002

Title: The NeuroD element is associated with selectivity for mineralocorticoid over glucocorticoid receptor binding in the brain

Authors: *L. T. C. M. VAN WEERT[1,3,4], J. C. BUURSTEDE[1], A. MAHFOUZ[2], P. S. M. BRAAKHUIS[1], J. A. E. POLMAN[5], H. C. M. SIPS[1], B. ROOZENDAAL[3], E. R. DE KLOET[5], N. A. DATSON[5], O. C. MEIJER[1];
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Abstract: In the limbic brain, mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) both function as receptors for glucocorticoids. MR and GR mediate distinct effects on cellular physiology via transcriptional mechanisms, but how target gene specificity comes about has remained enigmatic. We have detected DNA binding sites of MR and GR in the hippocampus of rats injected IP with corticosterone (3 mg/kg), using chromatin immunoprecipitation followed by sequencing (ChIP-Seq), in order to identify the extent of MR/GR binding selectivity and underlying mechanisms.
This dataset revealed 918 MR- and 1450 GR-specific binding sites. *De novo* motif analysis resulted in a similar binding motif for both proteins at 100% of the target loci, which matched the known glucocorticoid response element (GRE). An additional motif was found next to the GRE, that co-occurred near all MR-specific binding sites, but was absent for GR-specific or MR-GR overlapping sites.
Members of the NeuroD family of bHLH proteins were hypothesized to bind this additional motif and affect MR/GR signalling. Neurod1, Neurod2 and Neurod6, showed hippocampal expression and could act as DNA-binding transcriptional coactivators of MR/GR at the GRE in reporter assays in non-neuronal cells. Both N-terminal and C-terminal lacking receptor truncates could be potentiated, suggesting NeuroD factors interact with proteins in the MR/GR signalling complex rather than the receptors themselves. In conclusion, a NeuroD factor binding to an additional motif near the GRE seems to result in MR selective signaling in the limbic brain.

Disclosures: L.T.C.M. Van Weert: None. J.C. Buurstede: None. A. Mahfouz: None. P.S.M. Braakhuis: None. J.A.E. Polman: None. H.C.M. Sips: None. B. Roozendaal: None. E.R. de Kloet: None. N.A. Datson: None. O.C. Meijer: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.18/ZZ14

Topic: F.04. Stress and the Brain

Support: NSERC Grant 249853

NSERC Vanier CGS

Title: Mild chronic unpredictable stress is not sufficient toinduce depressive-like symptoms in rats

Authors: *C. J. FONTAINE, C. CHIU, S.-Y. YAU, A. R. PATTEN, B. R. CHRISTIE;
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Abstract: Depression is a mental disorder that is characterized by affective, cognitive, and physiological impairments that affects over 100 million people worldwide. Chronic unpredictable stress (CUS) is a common model for the induction of depression, however the initial development of depression-like symptoms under mild conditions remains controversial. In the present study we have used a mild CUS protocol for 2 or 4 weeks to examine key changes to gross physiology that have been associated with depression. These included monitoring for changes in body and adrenal gland weight, as well as for changes in systemic corticosteronelevels and levels of glutathione and adiponectin in the brain. We hypothesized that a depression-like state would be associated with reductions in body weight, glutathione and adiponectin as well as increases in adrenal gland weights and circulating corticosterone levels. Neither 2 nor 4 weeks following mild CUS is sufficient to produce depressive-like symptoms as described above. Interestingly, in contrast to our predictions, we saw increases in glutathione and adiponectin in the hippocampus following 2 weeks of CUS. Our data provide evidence for potential protective and compensatory actions of glutathione and adiponectin in the hippocampus under mild stress conditions.

Disclosures: C.J. Fontaine: None. C. Chiu: None. S. Yau: None. A.R. Patten: None. B.R. Christie: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.19/AAA1

Topic: F.04. Stress and the Brain

Title: Deletion of regulator of g protein signaling 2 promotes fear memory in pavlovian fear condtioning

Authors: *A. RAAB[1], L. HOMMERS[2], S. POPP[3], K.-P. LESCH[3], J. DECKERT[4];
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Abstract: Anxiety and depressive disorders result from a complex interplay of genetic factors, cellular signaling, personality traits as well as stressful life events and are common mutual comorbidities. Regulator of G protein signaling 2 (Rgs2), a protein negatively regulating G protein coupled receptor signaling by increasing the GTPase activity of the Gα protein subunit has been reported to be involved in human and rodent anxiety.
Here we investigate, whether homozygous Rgs2 deficient ($Rgs2^{-/-}$) mice show behavioral alterations related to human anxiety and depressive disorders.
Innate anxiety was evaluated using elevated plus maze, dark/light box and open filed tests. $Rgs2^{-/-}$ mice showed significantly increased avoidance behavior in all three tests, indicating heightened innate anxiety. Pavlovian fear conditioning (FC) revealed significantly faster learning in the conditioning session as well as significantly augmented short-term fear memory in context and cue tests 24 h later in $Rgs2^{-/-}$ mice. Long-term fear memory and extinction assessed by cue and context tests one and two weeks after conditioning, revealed faster extinction in $Rgs2^{-/-}$ mice. Acute stress via FC did not augment anxiety-like behavior but provoked locomotor suppression for both genotypes indicating increased cautious behavior. Chronic mild stress (CMS) potentiated anxiety-like behavior in wildtype mice in dark/light box and showed a gender specific effect in $Rgs2^{-/-}$ mice. While CMS led to an increase in anxiety-like behavior in male $Rgs2^{-/-}$ mice, female mice exhibited reduced anxiety-like behavior and increased locomotor activity. $Rgs2^{-/-}$ mice exhibited depressive-like behavior in forced swim test compared to wildtype controls, but CMS did not alter immobility times.
Expression of Rgs2 was analyzed 1h and 6h after FC. Mild dynamic changes of Rgs2 expression in wild type mice were detected, suggesting Rgs2 to act as an inducible modulator (intermediate early gene) in G protein pathways involved in fear memory. MicroRNA expression patterns were analyzed using small RNA sequencing in selected brain regions 1h and 6h after FC. Moreover,

Rgs2$^{-/-}$ mice showed significantly decreased levels of monoaminergic neurotransmitters as well as decreased expression of *ADRA2A, ADRA2B*, *ADRA2C, DRD2* and increased expression of *GABA2* and *DRD2* suggesting a regulatory influence of Rgs2 on the monoaminergic system. In conclusion, downregulation of Rgs2 leads to increased innate anxiety as well as learned fear and may result from deregulation of monoaminergic signaling.

Disclosures: A. Raab: None. L. Hommers: None. S. Popp: None. K. Lesch: None. J. Deckert: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.20/AAA2

Topic: F.04. Stress and the Brain

Support: Empire Clinical Research Investigator Program of NY State (ECRIP)

CWPW grant program

Children's Health and Research Foundation

Title: Molecular fundamentals of the impaired epinephrine response following recurrent hypoglycemia: a clue from microarray analysis

Authors: *B. B. NANKOVA[1], J. KIM[2], N. KUDRICK[2], E. F. LAGAMMA[3];
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Abstract: An attenuated sympathoadrenal response to hypoglycemia is a major component of Hypoglycemia-associated autonomic failure (HAAF), a well-known neuroendocrine complication in children and adults on insulin therapy for type 1 and type 2 diabetes. The molecular mechanisms underlying this defect are still elusive. Recently we reported an initial rise in tyrosine hydroxylase activity and gene transcription during HAAF, suggesting intact CNS - adrenal synaptic signaling. Yet, epinephrine responses were impaired and this correlated with reduced adrenal capacity to produce catecholamines indicating that peripheral components of the sympathoadrenal system may be directly affected by recurrent hypoglycemia (RH). We hypothesized that both, extensive cholinergic signaling between the splanchnic nerve fibers and

the adrenal medulla and cellular endoplasmic reticulum (ER) stress caused by prolonged/repeated glucose withdrawal, contribute to the defective adrenal epinephrine release (and production) in HAAF. To gain further insight into the molecular mechanism(s) mediating the blunted epinephrine responses following RH we utilized global gene expression profiling approach. SD rats were subjected to insulin-induced RH or received saline injections. After the antecedent treatments and overnight fast all animals underwent a hypoglycemic-hyperinsulinemic clamp (target glucose levels 45mg/dL). Set of animals from each group (n>6) were sacrificed before or during the clamp, total RNA isolated from individual AM samples and subjected to microarray analysis. Differentially expressed genes (DEGs) were identified and ranked according to significant up- or down-regulation of twofold or more (*P≤ 0.05). Based on comparison analysis of DEGs, sets of genes unique for RH were revealed. A complementary bioinformatics analysis confirmed activation of the unfolded protein response (UPR) - common cellular defense mechanism which allows for recovery of ER homeostasis, cell adaptation and survival, or in case of chronic or severe stress favors induction of apoptosis and cell death. Furthermore, the data point to at least three additional pathway/process networks altered in the AM following RH which may contribute to the impaired epinephrine secretion in HAAF: increased neuropeptide (PENK, NPY, GAL) signaling; altered ion homeostasis (Na+, K+, Ca^{2+}) and downregulation of multiple proteins involved in Ca^{2+}-dependent exocytosis. Given the pleiotropic effects of the UPR in different organs, involved in maintaining glucose homeostasis these findings indicate a plausible additional mechanism for maladaptive responses to recurrent hypoglycemia.

Disclosures: B.B. Nankova: None. J. Kim: None. N. Kudrick: None. E.F. LaGamma: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.21/AAA3

Topic: F.04. Stress and the Brain

Support: MIUR (PRIN 2009 prot.2009BRMW4W_001)

Fondazione Cariplo Prog. 2011-0635

ECNP Research Grant for Young Scientist 2010

MIUR (PRIN 2012 prot-2012A9T2S9)

Title: Acute stress is not acute: early and sustained effects of acute stress

Authors: *M. POPOLI[1], L. MUSAZZI[1], P. TORNESE[1], N. SALA[1], G. TRECCANI[1,2], D. TARDITO[1], M. SEGUINI[1];
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Abstract: Compelling evidence has shown that stress destabilizes structure and function of limbic/cortical areas, and represents a risk factor for stress-related neuropsychiatric disorders. In recent studies, our group contributed to the understanding of essential destabilizing effects of stress in prefrontal cortex (PFC). We showed previously that acute inescapable footshock (FS)-stress rapidly enhances depolarization-evoked glutamate (Glu) release/transmission in PFC, by increasing corticosterone (CORT) levels, stimulation of CORT synaptic receptors, and rapid (non-genomic) enhancement of the readily releasable pool (RRP) of presynaptic vesicles in perforated synapses, mediated by synapsin I phosphorylation. Moreover, we showed that stress at the same time dramatically increases (+42%) the total number of non-perforated synapses in prelimbic PFC. In addition, we observed significant atrophy/remodeling of apical dendrites as early as 24 hours after stress, sustained for up to 14 days. These findings provided new evidence that acute stress has both early and sustained effects on structure and function of excitatory circuitry. We show now that hyperactivation of glutamate release/transmission in PFC lasts for at least 24 hours after acute stress, with increased Glu release and RRP (up to 24 h), and synapsin I phosphorylation (up to 6 h). Mineralocorticoid receptor expression level in presynaptic membranes increased immediately after the stress protocol, while glucocorticoid receptor increased after 6 h.
In order to identify epigenetic changes responsible for early and sustained changes in synapses/circuitry, we measured the expression of 21 microRNAs (miRNAs), selected for their implication in the modulation of synaptic plasticity and antidepressant effect, in the PFC immediately after 40 minutes of acute FS-stress. We found 4 miRNAs significantly downregulated. Bioinformatic analysis results highlighted enrichment of miRNA targets in different pathways, some of them related to neuronal functions, synaptic plasticity and the stress response. Expression changes in gene targets were validated by qPCR and Western blot. Overall, our results show that acute stress may exert sustained effects in PFC, with relevance to stress-related disorders, such as depression, anxiety and PTSD.

Disclosures: M. Popoli: None. L. Musazzi: None. P. Tornese: None. N. Sala: None. G. Treccani: None. D. Tardito: None. M. Seguini: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.22/AAA4

Topic: F.04. Stress and the Brain

Support: NSF Grant IOS1456706

Title: Effects of acute systemic corticosterone treatment on clock gene expression in the male rat brain

Authors: *M. J. HARTSOCK, A. C. TOMCZIK, A. M. JANAS, L. E. CHUN, E. R. WOODRUFF, R. L. SPENCER;
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Abstract: Daily oscillations in gene expression, or circadian clocks, are present in cells throughout the body and serve to optimize cell function. These clocks consist of positive arm genes *Bmal1* and *Clock*, whose protein products induce transcription of negative arm genes *Per* and *Cry*. PER and CRY proteins then feed back to inhibit the positive arm, creating a molecular oscillation with a period of ~24 h. In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is a uniquely light-responsive brain region that entrains peripheral clocks to the solar day. The SCN regulates diurnal release of adrenal glucocorticoid hormones (CORT), which mediate circadian entrainment of many tissues in the periphery. Circadian clocks are also present in extra-SCN brain regions that receive no direct input from either light or the SCN, and it has been hypothesized that these extra-SCN brain clocks may also depend on CORT as a critical circadian entraining factor. Notably, the clock gene *Per1* has a CORT receptor response element in its promoter region, a potential mechanism for such entrainment. Presented here are two experiments examining whether CORT alone is sufficient to induce *Per1* expression in brain regions important for emotion control in male Sprague-Dawley rats maintained on a 12:12h light:dark cycle. In the first experiment, rats underwent adrenalectomy (ADX) or sham surgery, and ADX rats received CORT in the drinking water to maintain circadian CORT elevations at the start of the active phase, when water consumption is greatest. Five days later, all rats were administered CORT (2.5 mg/kg, ip) or vehicle during the inactive phase and killed 60 min later. Acute CORT increased *Per1* mRNA in the hypothalamus and neocortex, but only in ADX rats. In the second experiment, rats underwent ADX and were then supplemented with CORT in the drinking water for the first 2 h of the active phase. Three days later, rats were administered CORT (2.5 mg/kg, ip), vehicle, or no injection during the active or inactive phase and killed 30 min later. Acute CORT increased *Per1* mRNA in the neocortex in both CORT and vehicle-treated rats, but not in rats receiving no injection, suggesting that the stress of injection was sufficient to induce *Per1* expression even in the absence of stress-induced CORT levels. No time of day effects were observed. Our results align with our past work showing that 30 min of acute restraint stress rapidly increases *Per1* mRNA in the rat hypothalamus and neocortex, but that this induction is abrogated by ADX only in the hypothalamus. These findings suggest that acute stress may induce *Per1* expression differently in different brain regions, through both CORT-dependent and CORT-independent mechanisms.

Disclosures: M.J. Hartsock: None. A.C. Tomczik: None. A.M. Janas: None. L.E. Chun: None. E.R. Woodruff: None. R.L. Spencer: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.23/AAA5

Topic: F.04. Stress and the Brain

Title: Characterization of glucocorticoid-induced loss of DNA methylation of the stress gene Fkbp5 in neuronal cells

Authors: *O. H. COX, H. SONG, N. GADIWALLA, J. MENZIES, R. LEE;
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Abstract: Exposure to stressful and traumatic events especially during neurodevelopment leads to increased susceptibility to neuropsychiatric diseases. Emerging evidence suggests that exposure to stressful events is associated with molecular changes in genes that mediate or are targets of glucocorticoid (GC) signaling. In particular, the *FK506* binding protein 5 (*FKBP5*), a major regulator of the stress response and intracellular GC signaling, has been implicated in anxiety, depression, PTSD, and bipolar disorder. It has been shown to undergo epigenetic and transcriptional changes following exposure to stress. However, mechanisms that govern these changes are largely undetermined. To model the effect of GC exposure on the brain, the HT-22 mouse hippocampal cell line was treated with GCs for 7 days. Following treatment, cells were examined for epigenetic and transcriptional changes. *Fkbp5* exhibited a loss of DNA methylation and an increase in expression. The methylation marks persisted for one month following initial GC exposure in the absence of additional GCs, whereas expression returned to baseline levels soon after the initial exposure period. We then examined whether the GC-induced loss of methylation in the neuronal cells involved cell division. Interestingly, cells treated with mitomycinC or nocodazole, which inhibit cell division, prevented most of the GC-induced loss of methylation, implicating cellular division in GC-induced DNA methylation loss. We then characterized the functional significance of the GC-induced loss of DNA methylation. In the cell line, previous 7-day exposure to GCs caused a more robust expression of *Fkbp5* (45% increase over previously unexposed, p=0.008) when GCs were introduced a second time. Similarly, an *in vitro* reporter assay consisting of a CpG-free plasmid, *Fkbp5* intronic GRE (GC response element), and the luciferase gene showed significantly higher luminometric activity when the GRE was unmethylated (303% increase over the methylated GRE, p=0.0005). This suggests that previous exposure and loss of methylation can result in greater activation of *Fkbp5* in response to GC exposure. We are currently testing the interaction between the promoter and the GRE in the presence of GCs and are characterizing the role of the methyltransferase DNMT1 in the observed GC-induced methylation loss of *Fkbp5*. In summary, GCs can lead to persistent changes in DNA methylation and potentially affect gene function during second exposure events, conveying

future “risk” to a previously exposed individual. These findings may provide an important mechanistic understanding of the consequences of stress exposure in psychiatric disorders.

Disclosures: O.H. Cox: None. H. Song: None. N. Gadiwalla: None. J. Menzies: None. R. Lee: None.

Poster

253. Cellular Effects of Stress

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 253.24/AAA6

Topic: F.04. Stress and the Brain

Support: RO1MH103102

RO1MH100894

Title: Environmentally malleable epigenomic regions in the mammalian brain

Authors: *S. ODELL[1], S. L. KLEIN[2], A. SHARMA[2], E. MITCHELL[2], M. TOTH[2];
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Abstract: Environmental experience can shape behavioral responses via gene expression through epigenetic mechanisms, including DNA methylation and histone modifications. Early life adversity is a form of environmental experience that has long been studied in both humans and rodent models, due to its neuropsychiatric consequences in adulthood. Previously, the approach to investigate the epigenetics of early life adversity has been limited to the investigation of candidate genes. Our current work took an unbiased approach, looking at genome-wide changes in methylation in response to environmental challenge by enhanced reduced representation bisulfite sequencing (eRRBS). In order to specify the epigenetic signatures of early life adversity in mice, we used various pre/postnatal environmental insults including maternal anxiety (5-HT1AR deficit elicits anxiety-like behavior), maternal infection (injection of 50μg/mg of LPS once a day from E16 to E18), and maternal separation (3 hours/day from P3 to P14). In addition displaying model specific behaviors, all three of these adverse life situations lead to innate fear/anxiety in adult mice, demonstrated by decreased entrances and time spent in the open arms of the elevated plus maze, a behavior linked to the ventral dentate gyrus (vDG). Likewise we found these models share common epigenetic signatures in the vDG, defined as early life adversity differentially methylated regions (ELA-DMRs). We identified over a hundred ELA-DMRs, 200-300bp long regions in the mouse genome whose cytosine methylation status are inherently labile and responds with permanent changes in methylation to

at least two different early life adverse situations. We found our ELA-DMRs to have intrinsic DNA methylation-dependent enhancer-like activity in vitro by luciferase reporter assay. Further, network analysis reveals ELA-DMRs to be embedded in synaptic related genes, including enrichment in cytoskeletal rearrangement, synaptic plasticity cytoskeletal reorganization, transcription and apoptosis pathways. Further, *DNMT3a* knockout studies reveal that methylation patterns of ELA-DMRs in the vDG are *DNMT3a* dependent as well as developmentally delayed compared to global biomodal methylation. In conclusion our analysis provide evidence that ELA-DMRs and their experience dependent methylation shift is a strong candidate in connecting environmental input to gene expression and ultimately synaptic, neuronal network and behavioral changes.

Disclosures: S. Odell: None. S.L. Klein: None. A. Sharma: None. E. Mitchell: None. M. Toth: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.01/AAA7

Topic: F.08. Biological Rhythms and Sleep

Title: Physiological variations in the number of hypocretin and histamine producing neurons

Authors: R. MCGREGOR, L. SHAN, M.-F. WU, *J. M. SIEGEL;
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Abstract: Waking and sleep states are the result of the coordinated activities of different neuronal systems. Amongst them, arousal promoting cell groups like hypocretin (Hcrt) and histamine producing neurons play a critical role in waking whereas melanin concentrating hormone (MCH) expressing neurons are involved in sleep regulation. Consistent with their role in modulating each state, Hcrt and histamine neurons show the highest levels of activity during the active phase whereas the peak activity of MCH neurons has been reported to be prior to and during the inactive phase. In spite of the extensive evidence indicating circadian modulation of these systems, studies have so far assumed that the number of detectable neurons in each neurochemical group remains constant through the different behavioral states. To measure any variation in the number of immunohistochemically detectable neurons, we sacrificed groups of animals at 5 h into the light phase or 5 hours into the dark phase. We then processed the tissue for Hcrt, histidine decarboxylase (HDC) and MCH. We observed that the animals sacrificed during the dark phase showed a significantly greater number of Hcrt and histamine neurons compared to the animals sacrificed during the light phase. We did not observe differences in cell

size between conditions in Hcrt and HDC cells. The increase in Hcrt cell number was largely restricted to the medial hypothalamus. In contrast, we did not observe differences in the number of MCH neurons between the circadian phases. However, there was a significant increase in MCH cell size during the light phase. To further explore if there were more "undetected" Hcrt and MCH neurons we used colchicine to inhibit axonal transport. We observed that animals that received colchicine had a substantially greater number of Hcrt containing neurons compared to the highest number observed under physiological conditions. But we did not see an increase in the number of MCH cells with colchicine. This study shows that the cell number of Hcrt and HDC expressing neurons changes across the 24 hour period. We also find that the control of cell size and number varies across transmitter groups. Furthermore, we observed, as revealed by colchicine administration, that there is a greater number of neurons capable of Hcrt production than previously thought. These results have important physiological implications as well as suggesting the possibility of treatment of certain pathologies like narcolepsy, characterized in human by the loss of Hcrt neurons, by increasing peptide production in these "hidden" Hcrt neurons.

Disclosures: R. McGregor: None. L. Shan: None. M. Wu: None. J.M. Siegel: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.02/AAA8

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant 1R01NS091126

NIH Grant R01NS024736

Title: Orexin neurons are inhibited by local GABAergic neurons

Authors: *L. FERRARI, D. PARK, E. ARRIGONI;
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Abstract: The Basal Forebrain (BF) is a crucial region of the wake promoting systems in the brain and it contains a variety of cells including neurons that produce acetylcholine, glutamate and GABA. Recent work has shown that selective activation of GABAergic neurons in the BF through chemogenetics techniques produced sustained wakefulness associated to fast cortical rhythms in freely behaving mice (Anaclet et al., 2015 and Xu et al., 2015). The downstream circuit through which these neurons are able to produce wakefulness it is still unknown. Our

hypothesis is that BF GABAergic neurons can promote wakefulness by means of inhibition of GABAergic neurons in the Lateral Hypothalamus (LH), thus dis-inhibiting wake promoting neurons in the LH, such as orexin neurons. In this work we applied channelrhodopsin circuit assisted mapping (CRACM) to study if LH GABAergic neurons are able to directly inhibit orexin neurons. To test the presence of a functional connectivity between LH GABAergic neurons and orexin neurons we stereotaxically injected vGAT-cre mice in the perifornical region with a cre-dependent AAV ChR2 and an AAV-h-orexin-tdTomato (Saito et al. 2013), this resulted in the expression of channelrhodopsin (ChR2) in GABAergic cells and Td-tomato in orexin neruons. Four weeks after the AAV injections we prepared coronal brain slice and performed whole-cell recording from Td-tomato positive cells (orexin neurons) while stimulating local GABAergic neurons expressing ChR2 with blue light pulses. Photostimulation of perifornical GABAergic neurons expressing ChR2 evoked inhibitory postsynaptic current (IPSC) in 6 out of 9 orexin neurons. These photoevoked IPSCs were blocked 20uM of the $GABA_A$ receptor antagonist Bicuculline. In this work we have demonstrated that GABAergic neurons in the perifornical region inhibit orexin neurons through release of GABA and activation of postsynaptic $GABA_A$ receptors. We propose that LH GABAergic neurons inhibit wake active orexin neurons during sleep. During wakefulness LH GABAergic neurons are inhibited by wake promoting BF GABAergic neurons resulting in the dis-inhibition of the orexin neurons.

Disclosures: L. Ferrari: None. D. Park: None. E. Arrigoni: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.03/AAA9

Topic: F.08. Biological Rhythms and Sleep

Support: NIH K01AG041520

NIH NS052287

NIH NS084477

VAMC I01 BX000798

Title: Orexin gene transfer in pons projecting amygdala neurons suppresses cataplexy induced by predator odor in narcoleptic mice

Authors: *M. LIU[1], P. SHIROMANI[1,2], C. BLANCO-CENTURION[1], D. PELLURU[1], R. KONADHODE[1];

[1]Psychiatry and Behavioral Sci., Med. Univ. of SC, Charleston, SC; [2]Ralph H. Johnson VA Med. Ctr., Charleston, SC

Abstract: Narcolepsy, a sleep disorder associated with a massive loss of orexin neurons, is characterized by poor wake maintenance and sudden attacks of flaccid paralysis known as cataplexy. In both human and animal narcoleptics, cataplexy attacks could be triggered by strong emotions. The amygdala is important for regulating strong emotional responses and the loss of orexin may be critical for triggering cataplexy. We have shown that cataplexy attacks induced by predator odor can be blocked by orexin gene transfer into the amygdala in orexin-knockout mice. To further identify the specific amygdala-involved circuit responsible for emotion-induced cataplexy, we selectively expressed orexin in the amygdala neurons projecting to pons. In orexin-KO mice, recombinant adeno-associated viral vectors with Cre-driven expression of the mouse prepro-orexin gene, or GFP gene (rAAV-DIO-orexin or rAAV-DIO-GFP), were microinjected into the central nucleus of amygdala (CeA). Simultaneously rAAV-mCherry-IRES-WGA-Cre was injected into pontine areas including vlPAG and LC. Four weeks later sleep was recorded for 12h at night (no-odor). Subsequently mice were exposed to 1ml coyote urine upon lights-off, followed by 12h night sleep recording (odor night). Narcoleptic symptoms were assessed using Sleepsign software and video recordings. In rAAV-DIO-GFP control group, coyote urine odor dramatically triggered cataplexy attacks when compared to no-odor night ($n=7$, $p<0.05$). In rAAV-DIO-orexin group, Cre-driven orexin expression in CeA neurons significantly suppressed predator odor-induced cataplexy ($n=7$, $p<0.05$, compared to control group), while there was no significant effects on spontaneous cataplexy ($n=7$, $p>0.05$, compared to control group). These results indicated that amygdala-pons circuit mediates the emotion-induced cataplexy and could be served as a surrogate circuit to treat emotion-induced cataplexy.
Funded by the VAMC, NIA, NIMH, NINDS

Disclosures: M. Liu: None. P. Shiromani: None. C. Blanco-Centurion: None. D. pelluru: None. R. Konadhode: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.04/AAA10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant 2P01HL095491

Title: An *In vitro* study of pre and post synaptic cholinergic control of hypoglossal motor neurons in adult mice

Authors: *L. ZHU, L. L. FERRARI, D. PARK, N. L. CHAMBERLIN, E. ARRIGONI; Neurol., Beth Israel Deaconess Med. Center/ Harvard Med. Sch., Boston, MA

Abstract: In REM sleep, cranial respiratory muscles undergo a suppression of activity, with the genioglossus (GG) muscle showing the most dramatic suppression. A current hypothesis is that the loss of GG activity during REM sleep is mediated by a combination of 1) monoaminergic disfacilitation and 2) a direct cholinergic inhibition of hypoglossal motor neurons. Strikingly, blockade of cholinergic receptors in the hypoglossal motor nucleus fully restores REM sleep GG activity (Grace et al., 2013), suggesting that a cholinergic-mediated inhibition of hypoglossal motor neurons is largely responsible for the REM sleep suppression of GG activity. Previous *in vitro* electrophysiological work showed that hypoglossal motor neurons are excited by the cholinergic agonist carbachol via nicotinic receptors (Chamberlin et al., 2002). This work was conducted in neonatal rats, whereas, all the *in vivo* work has been done in adult animals.
In the current study, we tested the pre- and post-synaptic cholinergic control of hypoglossal motor neurons in brain slices from adult mice. We recorded from hypoglossal motor neurons in medullary slices of 18 6-8 week-old mice using whole-cell voltage-clamp mode. We found that bath application of the nonselective cholinergic agonist carbachol (15μM) (at -60mV in TTX) produced an outward (inhibitory) current mediated by muscarinic receptors in hypoglossal motor neurons. This inhibition was followed by a larger inward (excitatory) current mediated by nicotinic receptors.
We also expressed channelrhodopsin2 (ChR2) in glutamatergic hypoglossal premotor neurons of the intermediate medullary reticular formation (IRt) in 15 adult vglut2-cre mice. We photostimulated IRt axons/terminals with blue-light pulses and recorded photo-evoked excitatory postsynaptic current (EPSCs) in hypoglossal motor neurons. We found that photostimulation of the glutamatergic IRt input evoked AMPA-mediated EPSCs with short latency in hypoglossal motor neurons. Bath application of carbachol strongly inhibited the IRt glutamatergic excitation to hypoglossal nucleus via muscarinic receptors. Our results provide a possible mechanism for cholinergic inhibition of the hypoglossal motor neurons in REM sleep. We propose that direct cholinergic inhibition of hypoglossal motor neurons together with the cholinergic presynaptic suppression of the excitatory drive from the IRt hypoglossal premotor neurons can be responsible for the reduction in activity of hypoglossal motor neurons in REM sleep.

Disclosures: L. Zhu: None. L.L. Ferrari: None. D. Park: None. N.L. Chamberlin: None. E. Arrigoni: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.05/AAA11

Topic: F.08. Biological Rhythms and Sleep

Support: NIMH Grant 60670

Title: Malapropros noradrenaline impairs hippocampal plasticity and prevents memory consolidation

Authors: *M. A. FRAZER[1], A. SERGEEVA[2], B. GROSS[2], D. BAUER[2], K. SWIFT[2], G. POE[2]; [2]Anesthesiol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: The noradrenergic neurons of the locus coeruleus, which supply the forebrain with norepinephrine, fall silent prior to sleep spindle initiation, and are inhibited from firing during REM sleep. This decrease in noradrenaline allows for the depotentiation of previously potentiated circuits and overall bidirectional synaptic plasticity, resulting in the ability of new associations and information to be integrated into an existing memory network. However, some disorders, such as post traumatic stress disorder (PTSD), display a heightened noradrenergic system during sleep, resulting in aberrantly high levels of noradrenaline during sleep. It has been shown previously that high NE at the synapse results in hippocampal memory impairment, and we hypothesize that this impairment is due to NE acting to prevent depotentiation, leading to inflexible synaptic connections which precludes the incorporation of new memories into old schema during reconsolidation. We exogenously amplified the noradrenergic system during sleep and measured effects on hippocampal encoding during reversal learning, errors in memory consolidation, and effects on sleep spindles and REM architecture. Rats were instrumented for noradrenergic microinfusions to the hippocampus during sleep after learning, and were tested for the ability of to flexibly encode in an altered environment and for reversal learning and familiar reconsolidation. Aberrant presence of noradrenaline resulted in a hippocampal spatial map inflexible to novel information and impaired reversal learning, as evidenced by changes in hippocampal place cell stability and coherence. Reactivation episodes were also analyzed, and found to be altered in rats with inappropriately timed NE presence.

Disclosures: M.A. Frazer: None. A. Sergeeva: None. B. Gross: None. D. Bauer: None. K. Swift: None. G. Poe: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.06/AAA12

Topic: F.08. Biological Rhythms and Sleep

Support: NIH grant- 2PO1HL095491- 06

Title: CGRP neurons in the external lateral parabrachial nucleus and the pathways regulating hypercapnia induced cortical EEG arousal.

Authors: *S. KAUR, J. L. WANG, P. M. FULLER, C. B. SAPER;
Neurol., Beth Israel Deaconess Med. Ctr. and Harvard M, Boston, MA

Abstract: We have reported that glutamatergic signaling from neurons in the lateral parabrachial region mediates cortical arousal to hypercapnia. This region contains a population of neurons in the external lateral subnucleus (PBel) that express calcitonin gene-related peptide (CGRP) which has extensive forebrain projections and which we hypothesized may be necessary relay for the hypercapnic signal to cause arousal. To test this hypothesis, we conducted optogenetic inhibition of CGRP-PBel neurons and their terminal fields. To selectively inhibit CGRP-PBel, CGRPCre-ER mice (n=16) were injected on one side of the brain with an adeno-associated virus containing the gene for archaerhodopsin T TP009 (ArchT; n=12) in a Cre-inducible FLEX cassette AAV-FLEX-ArchT-GFP that expressed ArchT in CGRP positive PBel cells. In n=4 CGRPCre-ER mice, the control vector with no gene for ArchT (AAV-FLEX-GFP) was injected. On the other side we deleted the CGRP neurons by injecting a Cre dependent virus expressing the diphtheria toxin subunit A (AAV-FLEX-DTA) in all mice (n=16). All mice were instrumented for sleep recording and received unilateral implantation of an optic fiber above the PB. We investigated EEG arousals to 10% CO2 given for 30s every 300s, with and without inhibition of the PBel with a 593nm laser light. Laser light was on for 60 sec, beginning 20s prior and extending 10s after the CO2 stimulus. In 8 CGRP-Cre ER mice where the optical fiber targeted CGRPPBel, 593nm light increased the arousal latency significantly ($F_{3, 17}$= 31.2; P< 0.001) by fourfold 69.7 ± 6.7s (compared to no laser control-16.8 ± 0.6s) and in 49.8 ± 4.9% of the trials mice did not wake up to CO2 stimulus ($F_{3, 17}$= 31.1; P<0.001). To conduct optogenetic inhibition of the terminal fields of the CGRP-PBel neurons, CGRP-Cre ER mice were injected bilaterally with AAV-FLEX-ArchT-GFP in the PBel. The terminal fields of CGRP-PBel neurons such as central nucleus of amygdala (CeA) and substantia innominata in the basal forebrain (BF) were injected unilaterally (left side) with either 3% Ibotenic acid or combination of AAVCre + AAV-Flex-DTA (1:1) that caused unilateral cell deletion in the injected areas and the other side was implanted with optical fiber. Laser light induced Inhibition of the terminal fields at CeA (n=6) and BF (n=5), also increased the arousal latency to 30s CO2 significantly ($F_{3, 17}$= 22.0; P<0.001) to 48.2 ± 6.8 s and 54.7 ± 1.1 s ($F_{1, 7}$= 25.8; P<0.001) respectively. This inhibition of terminals in the CeA and BF also showed 32.55 and 52.4% of the trials respectively that failed to arouse to CO2. These results suggest that CGRP-PBel neurons mediate cortical EEG arousals to hypercapnia by its projections to the BF and CeA.

Disclosures: S. Kaur: None. J.L. Wang: None. P.M. Fuller: None. C.B. Saper: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.07/AAA13

Topic: F.08. Biological Rhythms and Sleep

Support: American Academy of Neurology/American Brain Foundation Clinician-Researcher Training Fellowship

NIH R01-NS085477

NIH R01-NS073613

Title: Activation of NOS1 neurons of the caudal hypothalamus produces prolonged wakefulness

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Abstract: The caudal hypothalamus is a long-appreciated component of the arousal system, but the precise neuronal populations, anatomical pathways and mechanisms underlying this are unknown. We recently showed that glutamate neurons of this region are wake-promoting when activated. A subpopulation of caudal hypothalamic glutamate neurons express nitric oxide synthase (NOS1). By stereotaxic microinjection of an adeno-associated viral vector into the the caudal hypothalamus, we were able to conditionally transduce the excitatory designer receptor (hM3-Dq) in NOS1 neurons in a NOS1-Cre mouse. After activation, mice showed prolonged wakefulness up to 6 hours. These results recapitulate the effects of activation of glutamate neurons of this region, suggesting that this NOS1 subpopulation of neurons may represent the wake-promoting subpopulation of caudal hypothalamic glutamate neurons. These neurons are a candidate component of the ascending arousal system.

Disclosures: N.P. Pedersen: None. C.B. Saper: None. P.M. Fuller: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.08/AAA14

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01MH099231

NIH P01NS083514

NIH T32 GM008962

Title: Sleep and wake differentially affect superficial and deep layers of mouse cortex

Authors: K. PEELMAN[1], C. M. FUNK[1], A. V. RODRIGUEZ[1], S. HONJOH[1], G. TONONI[1], *C. CIRELLI[2];
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Abstract: Cortical neurons tend to fire tonically during wake, while in NREM sleep they alternate between periods of strong firing and phases of neuronal silence. Several previous studies have found the mean cortical firing rate to be higher in wake and REM sleep than in NREM sleep. These general features of cortical neuronal activity have been described across many areas in both excitatory and inhibitory (fast spiking) neurons. It is also known that the sleep/wake effects on cortical activity can vary depending on neuronal subtypes, and that firing rates during wake are higher in the deep layers than in superficial layers. However, it is unclear as to whether sleep affects neuronal firing across layers in the same way. Here, we studied how wake, NREM sleep, and REM sleep influence the activity of single units in superficial and deep layers of several primary (V1, M1, S1) and secondary (V2, M2) cortical areas. Sleep/wake chronic polygraphic recordings were performed in freely-moving adult male mice (n=15) implanted with silicon probes spanning all cortical layers. Spike sorting was performed offline by superparamagnetic clustering of wavelet coefficients on multiunit clusters obtained over the light cycle, the major sleep phase in mice, and only well-isolated, stable single units were assessed. In excitatory cells (n=64), we found a significant decline in mean NREM firing relative to both active wake and REM sleep in both the superficial ($p<0.05$, paired t-test) and deep layers ($p<0.005$, paired t-test). The change in mean neuronal firing from active wake to NREM sleep was significantly more pronounced in the deep layers, which showed a 48% ± 17% (mean ± std) decrease in mean NREM firing relative to wake, whereas the superficial layers showed a 26% ± 19% decrease (super vs. deep NREM modulation, $p<0.005$, unpaired t-test). This pattern was prevalent across all cortical areas studied. By contract, preliminary observations in putative inhibitory neurons (n=14) showed comparable levels of decline in NREM sleep relative to active

wake across layers. Thus, across many cortical areas, the NREM sleep-dependent decline in excitatory neuronal activity is more pronounced in deep than in superficial layers.

Disclosures: K. Peelman: None. C.M. Funk: None. A.V. Rodriguez: None. S. Honjoh: None. G. Tononi: None. C. Cirelli: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.09/AAA15

Topic: F.08. Biological Rhythms and Sleep

Support: Department of Veterans Affairs

Title: Sleep-wake discharge activity of neurons in the parafacial zone in rats

Authors: *M. ALAM, A. KOSTIN, D. MCGINTY, R. SZYMUSIAK, N. ALAM;
Res. Service, VA Greater Los Angeles Healthcare Syst., North Hills, CA

Abstract: Introduction: Recent studies suggest that the medullary parafacial zone (PZ) plays a role in sleep-regulation. GABAergic neurons in the PZ express sleep-associated Fos-immunoreactivity and cell-specific lesions of this area cause suppression of nonREM sleep, but do not effect REM sleep. While these findings suggests that PZ neurons may be nonREM-sleep active, the activity profiles of PZ neurons across spontaneous sleep-wake cycles, especially during nonREM versus REM sleep remain unknown. The present study determined the discharge activity of PZ neurons during spontaneous sleep and waking in freely behaving rats. Methods: Male Sprague-Dawley rats were surgically implanted with EEG and EMG electrodes for chronic recording of sleep-wake states. Five pairs of 20um microwires were implanted through the barrel of a mechanical microdrive into the PZ for recording extracellular activity of the neurons across its dorsal-ventral extent. All recordings were conducted early in the light phase of a 12:12 light-dark cycle. Following isolation of single units, the discharge activity was recorded through 3-5 sleep-wake cycles. Results: Extracellular discharge activity was recorded from 41 neurons in the PZ. Based on their nonREM/wake, nonREM/REM, and REM/wake discharge ratios and a minimum 25% change criterion, a majority of neurons (n=22; 54%) were sleep-active. These included: a) 20 neurons that exhibited increased discharge during both nonREM (51±3%) and REM sleep (43±7%) as compared to waking. The mean nonREM/REM discharge ratio of these neurons were 1.09±0.05; and b) two neurons that exhibited >2 fold increase in discharge during REM sleep as compared to both waking and nonREM sleep. Wake-active neurons constituted 27% of the recorded neuronal population and included both wake/REM-active (n=7) and wake-

active (n=4) neurons. State-indifferent neurons constituted 20% (n=8) of the recorded neurons. Conclusion: These preliminary findings show that while PZ neurons exhibit several sleep-wake discharge profiles, a majority are sleep-active. These finding are consistent with a role of the PZ in sleep-regulation.

Disclosures: M. Alam: None. A. Kostin: None. D. McGinty: None. R. Szymusiak: None. N. Alam: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.10/AAA16

Topic: F.08. Biological Rhythms and Sleep

Title: Sleep restores slow intrinsic timescales of cortical dynamics after sustained wakefulness in rats

Authors: *C. MEISEL, D. PLENZ;
NIMH, Bethesda, MD

Abstract: Sleep is crucial for daytime functioning and well being. These observations suggest that sleep plays an important role in organizing cortical networks toward states where information processing is optimized. The neuronal correlates underlying the changes in cortical information processing, however, have been difficult to identify. The general idea that computational capabilities are maximized nearby critical states related to phase transitions or bifurcations led to the hypothesis that brain networks operate at or close to criticality. One of the important implications of being near criticality is that system dynamics recovers more slowly from small perturbations, a phenomenon called critical slowing down. The observation of slow timescales in brain dynamics at different spatial scales has recently led to a growing interest in understanding their underpinnings and how they might benefit optimal brain function and cognition, for example in terms of integrating information and improving the signal-to-noise ratio in short-term memory or decision-making processes at the neuron network level. Whether these slow timescales governing cortical dynamics change in a wake-time dependent way which could account for reduced network function, however, is still an open question.
Here, we show that cortical time scales change systematically at the network level during the wake/sleep cycle. While wake shortens timescales, sleep recovers slow dynamics.
We implanted microelectrode arrays into superficial layers of prefrontal cortex for chronic recordings of LFP and multiunit activity in the awake, behaving rat. Rats were sleep deprived for 6 h followed by a recovery sleep. We observed spiking activity to exhibit long timescales

characterized by a slow decay in the autocorrelation function which became progressively faster during sleep deprivation. Concomittantly, the recovery from large network synchronization events became faster with time awake as measured in spiking data and LFP. All observed changes were reversed by sleep. Experimental findings during sleep deprivation were closely matched by a neural network model which is gradually tuned away from an order-disorder phase transition.
Our results indicate that the long timescales at which cortex normally operates, are progressively disturbed during sustained wakefulness while sleep recovers them. Interpreted in a dynamical systems framework these observations provide support for an interesting hypothesis for a network-level function of sleep: to reorganize cortical networks towards a critical state and thereby ensure optimal function for the time awake.

Disclosures: C. Meisel: None. D. Plenz: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.11/AAA17

Topic: F.08. Biological Rhythms and Sleep

Support: Whitehall Foundation 2015-12-71

Brain and Behavior Research Foundation 23017

Guarantors of Brain

Title: Reduced effective connectivity between the frontal cortex and striatum during sleep

Authors: *M. J. REDINBAUGH[1], G. E. SPENCER[1], I. N. PIGAREV[2], Y. B. SAALMANN[1]; [1]UW Madison, Madison, WI; [2]Inst. for Information Transmission Problems (Kharkevich Institute), Russian Acad. of Sci., Moscow, Russian Federation

Abstract: Consciousness plays a pervasive role in how we function, and yet it remains unclear what neural mechanisms maintain consciousness and allow for transitions between awake and unconscious states. Mounting evidence associates loss of consciousness with decreased frontal-parietal and cortico-thalamic information transmission. However, not much is known about how deep brain structures influence this breakdown. The dorsal striatum is connected with frontal cortex and the thalamus through cortico-striato-thalamic loops, and lesion evidence implicates the striatum in alterations of the sleep/wake cycle, including sleep fragmentation. We hypothesize that the breakdown in communication in cortico-cortico and cortico-thalamic

circuits associated with loss of consciousness also occurs in cortico-striatal circuits. Thus, the striatum may influence thalamo-cortical processing across the sleep/wake cycle.
We performed simultaneous neural recordings (n=52) from the frontal eye field (FEF) and caudate nucleus (CN) in two cats during awake (task-free resting state), slow-wave sleep (SWS), and rapid-eye movement (REM) states. We recorded spikes and local field potentials (LFPs) in each brain area using a bipolar electrode configuration. To measure effective connectivity, we electrically stimulated FEF while recording from CN. To monitor the level of consciousness, we recorded the EEG across two exposed skull screws and tracked eye position. All recordings in awake, SWS and REM states took place under the same conditions: head-stabilized cats sat comfortably in a dark room (lights off) with no sensory stimulation. We analyzed neural data during stable eye epochs (excluding periods around eye movements).
Both FEF and CN LFPs had greater delta (1-4Hz) power in SWS relative to wake and REM, and greater gamma (>30Hz) power in wake and REM relative to SWS. There was also increased coherence between the LFPs in FEF and CN in the delta and alpha (8-13Hz) ranges during SWS (cf. awake and REM). Importantly, stimulating FEF elicited greater spiking activity in CN during the awake state compared with SWS. This suggests perturbed information transmission between FEF and CN during SWS.
Our results show that disruption of the pathway between cortical and striatal regions occurs during SWS. The mechanism underlying this disruption involves altered inter-areal synchrony, including unfavorable phase delays between regions, which interfere with the efficacy of information transmission. We suggest that the striatum, via cortico-striato-thalamic loops, plays a role in modulating thalamo-cortical processing and, consequently, the level of consciousness.

Disclosures: M.J. Redinbaugh: None. G.E. Spencer: None. I.N. Pigarev: None. Y.B. Saalmann: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.12/AAA18

Topic: F.08. Biological Rhythms and Sleep

Support: University of Missouri

Title: Basal forebrain cholinergic neurons are vital for sleepiness observed after alcohol consumption

Authors: *A. SHARMA, R. SHARMA, P. SAHOTA, M. THAKKAR;
Res., Harry S Truman Mem. Veterans' Hosp., Columbia, MO

Abstract: Purpose: Corticopetal wake-promoting basal forebrain region (BF) is implicated to mediate sleepiness following alcohol intake. However, the specific phenotype of the neuronal population mediating this effects is unknown. Since the BF cholinergic neurons are sole supplier of cholinergic inputs to several forebrain regions, including the cortex, we hypothesized that the cholinergic neurons of the BF may have a critical role in alcohol induced sleepiness.
Methods: To test our hypothesis, adult male Sprague-Dawley rats were instrumented with sleep recording electrodes and bilateral guide cannulas targeted toward the cholinergic zone of the BF. To verify alcohol induced sleep promotion, rats were administered alcohol [35% (v/v); 3 g/Kg; intragastric] at dark onset (pre-lesion). Subsequently, the animals were divided into two groups.
Lesions: Selective lesion of the BF cholinergic neurons was performed by bilateral administration of immunotoxin, 192-IgG-Saporin (0.28 μg/0.5μL/side) in the BF.
Shams: Bilaterally infusion with artificial cerebrospinal fluid (0.5μL/side).
Rats were left undisturbed for 3 weeks. Subsequently, alcohol induced sleepiness was re-examined (post-lesion) as described above. On completion, rats were euthanized, brains removed and processed for ChAT immunohistochemistry in the BF to verify selective lesion of the cholinergic neurons.
Results: ***Pre-Lesion:*** Robust sleep promotion was observed following alcohol administration.
Post-Lesion: As compared to controls, rats in the lesion group took significantly ($p<0.05$) longer time to fall asleep and spent significantly less time asleep following alcohol administration.
Conclusions: Our results suggest that the cholinergic neurons are the mediators of sleepiness following alcohol intake.

Disclosures: A. Sharma: None. R. Sharma: None. P. Sahota: None. M. Thakkar: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.13/AAA19

Topic: F.08. Biological Rhythms and Sleep

Support: INPRFM NC09-3240.2

Title: Electrical stimulation of the nucleus of the solitary tract: effects on electroencephalographic spectral power and the sleep-wake cycle in freely moving cats

Authors: *D. MARTÍNEZ-VARGAS, A. VALDÉS-CRUZ, V. M. MAGDALENO-MADRIGAL, R. FERNÁNDEZ-MAS, S. ALMAZÁN-ALVARADO;
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Abstract: The nucleus of the solitary tract (NTS) plays a determinant role in the antiepileptic effects of vagus nerve stimulation (VNS). One mechanism underlying the efficacy of VNS is the induction of cortical changes detected by electroencephalography (EEG), which can be mediated by the NTS through its projections to the cerebral nuclei responsible for modulating cortical activity. The NTS may alter the sleep-wake cycle and could alter cortical EEG activity by lesion or chemical stimulation in animals under acute conditions. Despite the key role of NTS on EEG and sleep-wake in acute conditions the effect of electrical stimulation of the nucleus of the solitary tract (ENTS) in freely moving animals is unknown. The aim of this work was analyze the effects of ENTS on the EEG spectral power and sleep-wake cycle in freely moving cats. Seven adult male cats were implanted stereotaxically into both lateral geniculate bodies of the thalamus, both amygdalae, right hippocampi and the left NTS. Also stainless steel epidural electrodes were implanted in the left (Cx Pf-L) and right (Cx Pf-R) pre-frontal cortices to record the EEG. The activity of EEG, hippocampus, amygdala, electromiogram and electrooculogram was recording. All of the animals underwent eight recording sessions of 23 hours; two sessions were conducted at baseline (BL), and six were conducted while ENTS was applied. The BL recordings were conducted firsty and the day after the BL recordings were finished, the stimulation threshold for the NTS was determined. The next day the recordings with ENTS began. In each recording, ten trains of stimulation (1 min On /5 min Off; pulse square waves, 0.5-ms; 30-Hz frequency and, intensity 200-400 μA) were applied to the awake cats starting at 10:00 a.m. Changes induced by ENTS on sleep-wake cycle and EEG spectral power of the Cx Pf-L, Cx Pf-R of the bands at 0.5-3.5 Hz, 3.5-8 Hz, 8-12 Hz, 12-14 Hz and 14-26 Hz during the 23 hours of recording were analyzed. ENTS increases the band power from 3.5-8 Hz, 8-12 Hz and 14-26 Hz for twelve hours. Furthermore, the ENTS modified the architecture of the sleep-wake cycle, the ENTS led to increases in the percentage and total time of wakefulness during the first six hours after ENTS. Following these effects, increases in the percentage and total time of rapid eye movement (REM) sleep values were observed. ENTS produces a long-lasting increase in the bands power of EEG at 8-12 Hz and 14-26 Hz, which favors wakefulness and REM sleep in freely moving cats. These effects are attributed to the possible influence of the NTS on the thalamocortical system, locus coeruleus and parabrachial nucleus that are involved in the regulation of wakefulness and REM sleep.

Disclosures: D. Martínez-Vargas: None. A. Valdés-Cruz: None. V.M. Magdaleno-Madrigal: None. R. Fernández-Mas: None. S. Almazán-Alvarado: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.14/AAA20

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R21CA191846

Title: Peripheral tumors disrupt normal sleep/wake states in mice

Authors: *J. C. BORNIGER, A. H. HINZEY, N. ZHANG, Y. M. CISSE, W. H. WALKER, II, M. M. GAUDIER-DIAZ, U. J. MAGALANG, M. B. LUSTBERG, R. J. NELSON, A. C. DEVRIES;
Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Adequate sleep is an important component in maintaining health and recovering from infection and injury. Breast cancer (BC) patients frequently experience sleep disruption, potentially exacerbating comorbid conditions and reducing post-treatment quality of life. It is not known whether the psychological burden of a cancer diagnosis, physiological alterations caused by tumors, cancer treatment(s), or other factors are primarily responsible for disrupted sleep in cancer patients. We hypothesized that changes in immune signaling in response to cancer and its treatment alter brain sleep circuitry. To probe this question, we measured sleep throughout the course of tumor development in female mice inoculated subcutaneously with 67NR non-metastatic mammary cancer cells. A subset of mice was challenged with a 6 hr sleep deprivation protocol to examine the effects of tumors on homeostatic responses to sleep loss. Approximately 3 weeks after tumor inoculation, central cytokine transcription was measured in brain regions related to vigilance state regulation (i.e., hypothalamus and brainstem). Peripheral tumors increased and fragmented sleep, particularly during the active phase, and this alteration was accompanied by marked elevation of IL-1β transcription within the brainstem. Furthermore, tumor-bearing mice showed an exaggerated response to sleep loss, sleeping more during recovery. These results suggest that peripheral tumors influence sleep, likely through a mechanism involving IL-1 in the brainstem; future studies are needed to determine whether psychological stress and subsequent treatment with chemotherapy or radiation exacerbates the phenotype we observed.

Disclosures: J.C. Borniger: None. A.H. Hinzey: None. N. Zhang: None. Y.M. Cisse: None. W.H. Walker: None. M.M. Gaudier-Diaz: None. U.J. Magalang: None. M.B. Lustberg: None. R.J. Nelson: None. A.C. DeVries: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.15/AAA21

Topic: F.08. Biological Rhythms and Sleep

Title: Altered sleep-wake behavior in a novel murine model of type 2 diabetes and Alzheimer's disease (db/AD mice)

Authors: ***M. SETHI**[1], L. GUERRIERO[1], C. WANG[1], R. BERNAT[4], A. M. HELMAN[2], T. MACHEDA[2], A. AGARWAL[4], M. P. MURPHY[2], M. J. DUNCAN[3], B. F. O'HARA[1]; [1]Dept of Biol., [2]Sanders-Brown Ctr. on Aging, Dept. of Biochem., [3]Dept. of Anat. & Neurobio., Univ. of Kentucky, Lexington, KY; [4]Signal Solutions LLC, Lexington, KY

Abstract: Sleep has been suggested to play a variety of roles ranging from learning, memory consolidation, and clearance of toxic metabolites such as Aβ, in addition to possible roles in basic energy metabolism and functional adaptions related to ecology. Sleep is also essential for optimal cognition over short time periods, but probably also over longer time periods of many years. Increasing data suggest a bidirectional role for sleep and Alzheimer's Disease (AD), with poor sleep promoting AD, and AD increasing sleep disruption. Insufficient sleep is also correlated with increased risk of obesity and diabetes, which in turn are increasingly linked to AD. Previous studies have shown that db/db mice, which have diabetes and obesity, have attenuated sleep wake rhythms in addition to increased sleep fragmentation and overall sleep duration. This is also true of several different AD mouse models. To examine the interaction of these diseases, a mouse model was created that combines the genetic mutations for diabetes (db/db) with a commonly used mouse model of AD (APP-PS1) that contains human autosomal dominant mutations in APP and PS1 that lead to early onset human AD, and AD-like pathology in the mouse. When combined, these mice also display severe cerebrovascular pathology, without increased Aβ deposition (Neidowicz et al., 2014). Cognitive impairments were more profound in these mice compared to db/db and APP-PS1 mice alone. Given the role of sleep in all of these disorders, we are examining multiple sleep and diurnal variables in the combined mouse model (db/AD) and the individual mouse models db/db and APP-PS1, and found significant alterations in normal sleep, and response to sleep deprivation, which may contribute to or interacts with disease pathology. These mice may also provide a useful tool to examine sleep interventions to slow disease progression.

Disclosures: **M. Sethi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal Solutions LLC. **L. Guerriero:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC. **C. Wang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC. **R. Bernat:** A. Employment/Salary (full or part-time): Employee at Signal Solutions LLC. **A.M. Helman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC. **T. Macheda:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC. **A. Agarwal:** A. Employment/Salary (full or part-time): Employee at Signal Solutions LLC. **M.P. Murphy:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC. **M.J. Duncan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Equipment from Signal Solutions LLC.

B.F. O'Hara: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.16/AAA22

Topic: F.08. Biological Rhythms and Sleep

Support: Research Grant Program at Escuela de Medicina, Universidad Anáhuac Mayab given to E.M.-R.

Title: Activation of PPARα enhaces wake-related molecules after total sleep deprivation in rats.

Authors: *A. POOT-AKE[1,2,3], K. GUZMÁN[5], G. ARANKOWSKY-SANDOVAL[6], R. JIMÉNEZ-MORENO[1,2,3], M.-R. ERIC[1,2,3,4];
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Abstract: The peroxisome proliferator-activated receptor alpha (PPAR) is a member of the nuclear receptor superfamily that has been suggested as a modulator of several physiological functions, including sleep. Endogenous ligands, such as oleoylethanolamide (OEA) activate PPARα leading to increase waking in rats. Although injections of OEA enhance wakefulness, no further evidence is available regarding whether activation of PPARα would modulate wake-related compounds after sleep deprivation. Thus, the aim of this study was to characterize if PPARα was able to promote wake-linked endogenous compounds such dopamine, norepinephrine, serotonin, and adenosine after total sleep deprivation (TSD) collected from nucleus accumbens (AcbC). Male Wistar rats (*n*= 20) were anesthetized, placed into the stereotaxic frame for implantation of a microdialysis guide-cannula aimed to the AcbC, and habituated to the experimental conditions for 7 days in the microdialysis bowl. Artificial cerebrospinal fluid was continuously perfused and dialysates were collected every hour (first 20 min) across the whole study. In the experimental day, TSD was carried out by maintaining rats on constant alertness across 6h from (08:00-14:00h). After TSD, animals received an intraperitoneal injection of vehicle, OEA, Wy14643 (agonist of PPARα), or MK-886 (antagonist of PPARα; 10, 20 or 30mg/Kg, each compound), and dialysates were collected across the next 4h. Samples were injected into HPLC and analyzed for detection and quantification of dopamine,

norepinephrine, serotonin, and adenosine. We found that activation of PPARα by OEA or Wy14643 enhanced wake-linked compounds after TSD. Opposite results were observed if MK-886 was administered. We conclude that nuclear receptor PPARα modulates neurochemical homeostasis after sleep deprivation.

Disclosures: A. Poot-Ake: None. K. Guzmán: None. G. Arankowsky-Sandoval: None. R. Jiménez-Moreno: None. M. Eric: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.17/AAA23

Topic: F.08. Biological Rhythms and Sleep

Support: NIH F31 HL128124

NIH P01 AG017628

Title: Exploring PPARg as a mediator of diet/energy balance effects on sleep/wake behavior

Authors: *I. J. PERRON[1], K. CHELLAPPA[2], A. PACK[1], S. VEASEY[1];
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Abstract: Our lab has previously found that energy imbalance (brought on by dietary manipulations) profoundly influences sleep/wake architecture in mice, independent of body weight. Specifically, mice in positive energy balance exhibit increases in total sleep time and sleep/wake fragmentation compared to mice in negative energy balance or maintained on a regular chow (RC) diet. Peroxisome proliferator-activated receptor gamma (PPARg) is a nuclear receptor that can directly influence the circadian clock, has elevated hypothalamic expression in obesity, and is sufficient to induce overeating and weight gain when activated. We hypothesize that PPARg within the hypothalamus serves as an energy sensor and contributes to energy balance effects on wakefulness. To determine the role of brain PPARg in mediating positive energy balance-induced changes to sleep, we implanted mice with an ICV guide cannula and EEG/EMG recording electrodes, and then fed them either RC or high-fat diet (HFD) for 4 weeks. After an undisturbed sleep recording (no injection), mice were randomized to the order of receiving two injections, the PPARg antagonist GW9662 (2 ug in 2uL) and vehicle (1:1 DMSO/saline, pH 7.5). These injections were given 3-4 days apart at ZT11 and sleep/wake behavior during the subsequent dark period was scored and analyzed. Our preliminary results

found that GW9662 did not affect sleep or wakefulness in either RC- or HFD-fed mice compared to vehicle. However, the vehicle condition decreased total wakefulness by approximately one hour in RC-fed mice (p=0.088) but not HFD-fed mice (p=0.520) compared to no injection, thus complicating the interpretation of GW9662 results. Thus, in light of the substantial effects of the vehicle on sleep/wake behavior, present attempts are directed towards using Cre/LoxP transgenic mice to test the importance of PPARg in mediating sleep/wake behavior.

Disclosures: I.J. Perron: None. K. Chellappa: None. A. Pack: None. S. Veasey: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.18/AAA24

Topic: F.08. Biological Rhythms and Sleep

Support: 5R25GM061151-12

NSF REU-CRIB Program Grant 1156810

Title: pumilio regulates sleep in *Drosophila melanogaster* through suppression of synaptic proteins

Authors: *L. DE JESUS[1], N. RODRIGUEZ[2], J. ALEMAN[2], J. ORTEGA[2], C. PACHECO[2], A. AVALOS[2], J. L. AGOSTO[2];
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Abstract: Numerous studies have shown that the brain's information encoding and storage mechanisms involve changes in the number and strength of its synaptic connections. According to the evidence supporting the Synaptic Plasticity Hypothesis (SHY), these changes result in a net increase in synaptic strength during wakefulness and a net depression during sleep. Therefore, SHY proposes that a key function of sleep is to decrease synaptic strength in order to prevent the saturation of the brain's storage capacity. Nevertheless, the mechanisms of how wakefulness increases synaptic strength, how sleep could be induced by such increases, and how synaptic strength is reduced by sleep remain to be elucidated. To answer these questions, we selected the neuronal homeostasis protein Pumilio (*Pum*) whose main function is to repress the translation of many synaptic proteins and decrease neuronal excitably during chronic patterns of neuronal activity. In this study, we show evidence indicating that *Pum* is recruited during sleep deprivation and prevent the uncontrolled synthesis of synaptic proteins at the translational and

transcriptional level. Interestingly, *Pum's* actions are more significant during chronic sleep deprivation (84 hours) than during acute deprivation (12 hours) and has little or no effect on normal daily sleep. At the behavioral level, *Pum* knockdown results in the abolishment, and even the reversal, of compensatory sleep responses to mechanical and pharmacological sleep deprivation. Consistent with the idea that these effects are due to exaggerated translation of synaptic proteins, exaggerating synaptic translation by overexpressing the eukaryotic initiation factor 4E (eIF4E), which is a known target of Pum repression, also decreased the compensatory sleep rebound. Based on these findings, we propose that the
synaptic strengthening, associated with extended wakefulness, triggers neuronal homeostasis mechanisms to avoid the saturation of the brain's storage capacity and induces sleep perhaps by decreasing the excitability of wake-promoting neurons.

Disclosures: L. De Jesus: None. N. Rodriguez: None. J. Aleman: None. J. Ortega: None. C. Pacheco: None. A. Avalos: None. J.L. Agosto: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.19/AAA25

Topic: F.08. Biological Rhythms and Sleep

Support: JSPS KAKENHI Grant Number 15J06369

Title: Systematic behavioral screening of Sleepy and Dreamless, newly identified mouse pedigrees with sleep abnormalities

Authors: *T. HONDA[1,2,3], T. FUJIYAMA[2], C. MIYOSHI[2], M. SATO[2], H. FUNATO[2,4], M. YANAGISAWA[2,5];
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Abstract: As a biggest unrevealed issue in sleep biology, the mechanism for homeostatic sleep/wakefulness regulation, as well as the neural substrate for "sleepiness", remains a mystery. To make a breakthrough in this question, we have initiated a large-scale forward genetic screen of sleep/wake abnormalities in mice, based on somnographic (EEG/EMG) measurements, the

gold standard in mammalian sleep/wake assessment. We have so far screened >7,000 heterozygous ENU-mutagenized mice and established 10 pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and whole-exome sequencing, we have so far identified three mutations: *Sleepy* and *Sleepy2*, causing marked hypersomnia (increased non-REM sleep), and *Dreamless*, causing short and highly fragmented REM sleep. Since these dominant mutations cause very strong phenotypes, we expect that the mutated genes play central role for regulating sleep/wake amounts. Furthermore, these mutant mice can be a sleep disease model to approach the relationship between sleep and other cognitive and mental functions such as learning and memory, depression, anxiety, sociability and more. Here, we examined the following series of behavioral analyses for both *Sleepy* and *Dreamless* mutant mice: 1) Morris water maze, 2) forced swim test, 3) tail suspension test, 4) open field test, 5) novel object recognition, 6) elevated plus maze, 7) social interaction test, 8) sucrose preference, 9) nest building, and 10) fear conditioning. We detected the significant behavioral phenotypes in both *Sleepy* and *Dreamless* mutant mice. These results of behavioral test battery provide us the landmark information to approach the link between sleep and other cognitive behaviors, as well as the further physiological functions of *Sleepy* and *Dreamless* genes.

Disclosures: T. Honda: None. T. Fujiyama: None. C. Miyoshi: None. M. Sato: None. H. Funato: None. M. Yanagisawa: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.20/AAA26

Topic: F.08. Biological Rhythms and Sleep

Support: DFG SFB 654 'Plasticity and Sleep'

Ev. Studienwerk Villigst

Graduate Training Center of Neuroscience (GTC) / International Max Planck Research School (IMPRS) at the University of Tübingen

Title: Sleep affects operant conditioning memory in *Aplysia* californica

Authors: *A. P. VORSTER[1], J. BORN[2];
[2]Inst. for Med. Psychology and Behavioral Neurobio., [1]Univ. of Tübingen, Tübingen, Germany

Abstract: Sleep is known to support memory consolidation. This has been shown for mammals and, more recently, also for invertebrates such as bees and Drosophila. Here, we investigated

whether sleep affects memory consolidation also in Aplysia californica which has an even simpler nervous system and is a well-studied model of synaptic memory formation. The animals were tested on an operant avoidance learning task ('learning that a food is inedible') three times (Learning, Retrieval 1, Retrieval 2), with a 17-h interval between tests. One group of animals had undisturbed sleep after Learning (sleep group), the other stayed awake, and recovered sleep after Retrieval 1. Compared with the wake animals, the sleep group displayed significantly better performance at Retrieval 1. Moreover, performance was correlated with prior sleep in the sleep group at Retrieval 1 as well as with prior recovery sleep in the wake animals at Retrieval 2.

Disclosures: A.P. Vorster: None. J. Born: None.

Poster

254. Sleep Behavior and Systems I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 254.21/BBB1

Topic: F.08. Biological Rhythms and Sleep

Support: RIKEN, SPDR

Title: CUBIC-MAP: Whole-brain profiling of patio-temporal cellular activity during sleep-wake cycles

Authors: *H. YUKINAGA[1], D. PERRIN[3,1], Q. ZHANG[4], G. A. SUNAGAWA[2], K. TAINAKA[4,1], H. R. UEDA[4,1];
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Abstract: To understand the neural architecture of sleep-wake cycles, we developed a method called CUBIC-MAP to image and analyze mouse brain activity at single-cell resolution. Specifically, we used Arc-dVenus transgenic (Tg) mice, which express the destabilized yellow fluorescence protein Venus under the control of the Arc gene promoter after neural activation. To visualize neural activity during sleep-wake cycles, we sampled Arc-dVenus Tg mice every six hours over 24 hours with chronic administration of two drugs that influence sleep and wake (methamphetamine and haloperidol). Using the CUBIC protocol developed in our laboratory to make tissues such as whole brains transparent, we observed fluorescence expression of Arc-dVenus Tg mice using a light-sheet fluorescent microscope. We developed an imaging and analysis pipeline (CUBIC-MAP) to visualize the link between neural activity and behavior during natural sleep-wake cycles and pharmacologically perturbed sleep-wake cycles. CUBIC-Map enables multiple brain comparisons and reveals the regions that are activated in natural and

pharmacological sleep and wake states. CUBIC-MAP will be a powerful tool not only for sleep-wake cycle research, but also for other applications in neuroscience.

Disclosures: H. Yukinaga: None. D. Perrin: None. Q. Zhang: None. G.A. Sunagawa: None. K. Tainaka: None. H.R. Ueda: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.01/BBB2

Topic: F.10. Food Intake and Energy Balance

Support: National Council of Science and Technology. Number. 261420.

National Council of Science and Technology. PhD Fellow for R.C-P. Number. 384091.

Title: Hypothalamic lipotoxicity coordinates insulin sensitivity inmale offspring of obese rats

Authors: R. CARDENAS-PEREZ[1], L. FUENTES-MERA[2], J. CORONA-CASTILLO[4], L. REYES CASTRO[5], E. ZAMBRANO[5], *A. CAMACHO[3];
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Abstract: Hypothalamic endoplasmic reticulum stress (ER) and mitochondria dysfunction contribute to insulin resistance generation during obesity and type 2 diabetes. ER and mitochondria interact through Mitofusin 2 (MTF2), which anchors in the outer mitochondrial and ER membranes regulating energy metabolism. We have reported that saturated lipids modulate hypothalamic MTF2 expression coordinating ER stress response and body insulin resistance (Díaz, B et al. 2015). Here we determine lipotoxic susceptivility of brain cell types coordinating insulin resistance by using in vitro and in vivo neuronal models. We found that lipotoxic insult induced by 24 h saturated fatty acid (palmitic) stimulation to microglia primary cultures promotes inflammation, evidenced by significant TNF alfa release when compare to palmitoleic, oleic and stearic acid. Also, confocal TMRM and ER-Tracker Green analysis in hypothalamic mHypoA-CLU192 cells showed that time-dependent palmitic stimulation induces early (1 h) mitochondrial mass and ER- Tracker reduction, which it is exacerbated at 12h, when we also found decrease in the mitochondrial membrane potential. Furthermore, we found that 24

h palmitic stimulation leads to decrease in 473 serine AKT phosphorylation, an effector of insulin resistance. Prevention of Insulin resistance during palmitic stimulation was performed by pre-incubation of the anti-inflammatory and the ER stress release reagents, sodium salicylate and 4 phenylbutirate, respectively. Finally, by using an in vivo model we demonstrated that maternal obesity in rats during pregnancy and lactation promotes hypothalamic lipotoxic insult affecting male offspring insulin sensitivity leading to plasma glucose increased. We conclude that lipotoxic insult promote time-dependent hypothalamic inflammation, mitochondria and ER dysfunction, which might lead to glucose insensitivity in male offspring.

Disclosures: R. Cardenas-Perez: None. L. Fuentes-Mera: None. J. Corona-Castillo: None. L. Reyes Castro: None. E. Zambrano: None. A. Camacho: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.02/BBB3

Topic: F.10. Food Intake and Energy Balance

Support: National Natural Science Foundation of China grants (81571125 and 91132712).

Title: Paraventricular thalamus drives high fat diet-induced compulsive eating behavior

Authors: *J. CHENG, C. LI, X. MA, Y. HE, H. ZHOU, G. XIAO, Y.-D. ZHOU;
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Abstract: Obese individuals exhibited increased activation of brain reward circuitry in response to palatable foods or food-related cues. Overconsumption of palatable foods triggers addiction-like neuroadaptive responses in the brain reward circuit and drives the development of compulsive eating behavior. However, how consumption of the energy-dense foods induces compulsive eating behavior remains unclear. Here, we demonstrated 1-week high-fat diet (HFD) consumption was sufficient to induce compulsive eating behavior in adult C57BL/6J mice. Remarkably, high-fat diet-induced compulsive eating caused a high level of c-Fos activation in the paraventricular nucleus of thalamus (PVT) in mice. Photostimulating PVT ChR2-expressing CaMKII neurons could effectively induce compulsive eating behavior in mice fed with regular chow. In contrast, inhibiting PVT CaMKII neurons by using the Designer Receptors Exclusively Activated by a Designer Drug (DREADD) method in HFD mice reversed their compulsive eating behaviors. Dorsal medial nucleus of the hypothalamus (DMH) was identified as a downstream target of PVT by using the adeno-associated virus anterograde tracing method. We further showed that activation of PVT-DMH pathway drove compulsive eating behavior in mice

fed with regular chow, while specifically inactivating this pathway reduced compulsive eating behavior in HFD mice. We thus conclude that HFD induces compulsive eating behavior by means of activating the PVT-DMH circuit. From an evolutionary perspective, we hypothesize that activation of the PVT-DMH circuit may play an important role in food seeking in a situation where food is scarce, and thus is important for survival. Keywords: Compulsive eating behavior, high-fat diet, paraventricular thalamus. Support: National Natural Science Foundation of China grants (81571125 and 91132712).

Disclosures: J. Cheng: None. C. Li: None. X. Ma: None. Y. He: None. H. Zhou: None. G. Xiao: None. Y. Zhou: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.03/BBB4

Topic: F.10. Food Intake and Energy Balance

Support: PROFOCIE 2015-2017

Title: Effects of a high-fat diet during gestation on learning/memory processes and leptin receptor expression on the hippocampus of Wistar rat pups

Authors: *N. Y. CORTÉS, C. R. VUELVAS-OLMOS, M. F. PINTO-GONZÁLEZ, O. GONZÁLEZ-PÉREZ, J. L. COLLÁS-AGUILAR, J. GUZMÁN-MUÑIZ, N. A. MOY-LÓPEZ; Neurosci., Univ. of Colima, Colima, Mexico

Abstract: Pregnancy is a critical period during which nutrition is a key factor for maternal and neonatal health. There are many dietary recommendations about pregnant women, however, food with high lipid levels are often preferred, and their consumption during pregnancy could influence on neurodevelopment and cognitive abilities of the neonate later in life. Therefore, our aim was to analyze the effects of a high-fat diet during gestation on spatial learning and memory, and on the expression of leptin receptors on hipocampal CA1 and CA3 areas. To accomplish this, 44 Wistar rats were used, 22 from control group (CG) which mother was fed balanced, diet and 22 from experimental group (EG) from a mother fed a high fat diet (HFD) with 42% more lipids during pregnancy. Weight gain was measured daily on both groups, starting at postnatal 2 (P2). Later, from P29 to P42, spatial learning and memory were evaluated using the Morris Water Maze (MWM). Groups were tested in 1 trial per day for 7 days for spatial learning, while spatial memory was tested after a week of rest on P42, with an only trial. After behavioral tests, brain tissue was obtained and then, hippocampal leptin receptor expression in hipocampal CA1

and CA3 was analyzed by optical density. Results showed a greater weight gain on EG without significantly different from CG. While on MWM, results indicated a significant reduction in escape latency (t=3.67, fd=42, p=0.001) and a better performance in memory (t=3.08, fd=42, p=0.004) in CG compared with EG, implying a decreased performance from rats whose mothers consumed a HFD during pregnancy. As for leptin receptor expression, significantly increased staining was observed on CA3 (U=0.000, p=0.001) and CA3 (U=0.000, p=0.001) hipocampal areas. These results imply that changes in leptin receptors may be caused by maternal nutrition during embryonic development, because HFD could increase adipocyte proliferation, releasing leptin into the bloodstream and interacting with astrocytes, which stimulate the neurons and could cause a decrease in the number of neuronal receptors, which could cause a decrease in hippocampal activity and therefore an affected efficiency on learning and memory processes, as we observed in EG performance in the MWM. Therefore, it is important to emphasize maternal nutrition for the proper neural and cognitive development of the infant.

Disclosures: N.Y. Cortés: None. C.R. Vuelvas-Olmos: None. M.F. Pinto-González: None. O. González-Pérez: None. J.L. Collás-Aguilar: None. J. Guzmán-Muñiz: None. N.A. Moy-López: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.04/BBB5

Topic: F.10. Food Intake and Energy Balance

Support: Netherlands Organization for Scientific Research (NWO/ALW Veni grant).

Title: Silencing hypothalamic inputs to the VTA differentially affects feeding and reward-seeking behavior

Authors: *G. VAN DER PLASSE, K. C. G. DE GIT, E. M. HAZELHOFF, M. C. M. LUIJENDIJK, R. A. H. ADAN;
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Abstract: Activity of ventral tegemental (VTA) dopamine (DA) neurons is important for reward-seeking, anticipation and motivated behaviors. These functions are under modulatory control of hypothalamic brain areas that allow flexible engagement of the DA system in behavior. However, despite recent technical advances that have identified specific inputs into the VTA, there is limited knowledge of the function of these projections.
In this study, VTA inputs from the dorsomedial hypothalamus (DMH) and zona incerta (ZI)

were targeted with a combination of CAV2-cre and a viral mediated silencing strategy. This approach induces irreversible silencing of the targeted pathway, rendering DMH-, and ZI inputs to the VTA non-functional.
Following viral expression we assessed the behavioral consequences of inactivation on functions that are known to be mediated by the VTA or hypothalamus. These behaviors include motivation for reward, food preference and locomotion, as well as food intake, body temperature and anticipation to food. Results indicate projection-specific modulation of motivated behaviour and food-intake.

Disclosures: G. Van Der Plasse: None. K.C.G. de Git: None. E.M. Hazelhoff: None. M.C.M. Luijendijk: None. R.A.H. Adan: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.05/BBB6

Topic: F.10. Food Intake and Energy Balance

Support: PHS grant DK 040498

PHS grant DK 081546

Title: Selective activation of catecholamine neurons in the caudal ventrolateral medulla using DREADD technology stimulates feeding.

Authors: *A.-J. LI, Q. WANG, S. RITTER;
Washington State Univ., Pullman, WA

Abstract: Previous results indicate that catecholamine (CA) neurons in caudal and rostral C1 in the ventrolateral medulla (VLM) are critical for glucoprivic control of feeding and adrenal medullary secretion, respectively. In the present study, we examined projections of Al/C1 neurons within the hindbrain that potentially contribute to glucoprivic feeding. We compared c-Fos expression in the VLM and dorsomedial medulla (DMM) in response to three treatments shown previously to stimulate food intake: selective activation of virally transfected A1/C1 CA neurons, systemic 2-deoxy-D-glucose (2DG) administration, and localized A1/C1 injections of 5-thioglucose (5TG). Designer receptors exclusively activated by designer drugs (DREADD) technology was used for selective activation of CA neurons. For this approach, a Cre-dependent DREADD construct, AAV2-DIO-hSyn-hM3D(Gq)-mCherry, was injected bilaterally into A1/C1 in Th-Cre^{+} rats. Immunohistochemical analysis of mCherry and dopamine beta-hydroxylase co-

expression showed highly selective and effective transfection of CA neurons in the injection sites. Systemic injection of the receptor agonist, clozapine-N-oxide (CNO), increased feeding (but not blood glucose) and activated CA neurons in the AAV-injected sites in VLM. In the DMM of the same rats, however, CNO specifically induced c-Fos expression in non-CA neurons in the subpostrema area (Sub-P) and dorsolateral solitary nucleus (Sol-DL). After systemic 2DG and local A1/C1 5TG injections, CA neurons were activated in the same VLM sites and non-CA neurons were activated in the same Sub-P and Sol-DL regions as seen after CNO injection. However, systemic 2DG and local 5TG injections also induced c-Fos in the hindbrain neurons not activated by CNO. Together these results suggest that (1) activation of A1/C1 neurons is sufficient to stimulate feeding and (2) that Sub-P and Sol-DL neurons are activated by signals originating from A1/C1 neurons and (3) that these downstream DMM sites may contribute to the glucoprivic feeding response and/or additional glucoregulatory responses.

Disclosures: A. Li: None. Q. Wang: None. S. Ritter: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.06/BBB7

Topic: F.10. Food Intake and Energy Balance

Support: NIH (U01 MH105941)

ADA (1-15-JF-52)

Swedish Society For Medical Research (SSMF)

Title: Pancreatic projections of glucose sensing CNS neurons

Authors: *A. ALVARSSON, M. BAYNE, S. A. STANLEY;
Diabetes, Obesity and Metabolism Inst., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: The central nervous system (CNS) plays a complex role in the control of metabolic functions. CNS glucose-sensing neurons respond to blood glucose levels and orchestrate counterregulatory responses to hypoglycemia. Although several brain regions are known to form synaptic connections with metabolic organs, the specific roles of glucose-sensing neurons in such projections have not yet been established. Glucose-sensing CNS neurons also appear to constitute distinct neuronal populations that mediate different physiological functions, but previous studies have been hampered by low expression levels of glucose sensors such as glucokinase (Gck). However, a recently generated transgenic mouse with cre-recombinase

expression in Gck-expressing neurons (Gck-cre/Rosa-tdTomato mice) allows the mapping Gck expression. Here we performed retrograde tracing studies to determine the neural pathways involving Gck-expressing neurons innervating the pancreas using Gck-cre/Rosa-tdTomato mice. PRV-GFP (5x100 nl) was injected into the pancreas of Gck-cre/Rosa-tdTomato mice via a Hamilton syringe. Five to eight days after PRV-GFP injections the mice were perfused and the brains dissected, sectioned and mounted for quantification using an optical microscope. The numbers of PRV-GFP infected neurons and neurons with overlapping Gck and GFP labelling were quantified. GFP labelling was seen throughout the hypothalamus, midbrain, hindbrain and cortex. Overlapping labelling between Gck-expressing and GFP-expressing neurons revealed that subsets of hypothalamic glucose-sensing neurons, particularly in the lateral hypothalamus and paraventricular nucleus of the hypothalamus, form synaptic connections that project to the pancreas. Taken together, these findings suggest that several brain regions, most notably in the hypothalamus, harbor glucose-sensing neurons that form polysynaptic connections to the pancreas, thus potentially playing roles in the control of pancreatic functions.

Disclosures: A. Alvarsson: None. M. Bayne: None. S.A. Stanley: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.07/BBB8

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant R01 DK105954

Title: TrkB-expressing neurons in the paraventricular hypothalamus regulate food intake through specific projections

Authors: *C. E. KINNEY[1,2], J. AN[1], G.-Y. LIAO[1], B. XU[1];
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Abstract: Brain-derived Neurotrophic Factor (BDNF) has recently been shown to play a role in the regulation of energy balance, specifically within the paraventricular nucleus of the hypothalamus (PVH). This nucleus acts as a site of integration for many signals from the periphery as well as within the brain, and as an output nucleus affecting both food intake and energy expenditure. Tropomyosin-related Kinase B (TrkB), the receptor for BDNF, is expressed by neurons in the PVH (PVH^{TrkB} neurons); and we show that loss of TrkB from these neurons in adult mice leads to a rapid & severe hyperphagic obesity phenotype. Furthermore, we use

projection-specific gene deletion to remove these receptors only from PVH^{TrkB} neurons that send axons to a specific downstream target nucleus. Using this method, we show an intermediate hyperphagic obesity phenotype for several projections, including to the parabrachial nucleus (PVH^{TrkB} → PBN) and lateral hypothalamus (PVH^{TrkB} → LH). These findings suggest that multiple projections act in concert to maintain energy balance, rather than a single projection being responsible for energy balance and the rest performing unrelated functions. Thus, targeting the whole population of PVH^{TrkB} neurons for the development of obesity treatments may exhibit efficacy with fewer side effects.

Disclosures: C.E. Kinney: None. J. An: None. G. Liao: None. B. Xu: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.08/BBB9

Topic: F.10. Food Intake and Energy Balance

Title: Effects of perinatal undernutrition on pyramidal neurons of the anterior cingulate and its correlation with the maternal response of rats

Authors: *M. ORTIZ VALLADARES, M. REGALADO, C. TORRERO, M. SALAS;
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Abstract: Perinatal undernutrition (PU) in the rat elicits long-term physical and behavioral consequences. The physiological and metabolic abnormalities associated with PU have been widely studied, but the negative consequences on the mother-litter behavior are still under investigation. The aim of this study was to analyze the pup retrieval and nest building responses of dams with the PU and its correlation with the morphology of the pyramidal neurons of the anterior cingulated cortex. The experimental underfed (UG) group consisted of undernourished dams fed with a caloric food restriction diet, while the control (CG) was fed ad libitum. At postnatal (PN) day 90 rats were mated, and after birth the retrieval of pups (body area, frequency and latency), and the nest building (shape and size) at PDs 4, and 12 of the lactation period were evaluated. The analysis of the pyramidal neurons was performed by using the Sholl method before the staining with the Golgi-Cox technique. We measured to the perimeter, and area of the perikarya and the dendritic arbor density. At PDs 4, and 12 the UG dams showed significant prolonged retrieving latencies, carrying the pups by improper body areas, and with deficient nest building organization, when compared with the CG dams. At PD 4 the UG dams showed significant reduction in the area and perimeter in the pyramids when compared with the CG dams. Moreover, the UG showed reduced dendritic arborization than the CG of dams. The

deficiencies in the maternal responsiveness of the UG mothers may partly reflect the synaptic and functional alterations at the telencephalic structures of the maternal circuitry, vulnerable to early food restriction in this case the anterior cingulate cortical pyramids. Partly supported by DGAPA/UNAM. IN200413 and CONACyT scholarship 660096 to M.O.V.

Disclosures: M. Ortiz Valladares: None. M. Regalado: None. C. Torrero: None. M. Salas: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.09/BBB10

Topic: F.10. Food Intake and Energy Balance

Support: Andalusian Council of Innovation, Science and Industry Grant HUM-6635

Title: Alterations in cortical thickness are related to the combined effects of age and BMI in adolescence and adulthood

Authors: *M. L. WESTWATER[1], R. VILAR-LÓPEZ[2], K. M. J. DIEDEREN[1], H. ZIAUDDEEN[1], P. C. FLETCHER[1], A. VERDEJO-GARCIA[2,3];
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Abstract: Overweight and obesity have been associated with adverse changes in the central nervous system, such as compromised white matter connectivity and reduced grey matter volume in regions involved in feeding behaviour. However, the association between excess weight and brain structure across human development remains understudied. Here, we examine the relationship between age, body mass index (BMI) and cortical thickness (CT) in adolescence and adulthood.
Seventy adolescents (M_{age}= 16.6, SD = 1.46, 35 female) and 75 adults (M_{age}= 33.3, SD = 6.36, 42 female) underwent structural MRI scanning. Exclusion criteria included psychopathology, metabolic syndrome, use of psychotropic medication and morbid obesity (BMI $\geq$ 40). Structural MRI data were collected on a 3T scanner, and FreeSurfer software was used to generate cortical surface reconstructions of MR images and obtain measures of CT. Multiple linear regression was used to examine the effects of age and BMI on global mean CT. As BMI is sex- and age-specific for teens, weight class (lean and excess) was used as a dichotomous variable in the adolescent group. For analyses of local CT, we examined the effects of age, BMI and weight class on thickness at each vertex of the subject's surface. Monte Carlo simulations were applied to

determine significant contiguous clusters that displayed significant ($p < 0.05$) vertex-wise thickness values that differed from zero. Sex, years of education and white matter surface area were entered as covariates in all models.
Among adolescents, we found a significant age by weight class interaction ($p < 0.05$) where excess weight moderated the association between age and global mean CT, resulting in increased CT with excess weight. Local analyses showed a similar interaction in a cluster within the right superiorfrontal cortex ($p < 0.05$), such that excess weight was associated with increased CT in this cluster. In adults, a significant age by BMI interaction ($p = 0.03$) was once again observed but in an inverse relationship: increasing age and BMI were associated with reduced global mean CT. Local analyses revealed reduced CT in four clusters within the left superiorparietal and superiorfrontal cortices ($p < 0.05$) and the right inferiorparietal ($p < 0.01$) and superiortemporal ($p < 0.05$) cortices. In keeping with the global findings, older subjects with higher BMIs showed the greatest reduction in CT in these areas.
Our findings show a differential relationship between increasing BMI and CT in adolescents and adults. The previously reported obesity-related cortical atrophy was observed only in adults, suggesting that excess weight may precede these changes, rather than emerge consequent to them.

Disclosures: M.L. Westwater: None. R. Vilar-López: None. K.M.J. Diederen: None. H. Ziauddeen: None. P.C. Fletcher: None. A. Verdejo-Garcia: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.10/BBB11

Topic: F.10. Food Intake and Energy Balance

Support: Oklahoma Center for the Advancement of Science and Technology HR12-196

Title: Temporal and regional changes in neuroimmune signaling with post-ovariectomy weight gain in rats

Authors: *K. S. CURTIS, K. MCCRACKEN, D. BUCK, W. BLAIK, B. BRANTLEY, R. L. DAVIS;
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Abstract: Laboratory rats rapidly and reliably gain body weight after ovariectomy without the need for special diets or genetic manipulations. The predictable time course of weight gain that occurs after ovariectomy allows us to investigate temporal and regional changes in neuroimmune

signaling known to occur in the CNS with obesity. Adult female rats were ovariectomized (OVX) and given *ad libitum* access to standard laboratory chow for 4 weeks (ad lib) or *ad libitum* feeding followed by access to chow for only 2 hours/day during the last 10 days (dieted). Rats (n = 4-5/group) then were euthanized and tissue punches taken from the hypothalamic arcuate (ARC) and paraventricular (PVN) nuclei, and from the hindbrain dorsal vagal complex (DVC). Interleukin-6 and monocyte chemoattractant protein-1 (IL-6, MCP-1) protein were determined by ELISA and toll-like receptor 4 (TLR4) by western blot from these punches. As expected, body weight increased during the 4 week-period in ad lib OVX rats and decreased in OVX rats that were dieted during the last 10 days. IL-6 and TLR4 expression in the ARC and the PVN were similar in the two groups; however, IL-6 levels and TLR4 expression in the DVC were greater in ad lib OVX rats that gained weight compared to dieted rats that lost weight. Expression of MCP-1 in the PVN was less in ad lib rats than in dieted rats, while MCP-1 in the ARC was greater in ad lib OVX rats. In other groups of ad lib and dieted OVX rats (n = 4-5/group), immunolabeling of astrocytes for glial fibrillary acidic protein (GFAP) revealed elevated GFAP in the ARC of ad lib rats that gained weight, compared to dieted rats that lost weight. Together, these findings show that post-OVX weight gain is associated with temporally- and regionally-specific patterns of cytokine/chemokine expression and glial activation. Importantly, examination of neuroimmune signaling comparatively early during this weight gain suggests that changes may occur earlier in the development of obesity than had previously been reported, and follow a regional and/or temporal sequence. For example, increased expression of IL-6 and TLR4 in the DVC may reflect altered signals from the periphery associated with greater food intake and initial weight gain after ovariectomy, whereas further increases in body weight may be necessary for any changes in the ARC and PVN. In contrast, changes in the 'pro-obesity' chemokine, MCP-1, and GFAP in the ARC and PVN suggest that modulation of these factors may contribute to the onset of weight gain. Thus, a temporal and regional sequence of neuroimmune signaling in interconnected CNS areas may be critical in post-ovariectomy weight gain.

Disclosures: K.S. Curtis: None. K. McCracken: None. D. Buck: None. W. Blaik: None. B. Brantley: None. R.L. Davis: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.11/BBB12

Topic: F.10. Food Intake and Energy Balance

Support: DGAPA/UNAM

IN200413

CONACyT scholarship 588926 to C.S.B.

Title: Effects of perinatal undernourishment on the maternal kyphotic posture of lactating rats

Authors: *C. A. SALCEDO BARRÓN, M. REGALADO, C. TORRERO, M. SALAS;
Inst. de Neurobiología, Univ. Nacional Autónoma De México, Queretaro de Arteaga, Mexico

Abstract: Among the most representative components of the maternal behavior that meet the purpose of the newborn nutrition, is the maternal kyphotic posture. During this posture the mother maintains an adequate environment for the protection, thermal regulation and breast-feeding of the progeny. Although the effects of perinatal malnutrition on the maternal behavior have been studied, however, little is known on the kyphotic long-term effects and its regulatory mechanisms elicited by perinatal undernutrition (PNU). The aim of this study was to analyze the kyphotic posture in lactating dams with PNU elicited by their own newborn pups. Wistar dams with PNU at 90 days of age were mated and pregnancy was confirmed by vaginal smears. At birth pups were culled to 10 pups per litter with food and water ad lib. Maternal testing was carried out between 1100-1300 h. Before testing the pups were removed for 4 h to increase the motivation for nursing, body weight, and temperature were obtained. During the test (1 h) the maternal response including the kyphotic posture were video-recorded in their home cage at postnatal days 4 and 12. After the test the litter was weighed and the temperature was taken again. The results showed that mothers with PNU significantly reduced the duration of kyphosis (high crouch) than control dams, choosing unconventional postures (prone and partial crouch) for breast-feeding. Moreover, the total nursing duration and licking of the pups were also reduced.
The findings suggest that PNU mothers have deficiencies in the mechanism underlying the kyphosis and/or that the sensory stimuli generated by the own pups to evoke the dam kyphotic position are suboptimal compared with the offspring of control dams. Furthermore, this condition may reflect the suboptimal growth of the progeny of the next generation.

Disclosures: C.A. Salcedo Barrón: None. M. Regalado: None. C. Torrero: None. M. Salas: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.12/BBB13

Topic: F.10. Food Intake and Energy Balance

Support: MEXT/JSPS KAKENHI Grant 16K18367 to T.O.

Title: Parcellation of human hypothalamus using high-resolution resting-state fMRI

Authors: *T. OSADA[1], A. OGAWA[1], M. TANAKA[1], M. HORI[2], S. AOKI[2], S. KONISHI[1]; [1]Dept Neurophysiol, Juntendo Univ. Sch. Med., Tokyo, Japan; [2]Dept Radiol, Juntendo Univ. Sch. Med., Tokyo, Japan

Abstract: The hypothalamus is a small structure (approximately 1 cm^3 per hemisphere in humans) that contains several nuclei. Due to its size, the precise functional architecture of the human hypothalamus is not well understood, and higher spatial resolution is required to investigate the hypothalamus using functional MRI. Here, we collected high-resolution functional MRI images (1.25 mm cubic voxel) from six young healthy subjects using multi-band EPI, and delineated boundaries in the hypothalamus using resting-state fMRI. Resting-state functional connectivity has allowed us to parcellate the cerebral cortex into putative functional areas based on the changes in the spatial pattern of connectivity in the cerebral cortex when a seed point is moved from one voxel to another (Cohen et al., Neuroimage, 2008; Hirose et al., Cerebral Cortex, 2013). The boundary mapping method, which has been applied to the 2D cortical surface, was extended to a 3D space in the present study, and the centers of parcellated areas in the hypothalamus were determined. Approximately ten areal centers were identified in the hypothalamus in each hemisphere, and most of them were located symmetrically in the left and right hemispheres. The symmetrical location of the identified centers confirms the reproducibility of the parcellation results. These results suggest the feasibility of the high-resolution 3D parcellation procedures for subcortical structures. It remains to be explored in the future what kind of functions each of the areal centers plays.

References:

Cohen AL, Fair DA, Dosenbach NU, Miezin FM, Dierker D, Van Essen DC, Schlaggar BL, Petersen SE. (2008). Defining functional areas in individual human brains using resting functional connectivity MRI. Neuroimage 41, 45-57.

Hirose S, Watanabe T, Wada H, Imai Y, Machida T, Shirouzu I, Miyashita Y, Konishi S. (2013). Functional relevance of micromodules in the human association cortex delineated with high-resolution fMRI. Cereb Cortex 23, 2863-2871.

Disclosures: T. Osada: None. A. Ogawa: None. M. Tanaka: None. M. Hori: None. S. Aoki: None. S. Konishi: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.13/BBB14

Topic: F.10. Food Intake and Energy Balance

Support: NIH RO1 DK089237

Title: Leptin specifies GLP-1 innervation to the paraventricular nucleus of the hypothalamus

Authors: *J. E. BIDDINGER[1], M. M. SCOTT[2], R. B. SIMERLY[1,3];
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Abstract: The nucleus of the solitary tract (NTS) plays a critical role in the central integration of nutritional signals from visceral organs and is the first central target of gastrointestinal vagal afferents that signal nutritional status from gut to brain. The paraventricular nucleus of the hypothalamus (PVH) receives ascending inputs from the nucleus of the solitary tract (NTS) that function to integrate neuroendocrine responses and autonomic regulation. Neurons that express glucagon-like peptide-1 (GLP-1) are located primarily in the NTS and send dense projections to the PVH. Moreover, nearly all GLP-1 neurons coexpress leptin receptors and are directly responsive to leptin. Although the physiological importance of GLP-1 projections to the PVH is well established, little is known about the development of this pathway and how environmental factors influence innervation of hypothalamic targets by GLP-1 neurons. The fat-derived hormone leptin functions during development to impact the organization of metabolic circuitry, but its possible role in impacting brainstem-hypothalamic connections is unknown. In order to determine if leptin is required for development of GLP-1 projections to the PVH, we used mice that express a fusion protein of synaptophysin and the fluorescent reporter tdTomato under control of the GLP-1 promoter in $Lep^{ob/ob}$ mice. In contrast to projections from the arcuate nucleus to the PVH, leptin-deficient $Lep^{ob/ob}$ mice show an increase in GLP-1 puncta, compared with densities observed in wild-type (wt) controls. To determine the role of leptin on the activity of GLP-1 neurons in the NTS and their postsynaptic neurons in the PVH in response to a visceral stimulus, $Lep^{ob/ob}$ and wt mice received peripheral injections of cholecystokinin (CCK). Compared with saline controls, IP injection of CCK resulted in increased cFos immunoreactivity in GLP-1 neurons and in PVH neurons in $Lep^{ob/ob}$ mice. We next tested the sufficiency of leptin receptors (LepRb) in directing these developmental events and determined that mice with functionally null leptin receptors ($LepRb^{TB}$ mice) also show an increase in GLP-1 fiber density in the PVH, indicating that LepRb are critical for normal development of GLP-1 neural circuitry. These results suggest that leptin acts directly on GLP-1 neurons in the NTS through the LepRb to specify the density of GLP-1 inputs to PVH neurons. Ongoing studies are defining the identity of

PVH neurons that receive enhanced inputs in the absence of leptin and are establishing the resulting physiological impact of these developmental events.

Disclosures: J.E. Biddinger: None. M.M. Scott: None. R.B. Simerly: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.14/BBB15

Topic: F.10. Food Intake and Energy Balance

Support: NIH Predoctoral Intramural Research Training Award (IRTA)

Title: Hunger-driven motivational state competition

Authors: *J. BURNETT[1,2,3], C. LI[1,2], E. WEBBER[1,2], E. TSAOUSIDOU[4,5,6,7], S. Y. XUE[1,2], J. C. BRUNING[4,5,6,7], M. J. KRASHES[1,2];
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Abstract: Motivational systems are most often studied in behavioral isolation, with strict control over internal and external variables for simplification. In this way the animal is encouraged to express goal-seeking behavior directed toward one goal. Using this strategy, a number of molecularly- circumscribed subsets of neurons have been attributed to govern defined behaviors. Starvation- sensitive Agouti-related peptide (AgRP) neurons expressed in the arcuate nucleus of the hypothalamus (ARC) are both sufficient and necessary to regulate food consumption. Given the strong feeding phenotypes elicited by ARC^{AgRP} activation, it may be possible that these neurons engage multiple motivational systems and serve a more generalized role in facilitating biological demands, beyond the acquisition and ingestion of calories. We sought to investigate the integration and competition between hunger and alternative motivational systems such as anxiety, fear, social interactions, thirst, and learned preferences.

Disclosures: J. Burnett: None. C. Li: None. E. Webber: None. E. Tsaousidou: None. S.Y. Xue: None. J.C. Bruning: None. M.J. Krashes: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.15/BBB16

Topic: F.10. Food Intake and Energy Balance

Support: NIH/NIDDK

Title: Hindbrain prolactin-releasing peptide (PrRP) neurons are not strongly connected to pre-motor circuits controlling jaw or tongue movements

Authors: *H. ZHENG, A. K. OWEN, J. R. STROTHER, L. RINAMAN;
Dept Neurosci, Univ. of Pittsburgh Dept. of Neurosci., Pittsburgh, PA

Abstract: PrRP is expressed by noradrenergic neurons in the caudal nucleus of the solitary tract (cNTS). In rodents, PrRP neurons express cFos in direct proportion to feeding-induced gastric distension, and central PrRP signaling limits meal size. PrRP neurons innervate brainstem regions that control oral consummatory behaviors, and thus may control feeding motor programs. To test this, transneuronal pseudorabies virus (PRV) transport was combined with immunofluorescent localization of PrRP to determine whether these cNTS neurons are synaptically linked to pre-motor neurons that control tongue or jaw movement. Sprague-Dawley rats were sympathectomized and the chorda tympani nerve severed to prevent central autonomic infection, then were injected with PRV into the anterior tongue or masseter muscles. Rats were perfused 3-4 d later, and brain sections processed to reveal PRV and PrRP. Infected motor neurons occupied the hypoglossal or trigeminal nucleus after tongue or masseter injection, respectively. Infected pre-motor neurons were located bilaterally within the cNTS, area postrema, medullary and pontine reticular formation, midbrain, hypothalamus, and limbic forebrain. Infected cNTS neurons often were intermingled with PrRP-positive neurons, but very few were double labeled. Cell counts in infected and non-infected cases suggest that PrRP immunolabeling was not reduced by PRV replication. We conclude that PrRP neurons are not synaptically linked to pre-motor circuits that control intrinsic tongue movements or jaw closing, although ongoing analysis suggests that PrRP neurons may innervate higher-order, later-infected forebrain pre-motor neurons.

Disclosures: H. Zheng: None. A.K. Owen: None. J.R. Strother: None. L. Rinaman: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.16/BBB17

Topic: F.10. Food Intake and Energy Balance

Support: NIH Intramural

Title: Manipulations of hypothalamic melanocortin circuitry produce lasting changes in body weight

Authors: *S. C. FUNDERBURK[1,2], C. LI[1,2], M. J. KRASHES[1,2];
[1]Natl. Inst. of Diabetes, Digestive, and Kidney Dis., NIH, Bethesda, MD; [2]Natl. Inst. on Drug Abuse, NIH, Baltimore, MD

Abstract: Balanced caloric intake and energy expenditure is critical to survival and optimal health; however, overconsumption and declining physical activity have led to a rapidly worsening obesity epidemic, prompting a need for greater understanding of the neural mechanisms controlling energy homeostasis. Within the arcuate nucleus of the hypothalamus (ARC), agouti-related peptide (AgRP) neurons promote hunger and reduce energy output. Optogenetic activation of ARC^{AgRP} neurons produces rapid, reversible increases in food intake, mediated by direct monosynaptic inhibition of melanocortin receptor 4 (MC4R) satiety neurons in the paraventricular nucleus of the hypothalamus (PVH). Recently, we have demonstrated that brief, repeated activation of ARC^{AgRP} neurons results in long-term increases in body weight, fat mass, food intake and energy expenditure. Likewise, long-term synaptic silencing of downstream PVH^{MC4R} neurons with the tetanus toxin light chain produces similar increases in body weight, fat mass, and food intake. Together, this suggests that repeated short-term activation of ARC^{AgRP} neurons can produce long-term plasticity in feeding neural circuits, likely including PVH^{MC4R} neurons, which can lead to long-term disruptions in energy balance.

Disclosures: S.C. Funderburk: None. C. Li: None. M.J. Krashes: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.17/BBB18

Topic: F.10. Food Intake and Energy Balance

Support: RO1 DK098747 (KS)

Title: GABAergic receptor neurons trigger consumption in *Drosophila melanogaster*

Authors: *S. K. CHEUNG, K. SCOTT;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Animals must regulate the amount of food consumed to ensure the appropriate intake of nutrients. Previous studies indicate that four GABAergic interneurons (DSOG1) in the *Drosophila melanogaster* brain are necessary to inhibit overconsumptive behavior. Here we identify the specific GABAergic receptor that is required for proper control of ingestion in Drosophila. Knockdown of this receptor in a driver line causes overconsumption of tastants, indicating that the line contains a set of neurons that regulates consumption in *Drosophila melanogaster*. These neurons are sufficient to drive consumption, as acute activation promotes consumption in flies. Complementary, acute silencing of these neurons causes decreased consumption of appetitive substances in motivated flies. Our study identifies neurons that are necessary and sufficient for *Drosophila* ingestive behavior and are most likely downstream of DSOG1 neurons.

Disclosures: S.K. Cheung: None. K. Scott: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.18/BBB19

Topic: F.10. Food Intake and Energy Balance

Support: Wellcome Trust RGAG/259

Bernard Wolfe Health Neuroscience Fund

Title: Obesity is linked to a global increase in cortical Gaussian curvature

Authors: *N. MEDIC[1,2], H. ZIAUDDEEN[1,2], L. RONAN[1], P. C. FLETCHER[1,2];
[1]Psychiatry, [2]Wellcome Trust-MRC Inst. of Metabolic Sci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: In this study, in two separate datasets of healthy subjects, we explored the association of obesity with global changes in a relatively unexplored measure of cortical morphology: intrinsic or Gaussian curvature. Using surface-based morphometry in FreeSurfer and Caret, we examined cortical thickness, surface area and two measures of cortical curvature: the local gyrification index (LGI) and Gaussian curvature. LGI describes cortical curvature at the centimetre level, and represents the degree of cortical folding. On the other hand, Gaussian curvature constitutes a feature intrinsic to the surface, independent of its spatial conformation, enabling the study of cortical shape at a more subtle, millimetre level. In the first dataset ($N = 52$, 26 lean [BMI $M = 22.08$ kg/m^2, $SD = 1.39$ kg/m^2], 26 obese [BMI $M = 33.73$ kg/m^2, $SD = 2.43$ kg/m^2]; 26 females; age $M = 25.44$, $SD = 5.32$) we found no differences between lean and obese subjects in global cortical thickness, surface area or LGI. However, global Gaussian curvature was increased in obese subjects, both on the pial ($t(47) = 4.27$, $p < .001$) and white matter surfaces ($t(47) = 4.51$, $p < .0001$). We replicated these findings in a larger dataset ($N = 203$, 68 lean [BMI $M = 22.43$ kg/m^2, $SD = 1.54$ kg/m^2], 68 overweight [BMI $M = 27.31$ kg/m^2, $SD = 1.3$ kg/m^2], 67 obese [BMI $M = 35.82$ kg/m^2, $SD = 4.73$ kg/m^2]; 79 females; age $M = 32.27$, $SD = 7.72$): again, we found no obesity-related changes in global cortical thickness, surface area or LGI, and a global increase in cortical Gaussian curvature (compared to lean subjects), both on the pial ($t(197) = 2.81$, $p < .01$) and white matter surfaces ($t(197) = 3.77$, $p < .001$). Overweight subjects from this dataset were not significantly different from their lean counterparts in any of the explored measures. In the second line the analysis (combined datasets), we demonstrated that the effect of obesity on Gaussian curvature is more strongly expressed on the white than the pial surface (surface-by-group interaction $F(2,760) = 20.49$, $p < .0001$). Further, in a post-hoc, exploratory analysis, we found that the obesity-linked increase in Gaussian curvature on the white matter surface is mediated by reduced intensity of the white matter in obese subjects, sampled 1mm below the white matter surface.
In summary, in two independent datasets comprising healthy subjects, we found a consistent and global obesity-linked increase in Gaussian curvature, expressed to a greater extent on the white matter surface, and possibly driven by reduced integrity of the white matter underlying the cortex.

Disclosures: N. Medic: None. H. Ziauddeen: None. L. Ronan: None. P.C. Fletcher: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.19/BBB20

Topic: F.10. Food Intake and Energy Balance

Title: Up-regulation of miR-210 in hypothalamus leads to increases in food intake and body weight in mice

Authors: *K. KIM[1], J. PARK[2], M.-S. KIM[3], H. NAM[2];
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Abstract: Hypothalamus plays a key role in metabolic regulation and organismal aging. In order to find a link between aging and regulatory RNA molecules we performed comprehensive transcriptome analysis of young and old hypothalamic tissues with interest in differentially expressed microRNAs. MicroRNAs are well known to regulate various functions through direct post-transcriptional repression of their targets in the central nerve system. We focused on miR-210 because our transcriptome data indicate that miR-210 was significantly up-regulated in aged hypothalamus and recent other reports suggest that this miR regulates energy metabolism by repressing mitochondrial regulatory proteins. To better understand significance of this up-regulation in hypothalamus we stereotaxically injected a AAV construct expressing miR-210 into ARC nucleus of 2 month old young mice hypothalamus. Within a month of injection, these injected mice showed significant increases in food intake and body weight, and a noticeable compromise in glucose tolerance compared to age-matched, control mice that received AAV vehicle. We are currently exploring molecular mechanism underlying this metabolic changes caused by this miR and its implication to aging process in hypothalamus and whole body.

Disclosures: K. Kim: None. J. Park: None. M. Kim: None. H. Nam: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.20/BBB21

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant F31NS089411

Title: A cholinergic basal forebrain feeding circuit modulates appetite suppression

Authors: *A. M. HERMAN[1], J. ORTIZ-GUZMAN[1], I. HERMAN[1], M. KOCHUKOV[2], K. QUAST[2], J. PATEL[3], J. SELEVER[2], J. CARLSON[1], B. ARENKIEL[2];
[1]Developmental Biol., [2]Mol. and Human Genet., [3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Irregular food intake, either consuming too much or too little, is a primary cause of obesity and other eating and metabolic disorders. Highly coordinated by the brain, food intake must be balanced with energy expenditure in order to maintain proper body weight homeostasis. To date, insight into the neural control of feeding has largely focused on signaling mechanisms classically associated with the hypothalamus, the major center in the brain that regulates body weight homeostasis. However, the role of non-canonical central nervous system signaling mechanisms in regulating feeding behavior has been largely uncharacterized. Acetylcholine has long been proposed to influence feeding behavior, due in part to the functional similarity between acetylcholine and nicotine, the addictive component in tobacco that acts as an appetite suppressant. It is worth noting that nicotine is an exogenous agonist for acetylcholine receptors, suggesting that endogenous cholinergic signaling may play a role in normal physiological regulation of body weight homeostasis. However, it remains unclear if cholinergic neurons in the brain regulate food intake, and how their circuits function to modulate feeding. Here, we report that cholinergic neurons of the basal forebrain potently influence food intake and body weight. While impairment of cholinergic signaling increases food intake and results in severe obesity, enhanced cholinergic signaling decreases food consumption. Accordingly, we found that this cholinergic population modulates appetite-suppression on downstream targets in the hypothalamus. Together, our data pinpoint the cholinergic basal forebrain as a major modulatory center underlying feeding behavior, highlighting a new role of acetylcholine in modulating food intake.

Disclosures: A.M. Herman: None. J. Ortiz-Guzman: None. I. Herman: None. M. Kochukov: None. K. Quast: None. J. Patel: None. J. Selever: None. J. Carlson: None. B. Arenkiel: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.21/BBB22

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DK075087

NIH Grant DK075088

Title: The role of melanocortin-4 receptors in the paraventricular hypothalamic circuits in homeostatic processes of appetite

Authors: *C. LI, M. KRASHES;
NIH/NIDDK, Bethesda, MD

Abstract: Nutrient intake is essential for survival and requires food seeking and consumption behaviors, however overeating has led to a growing obesity endemic. Thus, identifying the neural circuits controlling feeding is imperative. The agouti-related peptide (AgRP) neurons in the arcuate nucleus (ARC) of the hypothalamus promote feeding following acute soma activation. Moreover, stimulation of $AGRP^{ARC}$ terminal fields in the paraventricular nucleus of the hypothalamus (PVH) orchestrates food intake. Using optogenetics, we recently demonstrated that food intake driven by PVH terminal photostimulation of ARC^{AgRP} neurons is mediated by direct inhibitory synaptic connectivity onto satiety-promoting melanocortin-4 receptor (Mc4R) cells. In addition, we have also showed that photoactivation of PVH^{Mc4R} neurons alone promote satiety, reducing food intake in hungry mice, while chemogenetic silencing of this subpopulation increased motivated feeding in calorically replete animals. Currently we are monitoring *in vivo* physiological activity of PVH^{Mc4R} neurons via genetically encoded calcium indicator imaging strategies. Our data directly demonstrate that PVH^{Mc4R} neurons display state-dependent responses. These results strengthen the direct modulation, connectivity and function of the ARC^{AgRP} --> PVH^{Mc4R} circuit, helping reveal the precise wiring diagram of these complex neural networks directing motivated behaviors such as feeding.

Disclosures: C. Li: None. M. Krashes: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.22/BBB23

Topic: F.10. Food Intake and Energy Balance

Support: ANR-SERFEED

Title: How does the brain implement decision-making to eat: implication of the 5-HT4 receptors

Authors: *V. COMPAN;
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Abstract: Adaptive decision-making to eat is crucial for survival but in anorexia nervosa, the brain persistently supports reduced food intake despite a growing need for energy. How the brain persists in reducing food intake to the point of death despite the evolution of mechanisms to ensure survival by governing adaptive eating behaviors remains just as mysterious as the switch from anorexia to bulimia. Neural substrates belong to the reward-habit system and could differ from overeating-induced obesity. The contribution of serotonin receptors in eating is critical in humans and animal models. One possibility is that restrictive food intake critically engages decision-making (goal-directed) systems where the serotonin 4 receptors play a pivotal action in two critical brain structure of the reward system; The nucleus accumbens and the medial prefrontal cortex. These studies introduce the view that a persistent food restriction might mimic some aspects of addiction to drugs of abuse. Furthermore, novel molecular mechanisms influenced by the constitutive activity of the serotonin 4 receptors, in the nucleus accumbens, could underlie the switch from undereating to overeating in animal models.

Disclosures: V. Compan: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.23/BBB24

Topic: F.10. Food Intake and Energy Balance

Support: NIAAA AA020608

NIAAA AA006420

Title: Intermittent access to palatable food leads to compulsive self-administration subserved by anterior insula to ventral striatal projections

Authors: *S. R. SPIERLING, A. KREISLER, G. DE GUGLIELMO, O. GEORGE, E. ZORRILLA;
The Scripps Res. Inst., La Jolla, CA

Abstract: Palatable foods are hypothesized to elicit neurobehavioral changes known as “food addiction.” Compulsivity, a component of addiction, can be defined as perseveration of behaviors despite incorrect or adverse outcomes. The insular cortex subserves many visceral-emotional functions, including taste processing and implications in drug craving and relapse. The

anterior insular cortex (AIC) sends inputs to the nucleus accumbens core (AcbC), which subserves reward and reinforcement. AIC-AcbC projections have been implicated in compulsive-like drinking in a model of alcohol addiction [Nat Neurosci 2013; 16(8): 1094-100]. In rats, intermittent access to a preferred high sucrose, chocolate-flavored diet (P) leads to binge-like intake [Neuropsychopharm 2008 Feb; 33(3) :524-35]. This study tested the hypothesis that intermittent palatable food access leads to compulsive self-administration that is mediated by AIC-AcbC inputs. Female Wistar rats, matched for food intake and body composition, were assigned to 1 of 4 groups (n=7-10): 1) ad lib chow (C) 2) ad lib P 3) intermittent P for either 30 min or 4) 24 hr on 3 non-consecutive days/week (MWF), with C otherwise. On intermittent P days, all rats performed operant self-administration for food on a fixed-ratio 1 (FR) or progressive-ratio (PR) schedule. Compulsivity was assessed via: 1) PR session breakpoint and 2) FR self-administration despite intermittent footshock punishment. Independent of duration, rats receiving intermittent access to P, but not ad lib access to C or P, showed increased PR breakpoint and FR self-administration despite intermittent footshock, consistent with compulsive self-administration. Intermittent access rats showed a direct correlation of body fat and body weight gain with increased PR breakpoint, linking compulsive eating with weight gain risk. AAV5 virus expressing fluorescently labeled archeorhodopsin 3.0 T or non-opsin virus controls were stereotactically injected into the AIC. Fiber optic cannulae were implanted bilaterally into the AcbC to allow optoinhibition of AIC-AcbC projections. Each rat performed 1 FR and 1 PR session with (optoinhibition) or without (control) laser activation. AIC-AcbC optoinhibition significantly reduced the PR responding of intermittent access rats that developed compulsive-like, elevated PR performance, without affecting the lower PR performance of control ad lib access rats or non-compulsive-like intermittent access controls. FR responding was unaltered by optoinhibition. The results support the hypothesis that AIC-AcbC projections subserve compulsive palatable food self-administration in our intermittent-access model.

Disclosures: S.R. Spierling: None. A. Kreisler: None. G. de Guglielmo: None. O. George: None. E. Zorrilla: None.

Poster

255. Anatomy and Development of Central Pathway Regulating Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 255.24/BBB25

Topic: F.10. Food Intake and Energy Balance

Support: NIH/NIDDK

NIDDK/P&F

Title: The melanocortin_3 receptor controls autoinhibitory feedback from agrp neurons onto mc4r neurons in pvn

Authors: *M. GHAMARI-LANGROUDI;
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Abstract: The role of the melanocortin-3 receptor in energy homeostasis has remained an enigma. Global deletion of the receptor in the MC3R-/- mouse increases adipose mass in the absence of detectable hyperphagia or hypometabolism. Yet at the same time, the MC3R-/- mouse exhibits reduced fast-induced refeeding, and hypersensitivity to the anorexigenic and weight-reducing effects of cachexia., and MC3R-specific agonists stimulate food intake. Here, we demonstrate that the activation of MC3R stimulates food intake and is a presynaptic receptor on arcuate nucleus AgRP/NPY neurons, and regulates GABA release from these cells onto MC4R PVN neurons, creating an autoinhibitory feedback loop. This study demonstrates that MC3R in fact plays an anabolic role by driving food intake and suppressing energy expenditure. Thus, α-MSH released by POMC neurons to activate MC4R target neurons ultimately inhibits the firing of these same neurons by MC3R-mediated release of GABA onto these same neurons. The role of this regulatory loop in the whole animal is suggested by the fact that melanocortin agonist administration is initially equally effective in inhibiting food intake in WT and MC3R-/- animals, but exhibits more potent and sustained anorexigenic action in the MC3R-/- mouse.

Disclosures: M. Ghamari-Langroudi: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.01/BBB26

Topic: F.10. Food Intake and Energy Balance

Support: Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484

Fronteras de la Ciencia Grant 63

Productos Medix Grant 1275

Productos Medix y Problemas Nacionales 201501-464

Title: The combination of 5-HTP/carbidopa plus phentermine showed a synergistic effect on weight loss and decreases the locomotor activity induced by phentermine, and neural activity in nucleus accumbens shell correlates with the reduction of locomotor side effects

Authors: *C. I. PEREZ[1], B. KALYANASUNDAR[1], M. G. MORENO[1], S. A. SIMON[2], R. GUTIERREZ[1];
[1]CINVESTAV, Mexico City, Mexico; [2]Dept. of Neurobio., Duke Univ. Med. Ctr., Durham, NC

Abstract: Obesity is a major public health problem.Hendricks et. al., 2009 reported that physicians frequently prescribed a new drug combination of appetite suppressants for the treatment of overweight: phentermine (PHEN) plus 5-hydroxytryptophan/carbidopa (5-HTP/CB). 5-HTP is a precursor of serotonin, carbidopa helps to more 5-HTP available to enter the brain and PHEN acts inhibiting recapture of dopamine. Despite its common practice, it is not known if this combination induces a synergistic effect on body weight-lossand how it modulates single-unit activity in the nucleus accumbens shell (NAcSh).We used a daily i.p injections of PHEN and 5-HTP/CB either alone or in combination for 7 days. We found that the combination of 5-HTP/CB (31 and 75 mg/kg, respectively) plus PHEN (10, 15 and 20 mg/kg) promoted a significantly greater body weight-loss than these appetite suppressants alone. Importantly, this combination reduced locomotors side-effects induced by PHEN alone, especially during the last 4-7 days of daily treatment.These data indicates the existence of a synergistic effect of this combination in body weight and reduction of side-effects.In order to shed light of the neuronal correlates of this synergism, we recorded single-unit activity of NAcSh in rats while they received the combination 5-HTP/CB + PHEN (31, 75 and 15 mg/kg, respectively) and drugs alone. We found that PHEN alone exerts at the population activity level a strong inhibitory imbalance towards inhibition (n=197, inh 61%: act 8%) that correlated with the onset of locomotor side-effects, while 5-HTP/CB alone although it modulated spiking activity in the NAcSh (n=140, inh: 21%: act 21%) it does not induced any inhibitory imbalance at the population activity of the NAcSh and did not induce locomotor activity. PHEN with 5-HTP/CB attenuated the inhibitory imbalance induced by PHEN alone that correlated with the reduction of psychomotor activation induced by PHEN. These data indicates that the combination counterbalanced the inhibitory imbalance induced by PHEN, selectively increasing the percentage of neurons and magnitude of activated responses without altering neurons inhibited by PHEN. To confirm this data, we are now recording neuronal activity using a higher dose of 5-HTP (63mg/kg)/CB +PHEN.Our preliminary results indicate that these combinations of 5-HTP/CB+PHEN further attenuated locomotor side-effects and reversed the inhibitory imbalance induced by PHEN towards activation.This preclinical evidence indicates that this combination produced a synergistic effect in weight loss and found for the first time that NAcSh population activity explained their reduced locomotor side-effects.

Disclosures: C.I. Perez: None. B. Kalyanasundar: None. M. G. Moreno: None. S. A. Simon: None. R. Gutierrez: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.02/CCC1

Topic: F.10. Food Intake and Energy Balance

Support: NIH-NIDDK R01-DK102918

Postdoctoral Research Fellowship Neuroscience Institute,UTHSC

Title: Consumption of a high-fat diet increases persistent sodium currents in AgRP neurons in the arcuate nucleus of the hypothalamus.

Authors: *W. WEI[1], A. SMITH[1], K. O'CONNELL[1,2];
[1]The Univ. of Tennessee, Memphis, TN; [2]the Neurosci. Inst., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Excess food intake is the leading cause of obesity. Although the regulation of food intake is complex and involves multiple brain regions, the control of appetite begins in the arcuate nucleus of the hypothalamus (ARH). Within the ARH, neurons expressing Agouti-related peptide (AgRP) are of special importance, since they play a direct role in regulating appetite and food intake. Our previous work has shown that after high fat diet (HFD) consumption, the spontaneous firing frequency of AgRP neurons increases significantly, with an accompanying change in the action potential waveform. We reasoned that an altered subthreshold 'pacemaker' current might underlie the increased excitability of AgRP neurons after HFD consumption. The persistent sodium current ($I_{Na,P}$) is one of the pacemaker currents in many types of neurons. Using current- and voltage-clamp recording of AgRP neurons in acute brain slices, we compared the amplitude of $I_{Na,P}$ and voltage dependence of $I_{Na,P}$ between lean and HFD fed-mice. We found that application of 10 uM rizulole, a blocker of $I_{Na,P}$, induced membrane hyperpolarization and significantly decreased the firing rate of AgRP neurons from both HFD-fed mice and control diet (CD)-fed mice. Consistent with a increased role for $I_{Na,P}$ in neuronal excitability following consumption of HFD, AgRP neuronal inhibition by riluzole was greater in neurons from HFD mice compared to controls. We also found that the peak amplitude of $I_{Na,P}$ is larger in AgRP neurons from HFD-fed mice than from control mice. Using single-cell RT-PCR, we confirmed expression of Nav1.7 and Nav1.9 subunits in AgRP neurons, both of which exhibit a gating mode that generates a persistent current and may represent a putative molecular correlate of $I_{Na,P.}$ Taken together, HFD consumption leads to a significant increase in the amplitude of $I_{Na,P}$ in AgRP neurons, which may contribute to the mechanism of increased spontaneous firing frequency of AgRP neurons following HFD consumption.

Disclosures: W. Wei: None. A. Smith: None. K. O'Connell: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.03/CCC2

Topic: F.10. Food Intake and Energy Balance

Support: University of Illinois

Title: Alcohol injection but not voluntary drinking robustly suppresses appetite in rats

Authors: *N. NELSON;
Neurosci. & Psychology, Univ. of Illinois At Urbana-Champaign, Champaign, IL

Abstract: In addition to being the most commonly abused substance in most parts of the world, alcohol is also a calorie containing (7 kcal/g) but nutritionally poor food source. On heavy drinking occasions, alcohol-derived calories could account for 10 - 27% of the recommended daily energy intake in adults. Consumption of alcoholic beverages has been proposed to be a risk factor for obesity; yet, other researchers believe the contrary. To that end, it is vital to understand the extent to which alcohol administration affects body weight (BW) and appetite. Our prior work revealed that IP alcohol administration dose-dependently reduces food intake, preference for a 45% high fat diet, and weight gain in male *Long-Evans* rats. Here we hypothesize that the effects of alcohol on energy homeostasis would depend on the route and pattern of administration. In experiment 1, male *Long-Evans* rats were injected (IP) on Mon, Wed and Fri (3 weeks) with 3 g/Kg/day (30% V/V in saline) alcohol doses administered all at once (EtOH_S) or in 2 separate 1.5 g/Kg injections that were spaced 3h apart (EtOH_D). In experiment 2, male and female *Long-Evans* rats voluntarily consumed 5% and 10% alcohol solutions sweetened with 0.1% saccharine (a non-calorie sweetener) on Mon - Fri (7.5 h/day) for 1 week and 7 weeks, respectively. Blood alcohol concentrations (BAC) were determined from plasma derived from tail blood in both experiments. Results of experiment 1 indicate that 3 g/Kg/day alcohol led to BACs of 246 ± 40 mg/dL (EtOH_S) and 120 ± 10 mg/dL (EtOH_D) 7.5h after administration and acutely suppressed energy intake and BW in both groups, but to a greater extent in the EtOH_S group. Results of experiment 2 indicate that although male and female rats would voluntarily ingest around 3g/Kg alcohol within 7.5h, acute BW and appetite suppressions were not observed. Furthermore, the pilot data show that BACs at 1h and 9h after alcohol presentation were 79 ± 15 mg/dL and 6 ± 0.1 mg/dL, respectively. Hence, the BAC achieved during voluntary drinking was never as high as those of IP injection. Together, these data show that 3 g/Kg IP alcohol produces robust appetite and BW suppression due to the higher BAC attained than voluntary drinking. Such results suggest that BAC attained and the maintenance of high BAC are the determining factors for how alcohol consumption affects acute appetite and long term energy balance.

Disclosures: N. Nelson: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.04/CCC3

Topic: F.10. Food Intake and Energy Balance

Support: AHA Grant-in-Aid

Title: The glucose sensitivity of lateral hypothalamic area (LHA) orexin glucose-inhibited (GI) neurons may influence reward-based feeding via modulation of ventral tegmental area (VTA) dopamine (DA) neurons

Authors: S. B. TEEGALA[1], Z. SHENG[1], U. KHAN[1], M. P. THOMAS[2], *V. H. ROUTH[1]; [1]Dept Pharmacol & Physiol, RBHS: New Jersey Med. Sch., Newark, NJ; [2]Biol. Sci., Univ. Northern CO, Greeley, CO

Abstract: Lateral hypothalamic area (LHA) orexin neurons are inhibited by glucose (glucose-inhibited; GI). We found that metabolic state regulates the glucose sensitivity of LHA orexin-GI neurons. Fasting and the "hunger" hormone ghrelin enhance activation of these neurons in low glucose, whereas the satiety hormone leptin does the converse. LHA orexin neurons project to ventral tegmental area (VTA) dopamine (DA) neurons where orexin induces glutamate plasticity. VTA DA neurons are involved in reward-based feeding behavior. We hypothesized that activation of LHA orexin-GI neurons in low glucose persistently enhances glutamate neurotransmission onto VTA DA neurons and reinforces reward-based feeding behavior. We first measured spontaneous N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated glutamate postsynaptic currents on VTA DA neurons using voltage clamp recording in horizontal mouse brain slices containing the LHA. Lowering glucose from 2.5 to 0.25 or 0.7 mM increased NMDA (n=8; $P<0.001$) and AMPA (n = 7; $P<0.0001$) receptor-mediated current amplitude; effects persisted for at least 1 hour after return to 2.5 mM glucose. When glucose was lowered in the presence of the orexin A antagonist SB334867 (10μM) NMDA current amplitude was not increased (n=5; $P>0.05$). This suggests activation of orexin-GI neurons mediated the effect of low glucose. A 24 hour fast increased the AMPA/NMDA current amplitude ratio, a measure of in vivo glutamate plasticity (n=7, P=0.03). Next, we dialyzed the LHA of rats with glucose concentrations seen in the brain during fasting, euglycemic and hyperglycemic conditions (0.7, 2 and 4 mM, respectively) and measured conditioned place preference (CPP). Rats were food restricted to 85% of their body weight, bilaterally implanted with microdialysis guide cannula aimed 1mm above the LHA (AP: -2.8,

ML: +/- 3.1; DV: -8.1; 15^{o}) and maintained at 85% body weight. They were then conditioned with 5g of a positive reinforcer (chocolate) on alternate days for 6 days using a two compartment CPP apparatus. Chocolate was placed on the side opposite to their initial preference. On day 7, glucose was dialyzed into the LHA and the time spent in each compartment in the absence of chocolate was measured. The difference in time for the chocolate conditioned vs initially preferred compartment was calculated as an index of CPP. There was a significant negative correlation between glucose concentration and CPP ($P<0.05$, $R^2=0.66$, $n=6$). Our data suggest that the glucose sensitivity of LHA orexin-GI neurons may link metabolic status to reward based-feeding by altering glutamate plasticity on VTA DA neurons.

Disclosures: S.B. Teegala: None. Z. Sheng: None. U. Khan: None. M.P. Thomas: None. V.H. Routh: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.05/CCC4

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DK083452

College of Veterinary Medicine WSU

Title: Leptin receptor-expressing neurons in the nucleus of the solitary tract receive mono-synaptic input from CCK-sensitive visceral afferents.

Authors: *D. NEYENS, S. APPLEYARD, H. ZHAO, S. PAGE;
Integrated Physiol. and Neurosci. (IPN), Washington State Univ., Pullman, WA

Abstract: Leptin is an essential anorexigenic hormone that regulates energy homeostasis; it is produced primarily by white adipose tissue and circulates at levels that reflect fat mass. Historically, research has focused on the effects of leptin in the hypothalamus, but leptin has also been shown to act in the brainstem to reduce food intake. The nucleus of the solitary tract (NTS) is an important hub of integration for direct and indirect controls of food intake, as it receives input from visceral afferents in the gut as well as descending input from forebrain structures that control feeding behavior. Injections of leptin into the 4th ventricle or NTS significantly reduce food intake; however, the cellular mechanisms underlying this effect are not well understood. A subpopulation of NTS neurons express leptin receptors (LepRs), but the electrophysiological profile of these LepR-expressing neurons has not been well-characterized. Here we use patch-

clamp techniques and selective stimulation of the vagus-containing solitary tract in horizontal brain slices to characterize how LepR neurons process visceral afferent information. We selectively recorded from LepR-expressing NTS neurons using a transgenic mouse line in which the LepR promoter drives Cre-dependent expression of td-Tomato (LepRCre-tdTomato). The majority of LepR neurons in the NTS receive at least one direct, monosynaptic input from solitary tract afferents, as defined by low jitter or variability (<200 μs) in the latency of evoked excitatory post-synaptic currents (EPSCs) and no failures. Nearly all LepR neurons also received polysynaptic inputs, suggesting these neurons can integrate multiple excitatory inputs following visceral afferent stimulation. The gut peptide cholecystokinin (CCK) significantly increased (~340%) the frequency of spontaneous excitatory inputs onto about half of LepR neurons tested, suggesting that NTS LepR neurons are targets for CCK-sensitive afferents innervating the lower GI tract. Interestingly, almost all LepR neurons (~99%) express post-synaptic NMDA receptors, as indicated by Mg-sensitive currents blocked by the antagonist DCPene (10 μM). NMDARs are critical for vagus-mediated satiety reflexes, and our lab has shown that NMDARs help maintain fidelity of transmission during high-frequency stimulation of the solitary tract. Taken together, these data suggest that LepR-expressing NTS neurons integrate inputs from CCK-sensitive and CCK-insensitive vagal afferents, and NMDAR-mediated currents may be essential to their function in the NTS.

Disclosures: D. Neyens: None. S. Appleyard: None. H. Zhao: None. S. Page: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.06/CCC5

Topic: F.10. Food Intake and Energy Balance

Title: MRAP2 is essential to central ghrelin activity

Authors: *D. SRISAI, J. A. SEBAG;
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Abstract: The Melanocortin Receptor Accessory Protein 2 (MRAP2) is a single-pass transmembrane protein essential for the control of energy homeostasis. MRAP2 is highly expressed in the central nervous system, including the arcuate nucleus of the hypothalamus (ARC) and the paraventricular nucleus of the hypothalamus. It acts by regulating the activity of several central G-protein coupled receptors (GPCR) that control food intake and energy expenditure, including the Melanocortin-4 receptor and the Prokineticin Receptor 1. In this study we identify a role for MRAP2 in the regulation of the Ghrelin receptor GHSR1a. Ghrelin is a

hormone mainly secreted by the stomach that signals hunger to the brain. The ghrelin receptor is mainly expressed in the hypothalamus, especially in AGRP neurons of the ARC, where its activation leads to hunger and increased food intake. Here we demonstrate that MRAP2 KO mice have a defect in hunger sensing and show that fasting fails to activate the orexigenic AGRP neurons in the hypothalamus of mice lacking MRAP2. We also show that MRAP2 is essential for ghrelin-stimulated intracellular signaling and that MRAP2 forms a complex with the ghrelin receptor. Also, whereas the intra-cerebroventricular injection of ghrelin significantly induces food intake in WT satiated mice, the orexigenic effect of ghrelin is largely lost in MRAP2 KO mice. These results suggest that MRAP2 is essential for the central actions of ghrelin and further demonstrate that MRAP2 is a master regulator of the energy homeostasis machinery.

Disclosures: D. Srisai: None. J.A. Sebag: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.07/CCC6

Topic: F.10. Food Intake and Energy Balance

Title: Fasting promotes liver oleoylethanolamide signaling through a histamine-dependent mechanism

Authors: *A. MISTO[1], G. PROVENSI[2], V. VOZELLA[1], B. PASSANI[3], D. PIOMELLI[1,4]; [1]D3, Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Dept. di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, [3]Dept. di Scienze della Salute, Univ. di Firenze, Firenze, Italy; [4]Dept. of Anat. and Neurobio., Univ. of California, Irvine, CA

Abstract: The fatty acid ethanolamide, oleoylethanolamide (OEA), is a lipid mediator that regulates feeding (Rodriguez de Fonseca et al., 2001; DiPatrizio et al., 2015; Piomelli 2013) and stimulates lipolysis and fatty acid oxidation in adipocytes and hepatocytes (Guzman et al., 2004) through activation of nuclear peroxisome proliferator-activated receptor-alpha (PPAR-α) (Fu et al., 2003). Fasting is accompanied by a substantial elevation in liver OEA levels (Fu et al., 2007), but the biochemical mechanism and physiological implications of this effect are unknown. The neurotransmitter histamine plays an important role in the control of feeding behavior and mediates, in part, the anorexic effects of OEA (Provensi et al., 2014).
In the present report, we show that histamine is obligatory for food deprivation-induced OEA accumulation in mice liver through a mechanism that requires activation of H_1-type histamine receptors.
Male HDC-/- mice (mice deficient in the histamine-synthesizing enzyme histidine

decarboxylase), and wild-type littermates (129/Sv background), male C57BL/6J-Kit$^{W\text{-}v}$/J mice (mice mast cells deficient) and male C57BL/6J mice were subjected to 3 different conditions: a) free-feeding (FF); b) 12h food-deprivation (FD) and c) 1h refeeding after 12h food-deprivation (RF).
Alpha-fluoromethylhistidine (α-FMH), a suicide inhibitor of HDC, was administered by intracerebroventricular (i.c.v.) infusion (5µg/5µl), while histamine receptor antagonists (fexofenadine and famotidine, 10 mg/kg and ciproxifan, 3mg/kg) were administered by intraperitoneal injection during FD or RF. Histamine was administered by intrahepatic (IH) injection (2-4mg/kg for 30min and 1h) during FF. Endogenous OEA, fatty acid 18:1 and N-oleoyl-PE levels were quantified after lipid extraction by liquid chromatography/mass spectrometry.
We found that: 1) fasting stimulates liver OEA mobilization in wild-type mice, but fails to do so in mutant HDC-/- mice; 2) the H_1 receptor antagonist, fexofenadine, prevents the effect of fasting on OEA accumulation in wild-type mice; 3) the H_2- and H_3-receptor antagonists, famotidine and ciproxifan, and i.c.v. infusion of α-FMH do not affect fasting-induced OEA accumulation in wild-type mice; 4) histamine administration stimulates hepatic OEA production in dose-and time-dependent way; 5) histamine effect is selective only for OEA not for oleic acid.
In conclusion, our results suggest that fasting stimulates peripheral histamine-dependent signaling, which promotes OEA mobilization in mice liver through activation of H_1, but not H_2 or H_3 receptors. The mechanism underlying this novel function of histamine is under investigation.

Disclosures: A. Misto: None. G. Provensi: None. V. Vozella: None. B. Passani: None. D. Piomelli: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.08/CCC7

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DK083452

WSU ADARP

Title: Endogenous cholinergic activation of NTS catecholamine neurons depends on glucose concentration.

Authors: *S. PAGE, S. M. APPLEYARD;
Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Visceral afferents carrying satiety information from the gastrointestinal system terminate primarily on neurons in the nucleus of the solitary tract (NTS). Catecholamine neurons in the NTS (NTS-CA) play a critical role in integrating and relaying information regarding satiety, cardiovascular activity, and stress to other brain nuclei. Nicotine is a potent suppressor of appetite, and smoking cessation is associated with heightened appetite and weight gain. We have previously demonstrated that nicotine activates NTS-CA neurons through a pre-synaptic mechanism by binding to nAChRα7, increasing the rate at which glutamate is released from visceral afferent terminals. Further, our lab showed that, similar to the 5-HT3 receptor, activation of visceral afferent terminals through nAChRα7 is rapidly modulated by changes in extracellular glucose concentration. For the current study, we determined whether NTS-CA responses to native cholinergic inputs, which release the endogenous nAChR agonist acetylcholine (ACh), are also regulated by changes in glucose. To study NTS-CA neuronal responses to endogenous ACh, we used a line of transgenic mice that express enhanced green fluorescent protein under the control of the tyrosine hydroxylase promoter (TH-EGFP), and crossed it with a line that expresses light sensitive channelrhodopsin-2 selectively in choline acetyltransferase-positive neurons (ChAT-ChR2). The resulting offspring expressed both transgenes (TH-EGFP/ChAT-ChR2), allowing us to simultaneously identify NTS-CA neurons and photo-stimulate local ACh release during whole-cell recordings. It was found that whole-field laser illumination of horizontal brain slices increased the frequency of glutamatergic spontaneous excitatory post-synaptic currents (sEPSC) in NTS-CA neurons. This response was absent in animals lacking ChR2, and was completely blocked by alpha7 nicotinic ACh receptor (nAChRα7) selective antagonists MG624 (20μM) and methyllycaconitine (100μM). sEPSC responses were reproducible within the same cell by repeating stimulation in 30-second intervals. We found that reducing the concentration of bath glucose from 10mM to 2mM decreased the magnitude of endogenous ACh responses by ~32%. The effect of 2mM glucose on ACh responses was fully reversed by restoring glucose to 10mM. The results of this study indicate that endogenous acetylcholine activates NTS-CA neurons through indirect actions on nAChRα7 to increase glutamate release from pre-synaptic visceral afferent terminals, and that this action is dependent on local glucose levels.

Disclosures: S. Page: None. S.M. Appleyard: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.09/CCC8

Topic: F.10. Food Intake and Energy Balance

Support: Commonwealth Health Review Board #349-02-15

Howard Hughes Medical Institute Precollege & Undergraduate Science Education program to Washington & Lee University

Lenfest Sabbatical Award

Lenfest Summer Research Award

Title: Access to snacks from weaning onward in female rats causes obesity, insulin insensitivity and sustained leptin resistance in adulthood.

Authors: L. N. DELA CRUZ, R. C. CLAWSON, T. J. WENGER, S. T. ALLEN, *H. I'ANSON;
Biol. Department, Howe Hall, Washington & Lee Univ., Lexington, VA

Abstract: Approximately one third of the US adolescent population is obese or overweight. Therefore, we developed a rat model that mimics snacking behavior to investigate the mechanisms leading to childhood obesity. We hypothesized that giving constant access to energy from snacks after weaning causes an early onset of obesity and related disorders. At 22 days of age, we divided 24 weanling female Long-Evans rats into three groups (n=8 per group): chow only (Control), healthy snack (HS), and unhealthy snack (US). HS and US rats were sequentially given a rotation of three snacks at 10am and 3pm daily (HS: Kashi GoLean cereal, unsalted almonds, or Cheerios; US: Keebler's Sandies, Tostitos, and peanut butter chips). All groups received *ad libitum* chow and water. Food intake, snack intake, and body weight (BW) were monitored daily. A leptin challenge was administered at the time of puberty (Days 34-38) and during adulthood (Days 69-73) to investigate possible leptin resistance during development or in response to snacking. To determine insulin sensitivity, a glucose tolerance test (GTT) was performed after an overnight fast on Days 84-85. On Days 90-93, rats were terminated, blood and tissue samples collected, and abdominal fat pads removed and weighed. At this time, HS and US rat BWs were significantly more than Control rats, and US rats grew faster than HS rats. Total caloric intake did not differ between snacking groups, though control rats consumed more calories later in the study. Control rats had significantly less visceral fat than both snacking groups, which had similar fat pad weights. At the time of puberty, neither snacking nor Control rats decreased food intake in response to either leptin dose (1mg/kg or 10mg/kg ip). As adults, only the Control rats decreased their food intake in response to leptin (1 or 5 mg/kg ip). These data suggest that leptin resistance during development may create greater vulnerability to poor dietary choices. Snacking adults remained leptin resistant, likely contributing to their defense of a higher body weight. The GTT resulted in a similar initial insulin release in all groups, but serum insulin levels remained elevated in both snacking groups for more than two hours while insulin in control rats returned to basal levels within thirty minutes of glucose gavage. These data support our hypothesis that constant access to energy from snacking induces early onset of obesity in rats and accompanying markers of metabolic syndrome. Further, we conclude that

young rats, and probably humans, are vulnerable to snacks from weaning due to inherent leptin resistance during development that is sustained into adulthood.

Disclosures: L.N. dela Cruz: None. R.C. Clawson: None. T.J. Wenger: None. S.T. Allen: None. H. I'Anson: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.10/CCC9

Topic: F.10. Food Intake and Energy Balance

Title: Effects of peripherally and centrally evoked swallow on the jaw-opening reflex responses

Authors: *T. SUZUKI, S. SAKAI, K. TSUJI, J. MAGARA, T. TSUJIMURA, M. INOUE; Div. of Dysphagia Rehabil., Niigata Univ. Grad. Sch. of Med. and, Niigata-Shi, Japan

Abstract: Purpose: We previously demonstrated that the jaw-opening reflex (JOR) response evoked by the low-threshold trigeminal afferents was inhibited during swallowing evoked by superior laryngeal nerve (SLN) stimulation, suggesting that the oral sensory transmission is inhibited by activation of swallowing neural network. The aim of this study was to investigate whether the JOR response was modulated during swallowing evoked by not only peripherally but also centrally evoked swallow. Materials and Methods**:** The experiments were carried out on anesthetized rabbits with urethane (1.0 g/kg, iv). Electromyographic activity was recorded from the digastric and mylohyoid muscles on either side. To evoke the JOR, the inferior alveolar nerve (IAN) was stimulated (single pulse, 0.2msec pulse duration). Stimulus intensity was set at 2.0 times (T) the threshold for evoking the JOR. To evoke swallowing, either the SLN or cortical swallowing area was repetitively stimulated; 30 Hz, 0.2 msec pulse duration for the former and 30 Hz, 0.5msec pulse duration for the latter. Stimulus intensity of SLN and cortex was set between 0.8-4 T and 0.8-1.4 T the threshold for evoking the swallowing at least once for 10 sec, respectively. To reduce the effect of saliva, bilateral submandibular glands were removed and parotid ducts were ligated. In a recording session, the JORs were evoked by the IAN stimulation at 1 Hz for 30 sec. In the middle 10 sec, SLN or cortex was simultaneously stimulated at either intensity. The mean amplitudes and latencies of the JOR were compared among the conditions; before, during and after SLN or cortical stimulation. Results & Discussion**:** Stimulus threshold ranged between 0.05 and 0.17 mA for IAN, between 0.04 and 0.6 mA for SLN and between 0.25 and 1.2 mA for cortex. Amplitude of the JOR was smaller and latency was longer during SLN or cortical stimulation than before stimulation. Furthermore, the inhibitory effects tended to increase as the stimulus intensity of the SLN and cortex increased. The results suggested that the

oral sensory transmission is inhibited during swallowing evoked by either stimulation of SLN or cortex.

Disclosures: T. Suzuki: None. S. Sakai: None. K. Tsuji: None. J. Magara: None. T. Tsujimura: None. M. Inoue: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.11/CCC10

Topic: F.10. Food Intake and Energy Balance

Title: Acute hypoglycemic and hyperglycemic states induce specific changes in cervical vagus nerve activity

Authors: *E. A. BATTINELLI[1,3], J. NEWMAN[1], T. TSAAVA[1], H. SILVERMAN[1,3], M. CUCINO[3], C. BOUTON[2], S. S. CHAVAN[1], K. J. TRACEY[1,3];
[1]Lab. of Biomed. Science, Ctr. for Bioelectronic Med., [2]Ctr. for Bioelectronic Med., Feinstein Inst. at Northwell Hlth., Manhasset, NY; [3]Hofstra Northwell Sch. of Med. at Hofstra Univ., Hempstead, NY

Abstract: Food intake and energy balance are regulated by crosstalk between the central nervous system and visceral organs. Physiological homeostasis is maintained by reflex mechanisms in which afferent signals are interpreted by the central nervous system and efferent responses are initiated to maintain energy homeostasis. The vagus nerve, with its anatomical distribution and known contribution to metabolic homeostasis, is a conduit that conveys messages regarding changes in peripheral glucose levels to the brainstem. Here, we have recorded and analyzed extraneural activity in the mouse cervical vagus nerve in response to both acute hypoglycemic and hyperglycemic states (a bolus of insulin or glucose, respectively), using both manual and automated wavelet-based analysis techniques. In hypoglycemic animals, a transitory increase was observed in both heart rate and respiratory rate. Whereas in hyperglycemic animals, a transient decrease followed by an increase was observed in heart rate, with no significant changes in the respiratory rate. Administration of insulin and glucose both induced significant increases in vagus nerve activity as compared to quiescent baseline activity, with an average peak rate of 70 events per second during the response compared to the baseline average of ~6 events per second. Induction of acute hypoglycemia induced an immediate response with a latency of 200 seconds that lasted for ~200 seconds. We also observed delayed longer-lasting responses in hyperglycemia (latency of 500 seconds and a total duration of 400 seconds). Together, these studies demonstrate that acute changes in the blood glucose levels mediate

altered heart and respiratory rates associated with specific alterations in the cervical vagus nerve activity.

Disclosures: E.A. Battinelli: None. J. Newman: None. T. Tsaava: None. H. Silverman: None. M. Cucino: None. C. Bouton: None. S.S. Chavan: None. K.J. Tracey: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.12/CCC11

Topic: F.10. Food Intake and Energy Balance

Support: State University of New York

Title: An inhibitory septum to lateral hypothalamus circuit that mediates stress induced anorexia

Authors: *P. SWEENEY, Z. XU, Y. YANG;
SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Changes in food intake in response to environmental alterations, such as stress, are coordinated by hormonal interactions with the central nervous system. However, the cellular and circuit mechanisms governing stress-induced anorexia are largely underexplored, and effective treatments remain lacking. Here, by using the cell-type-selectivity of genetic methods, circuit mapping, and behavior assays, we decipher a cell-type-specific inhibitory septum to lateral hypothalamus (LH) circuit that contributes to stress-induced suppression of feeding. We find that chemogenetic inactivation of septal GABAergic neurons in the dorsal and medial septum (LSd/MS) attenuates the anorexigenic effects on food intake induced by physical restraint-based stress. Supportively, we find that chemo-/optogenetic activation of LSd/MS GABAergic neurons or their inputs in the LH reduces food intake. Furthermore, we decipher a putative neural circuit that contributes to stress induced anorexia consisting of LSd/MS GABAergic neuronal inputs to a subset of GABAergic neurons localized in the LH, an area involved in homeostatic and hedonic control of energy states. Collectively, our data demonstrate a novel circuit mediating stress-induced anorexia, one that may serve as a therapeutic target for the treatment of emotion-related eating disorders, such as anorexia nervosa.

Disclosures: P. Sweeney: None. Z. Xu: None. Y. Yang: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.13/CCC12

Topic: F.10. Food Intake and Energy Balance

Support: 243335

Title: TNFα contributes to the development of hyperalgesia in overweight ovariectomized rats

Authors: *O. A. JARAMILLO-MORALES[1], J. V. ESPINOSA-JUÁREZ[2], F. J. LÓPEZ-MUÑOZ[2];
[1]Cinvestav, DF, Mexico; [2]Cinvestav, Mexico city, Mexico

Abstract: Menopause is associated with greater perception to pain and an increase in abdominal fat. Additionally, it is known that obesity and overweight is more prevalent in women than in men. Weight gain is related to changes in the perception of pain; however, its mechanism is not clear. **Objective**. The aims of this study were to analyze the behavioral responses of hypoestrogenic, overweight Wistar rats to thermal stimuli and to analyze the leptin and TNFα levels in this population. **Methods**. Animals with hypoestrogenism induced by bilateral ovariectomy were used. Animals received either a hypercaloric diet (30% sucrose) or regular water with standard laboratory food *ad libitum* for 4 weeks; the thermal nociception and body weight were measured during this period. Four weeks after treatment, the TNFα and leptin levels and the abdominal fat weight were measured in both groups. Nociception was assessed using the "Plantar test". **Results.** Overweight ovariectomized Wistar rats displayed significantly higher body weight and abdominal fat weight than did the control group. A hyperalgesic response was observed in animals fed sucrose. Thermal latency was also significantly decreased during the 4th week in these animals compared to that of controls. There were no differences in leptin levels, but the TNFα levels were altered between groups. **Conclusions**. Our data indicate that increased body weight and abdominal fat and increases in TNFα are associated with the hyperalgesic responses observed in ovariectomized overweight female rats.

Disclosures: O.A. Jaramillo-Morales: None. J.V. Espinosa-Juárez: None. F.J. López-Muñoz: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.14/CCC13

Topic: F.10. Food Intake and Energy Balance

Title: A genetic approach to pinning down the wanderer: Mapping vagal afferent innervation of the mouse small intestinal mucosa

Authors: *H. SERLIN, E. A. FOX;
Psychological Sci., Purdue Univ., West Lafayette, IN

Abstract: Vagal afferents that supply the small intestine mucosa play a key role in signaling satiation and regulation of digestive functions (e.g., Yox AJP, 260:R503, 1991). Little is known about the morphology or distribution of this innervation. A major obstacle has been selectively labeling a large proportion of vagal mucosal afferents independent of other extrinsic and intrinsic innervation (Fox Auton Neurosci, 126-7:9, 2006). A recent breakthrough revealed that Nav1.8Cre-Tomato control (NavCreTom-CON) mice express Tomato protein in ~80% nodose and dorsal root ganglion (DRG) cells and in mucosal afferents throughout the intestines (Gautron, 519:3085, 2011). Subdiaphragmatic vagotomy in NavCreTom-CON mice (NavCreTom-VGX) resulted in complete loss of this mucosal innervation, suggesting the innervation was all of vagal origin. However, this study relied on visualization of native Tomato fluorescence and qualitative analysis. To quantitatively explore the distribution, more thoroughly evaluate the vagal origin, and examine the morphology of Tomato-containing nerve terminals throughout the entire small intestine, innervation at 13 sites along the small intestine of NavCreTom-CON and NavCreTom-VGX mice were compared. Also, Tomato protein was stained to amplify its native fluorescence signal for more sensitive visualization. CON and VGX tissue was harvested, processed and mucosal innervation quantified in parallel. After perfusion fixation, 1 cm length blocks of small intestine from a random subset of 5 out of the 13 sampling sites from each mouse were harvested, frozen, cross-sectioned, and stained. Several features of nerve terminals in villi or adjacent to crypts were quantified blind. Preliminary data from NavCreTom-CON mice identified a large proximal to distal decrease in the mean number of fibers entering villi (5 + 0.24 vs 1 + 0.11), of fiber line crossings per villus width at villus mid-height (4 + 0.17 vs 0.9 + 0.12), and of crypts in close apposition with nerve fibers (75 + 0.05 vs 25 + 0.02). All of these measures were reduced in VGX mice with only 22% proximal and 13% distal villi exhibiting stained fibers, compared to 92% and 50%, in controls. Interestingly, the remaining fibers in VGX mice showed stable numbers in the 3 measures above along the entire length of the small intestine rather than a proximal-distal gradient (0.4 + 0.07 vs 0.3 + 0.08, 0.3 + 0.08 vs 0.6 + 0.17, 29 + 0.03 vs 13 + 0.02, respectively). These findings suggest labeled afferents in the small intestinal mucosa are mainly vagal in origin. Thus, this mouse model will be

valuable for studying the morphology, plasticity, and function of vagal mucosal afferents at all levels of the small intestine.

Disclosures: H. Serlin: None. E.A. Fox: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.15/CCC14

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DK105510

Title: Stimulation of AgRP neurons eliminates the effects of appetite suppressing compounds

Authors: R. A. ESSNER, *M. CARTER;
Williams Col., Williamstown, MA

Abstract: To maintain energy homeostasis, the brain controls appetite by detecting circulating anorexigenic (appetite suppressing) and orexigenic (appetite promoting) factors in the bloodstream, and integrating these signals to affect food-seeking behavior. Endogenous anorexigenic hormones include amylin, secreted by the pancreas, and cholecystokinin (CCK), secreted by the small intestine. In addition, exogenous compounds, such as lithium chloride (LiCl), a salt that creates gastric discomfort, and lipopolysaccharide (LPS), a bacterial cell wall component that induces sickness, have appetite suppressing effects. Within the arcuate nucleus of the hypothalamus, agouti-related peptide (AgRP)-expressing neurons make up one key population of orexigenic neurons. Recent studies have shown that optogenetic or pharmacogenetic stimulation of AgRP neurons is sufficient to cause a rapid and reversible increase in food intake in mice. Here, we show that optogenetic activation of AgRP neurons is sufficient to overcome the appetite suppressing effects of amylin, CCK, and LiCl. Interestingly, AgRP neuron stimulation was completely unable to overcome the appetite suppressing effects of LPS. We also show the effects of AgRP stimulation on expression of Fos, an indirect marker of neural activity, in brain regions that suppress appetite following administration of anorexigenic compounds. Taken together, these results show that appetite-suppressing compounds operate in different ways, some of which can be overcome with AgRP neuron stimulation and others that cannot.

Disclosures: R.A. Essner: None. M. Carter: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.16/CCC15

Topic: F.10. Food Intake and Energy Balance

Support: FC-UNAM2016

Title: Metabolic triglycerides and glucose circadian rhythms are altered by perinatal cafeteria diet in rat

Authors: *D. J. BUSTAMANTE VALDEZ, P. DURAN;
LBAE Biol Cel, Facultad de Ciencias, UNAM, Ciudad de Mexico, Mexico

Abstract: The Hypothalamic Suprachiasmatic Nucleus (SCN) known to be the circadian master clock in mammals also receives humoral and neural information about the metabolic status of the body. The quantity and quality of food intake is related with the correct use of metabolites that are involved in the energy balance. Proper nutrition is one of the factors necessary for the health and development of living beings The intrauterine environment in which grows a fetus is one of the most important factors for the optimal development of this, depends in part on the external stimuli which receive, including the environment nutrition; as an disproportion in the quantity or quality of the nutrients can affect critical periods of development, triggering irreversible physiological or metabolic changes. Epidemiological studies in humans as well in animal models report that an overnutrition due to hypercaloric diets (vgr cafeteria diet) has been associated with predisposition of metabolic syndrome and diseases. The aim of the present study was to evaluate and to establish the differences in the daily profiles, amplitude and acrophase of glucose and plasmatic triglycerides, as well as assess the plasma concentrations of total triglycerides after a test of prolonged fasting in male and female rats of 40 and 90 days of age. Sprague-Dawley female rats were randomly divided in 2 nutritional protocols (control-CO and hypercaloric-low protein malnutrition-MHp) maintained with food and water ad libitum three weeks before mating and during gestation and lactation. All studies were performed in the juvenile male and female offspring. Postnatal body weight and size from birthing trough adulthood were recorded. Results shows that MHp presented a decrease in weight and size, as well as an area significantly lower in the glucose tolerance test from juvenile to adult ages. Preliminary Cosinor analysis of glucose and plasmatic triglycerides daily rhythm shows a reduction in the amplitude and a delay in the acrofase for total triglycerides and glucose for females and males at 40d of age, as well as for total triglycerides daily profile for males of 90d. We performed also a fasting test where a significant reduction of total plasmatic triglycerides in plasma for the MHp was observed. Therefore it can be inferred that the disparity presented in the MHp group in comparison with the CO in the daily rhythm of glucose and triglycerides processes, as well as the delay in the growth

is due to the exposure of hypercaloric and protein known as "cafeteria diet". Therefore nutritional conditions during the early stages of development has an influence on the energy balance in later stages.

Disclosures: D.J. Bustamante Valdez: None. P. Duran: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.17/CCC16

Topic: F.10. Food Intake and Energy Balance

Title: Voluntary wheel running and removal produces alterations in locomotor behavior and improvements in diabetic symptoms in mice

Authors: *N. L. ARRUDA, A. HATZIDIS, J. A. HICKS, I. DE PINA MONTEIRO, R. R. GELINEAU, A. V. CUSHMAN, M. H. CHASSE, J. A. SEGGIO;
Dept. of Biol. Sci., Bridgewater State Univ., Bridgewater, MA

Abstract: Both exercise and diet can have profound effects on behavior and health. Consumption of high fat diets can lead to alterations to a variety of different behaviors, including anxiety and locomotion, in addition to producing obesity and other health problems. Alterations to locomotor activity, even under entrained 24 h LD cycles, can produce alterations to feeding behaviors and energy balance, which can exacerbate the negative health consequences of a poor diet. Conversely, exercise has been well characterized to produce healthy outcomes and improve memory; however, the extent to which exercise can alleviate the behavioral and physiological problems caused by poor diet might be small. This study investigated the effects of running wheel access and high fat diet consumption and its subsequent removal on circadian locomotor activity patterns, anxiety behaviors, and diabetic phenotype in B6 mice. These experiments may elucidate whether exercise or calorie reduction has a greater effect on improving health. Mice were placed into either running-wheel cages or cages without a running wheel, and initially, mice were given access to either regular chow or 60 percent fat diet. Measures of health and behavior included: glucose tolerance, body mass, insulin, leptin and triglyceride levels, anxiety behaviors, and circadian locomotion. Baseline measurements for all parameters were taken prior to food switch. After 10 weeks on the high-fat diet, half of the mice given 60 percent fat diet had their diets replaced with regular chow, and after 5 weeks, their behaviors and health parameters were measured again. Prior to the switching of the high fat diet to regular chow, all high fat diet mice exhibited similar levels of insulin and leptin and body mass to each other and were significantly increased compared to mice consuming regular chow. Additionally, wheel running and high fat

diet consumption also produced changes in behavior during the open field, but not the light dark box. Removal of the high fat diet significantly improved body mass, glucose tolerance, and hormone levels compared to mice on continuous high fat diet access. Additionally, open field behaviors and circadian locomotor activity in mice with the food switch exhibited behaviors similar to controls, but different compared to mice continuously fed high fat diet. These results voluntary wheel running can improve, but not completely overcome, the physiological and behavioral deficits caused by high fat diets, and that replacement of a poor diet with a healthier one can produce larger improvements in anxiety and overall health more so than exercise alone.

Disclosures: N.L. Arruda: None. A. Hatzidis: None. J.A. Hicks: None. I. De Pina Monteiro: None. R.R. Gelineau: None. A.V. Cushman: None. M.H. Chasse: None. J.A. Seggio: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.18/CCC17

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DK091710

Title: Morning pulsed leptin infusion reduces body weight but avoids onset of leptin resistance

Authors: P. J. SCARPACE[1], K. Y. E. STREHLER[1], M. K. MATHENY[1], N. KIRICHENKO[1], Y. SAKARYA[1], E. BRUCE[1], C. S. CARTER[2], D. MORGAN[3], *N. TUMER[1];
[1]Pharmacol., [2]Aging, [3]Psychiatry, Univ. Florida, Gainesville, FL

Abstract: Leptin administration results in leptin resistance presenting a significant barrier to therapeutic use of leptin. We hypothesized that pulsed central leptin infusion at specific times of the day would evoke favorable body weight reductions while tempering the development of leptin-induced leptin resistance. Methods: The first experiment examined leptin responsiveness to central leptin infusion, comparing pulsed versus constant infusion of 3 ug/day leptin, and the second experiment compared a 2 hr morning versus a 2 hr evening pulsed central leptin infusion of a submaximal dose of 0.25 ug/day. Leptin-induced pSTAT3 signaling was assessed prior to death. Results: Pulsed infusion of the supramaximal dose of 3 ug/day was not different than constant infusion with both leptin treatments resulting in significant 60 g decreases in body weight and 30% decreases in food intake ($p<0.001$). Both infusions were associated with leptin resistance with 30% and 60% decreases in STAT3 phosphorylation, respectively, for pulse and constant infusions. The 2hr morning pulsed central infusion of the submaximal leptin dose reduced food intake only over the subsequent immediate meal period and was associated with a

30g body weight reduction ($p<0.001$), but resulted in cellular leptin resistance (50% decrease in STAT3 phosphorylation, $p<0.001$). The evening pulsed infusion did not decrease food intake but reduced body weight to same extent as the morning pulsed infusion, yet maintained full leptin signaling. Conclusion: Pulsed infusion of a high dose of leptin induces leptin resistance as does pulsed morning infusion of a submaximal leptin dose. In contrast, a pulsed infusion prior to the dark phase reduces body weight with minimal reduction in food consumption and maintains full leptin signaling. The positive benefit for pulsed delivery remains speculative, yet potentially may provide an alternative mode of leptin therapy.

Disclosures: P.J. Scarpace: None. K.Y.E. Strehler: None. M.K. Matheny: None. N. Kirichenko: None. Y. Sakarya: None. E. Bruce: None. C.S. Carter: None. D. Morgan: None. N. Tumer: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.19/CCC18

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DA034009 to NVD

Supplement to DA034009 to DA

Title: Dysregulation of gut-brain endocannabinoid signaling and expression of the endocannabinoid system in mice maintained on Western Diet.

Authors: D. ARGUETA, *N. V. DIPATRIZIO;
Div. of Biomed. Sci., Univ. of California Riverside Sch. of Med., Riverside, CA

Abstract: The endocannabinoid (eCB) system is an important regulator of feeding and energy balance. We previously reported that levels of the eCB, 2-arachidonoyl-*sn*-glycerol (2-AG), are elevated in the jejunum mucosa of male rats that were food deprived for 24 h (FD), when compared to ad-libitum free-feeding controls (FF). This local signaling event in the small intestine drives refeeding after a fast and is dependent on cholinergic neurotransmission (DiPatrizio et al, *Amer J Phys* 309 (2015) 805-13). Similarly, it is suggested that oral exposure to dietary fats is a driving force in gut eCB production, which is also regulated by cholinergic signaling (DiPatrizio et al, *Proc Natl Acad Sci USA* 108 (2011) 12904-08). Our current findings reveal that a Western Diet (i.e., high levels of fats and sucrose) induces obesity (DIO, 60 day access) in male mice and is associated with dysregulated 2-AG signaling in jejunum mucosa.

DIO mice exhibit increases in levels of jejunal 2-AG under FF conditions that are comparable to levels found under FD in control mice maintained on standard lab chow. Levels of 2-AG are not increased any further under FD condition, when compared to FF, which provides evidence of impaired fasting-induced eCB signaling in the upper small intestine in DIO. Gene expression analysis (i.e., qRT-PCR) of cannabinoid CB_1 receptors and diacylglycerol lipase – an enzyme associated with the production of 2-AG – reveals an increase in their expression in response to fasting in mice fed a standard diet, an effect absent in DIO mice. Furthermore, FD in lean mice failed to affect levels of 2-AG in plasma. In contrast, FF and FD DIO mice exhibited a large increase in 2-AG levels. Collectively, this work suggests that *(i)* eCB signaling in the gut is initiated under several behavioral and metabolic conditions in rodents, and *(ii)* under conditions of DIO, 2-AG biosynthesis and possibly its signaling capacity at CB_1 receptors are dysregulated. Thus, these investigations advance our understanding of gut-brain eCB signaling and suggest potential new pharmacological avenues for appetite control and the treatment of obesity.

Disclosures: D. Argueta: None. N.V. DiPatrizio: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.20/CCC19

Topic: F.10. Food Intake and Energy Balance

Title: Neuronal activity in the mouse brain during endotoxemia

Authors: *T. TSAAVA[1], B. E. STEINBERG[2], M. GUNASEKARAN[1], E. A. BATTINELLI[1,3], M. J. TAYLOR[1], S. S. CHAVAN[1], K. J. TRACEY[1];
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Abstract: The central nervous system maintains body homeostasis and regulates organ physiology. When changes in the environment disrupt this equilibrium, the brain senses the perturbation and coordinates a neurophysiological response to regain homeostasis. Selective regions of brain are activated in response to specific challenges such as pain, tissue injury and inflammation during bacterial infection. The current study was designed to identify specific deep brain regions that are activated in response to systemic inflammation. We used lipopolysaccharide (LPS)-induced endotoxemia to induce systemic inflammation, and immediate early gene *c-fos* expression as a neuronal activation marker to identify the deep brain structures. Adult male BALB/C mice were habituated by daily handling to reduce background c-Fos

expression prior to LPS challenge. Following habituation, animals received intraperitoneal administration of LPS (2 mg/kg), and brain c-Fos expression was analyzed after 90 min by immunohistochemistry. c-Fos immunoreactive cells were observed in nuclear groups of hypothalamus, medulla and pons. These findings suggest that short-term systemic inflammation results in activation of deep brain regions responsible for modulating physiological responses during illness. The future work will generate a detailed map of the specific brain nuclei acutely activated by LPS and compare it to the activation of other pathogen associated molecular patterns.

Disclosures: T. Tsaava: None. B.E. Steinberg: None. M. Gunasekaran: None. E.A. Battinelli: None. M.J. Taylor: None. S.S. Chavan: None. K.J. Tracey: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.21/CCC20

Topic: F.10. Food Intake and Energy Balance

Support: JDRF Grant 3-PDF-2014-115-A-N

NINDS RO1 NS029728

Title: Catecholaminergic innervation and the neuronal activation of hypothalamic glucose sensitive regions during rapid- and slow-onset hypoglycemia in adult male rats.

Authors: *A. JOKIAHO[1], A. G. WATTS[2];
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Abstract: Hypoglycemic counterregulation is mediated by glucosensors located in the hypothalamus, hindbrain, and portal-mesenteric veins. But which are engaged is rate-dependent, with portal vein sensors being obligatory for slow- but not rapid-onset hypoglycemia. Slow-onset hypoglycemia is particularly prevalent with insulin therapy in type 1 diabetes. We have previously shown that hindbrain-to-hypothalamus catecholaminergic (CA) projections are required for sympathoadrenal responses to slow- but not rapid-onset hypoglycemia, and that rapid- but not slow-onset hypoglycemia significantly increases CA/Fos colocalization in the ventrolateral medulla. These results show that the organization of a hypoglycemia-responsive brain networks is rather complex, and involves a set of what are likely parallel but interactive networks, each of which is responsible for controlling epinephrine, glucagon, and glucocorticoid

responses. We now examine how various forebrain cell groups known to be important for glycemic regulation respond to , and how these responses are impacted by removing hindbrain-to-hypothalamus CA projections using injections of the immunotoxin, saporin conjugated to anti-DBH (DSAP) into the hypothalamic paraventricular nucleus (PVH). These injections remove CA inputs to the PVH and other regions within the medial hypothalamus. We then examined whether DSAP lesions affected Fos responses to slow- and rapid-onset insulin-induced hypoglycemia in key forebrain regions. We found that removing CA innervation differentially influences regional hypothalamic Fos responses to slow- and rapid-onset insulin-induced hypoglycemia. Rapid-onset hypoglycemia produced significantly greater Fos activations in the medial and lateral parvocellular and lateral parts of the PVH, parts of the lateral hypothalamus (LHA), the bed nucleus of the stria terminalis that was significantly reduced in all these regions with DSAP lesions. Of particular interest was the altered Fos in LHA regions that contain orexin neurons. We found that 27% of Fos activated neurons colocalized with orexin neurons in rapid-onset hypoglycemia, but this colocalization was significantly reduced by DSAP lesions. Furthermore we used a retrogradely transported polysynaptic neurotropic virus (PRV-152) injected into adrenal gland to show that 25% of PRV-labeled neurons in the LHA colocalized with orexin neurons. These results show that hindbrain-to-hypothalamus CA projections provide hypoglycemia-related information to regions of the forebrain in a rate-dependent way, with orexin neurons playing a particularly prominent role for sympathoadrenal responses.

Disclosures: A. Jokiaho: None. A.G. Watts: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.22/CCC21

Topic: F.10. Food Intake and Energy Balance

Title: Combined consumption of alcohol and a high fat diet: sex differences in anxiety, feeding and drinking behavior

Authors: *R. R. GELINEAU, N. L. ARRUDA, J. A. HICKS, I. DE PINA MONTEIRO, A. HATZIDIS, J. A. SEGGIO;
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Abstract: Poor diets and alcohol are known to affect both behavior and overall health. Additionally, there are sex differences in how males and females respond physiologically and behaviorally to alcohol and high fat diets; however, few studies have examined simultaneous access of moderate alcohol consumption and high fat diet. This project investigated how joint

high fat and ethanol access affected feeding, drinking and anxiety behaviors in male and female B6 mice. Each sex of mice were separated into three groups; 60 percent fat diet, 10 percent fat diet, and regular chow and each group was paired with either water or forced 10 percent alcohol. Weekly body weight, food and fluid intake measurements were recorded. Anxiety behaviors were measured using the open field and light dark box. Hedonic substitution was tested to determine if the addition of a high fat diet would affect alcohol or preference and vice versa. Overall, fluid consumption was greater in mice fed regular chow compared to the 60 percent and 10 percent diets for both sexes. The combined high fat diet and alcohol mice drank more fluid than their water consuming counterparts, a result not seen in regular chow and 10 percent fat diet consuming mice in males. Alcohol drinking females consumed more kilocalories than water drinking females, which then consumed more ethanol than males overall. There is no evidence of hedonic substitution for either sex. In the open field, there were differences between the high fat and regular chow with respect to explorative behaviors, but not between the high fat and 10 percent fat diets for males. For the females, the only behavioral differences were in the open field assay with high fat diet exhibiting reduced velocity than the rest of the groups. Additionally, females consuming high fat diet exhibited increased center zone time compared to both males on high fat diet and females consuming regular chow. There were no differences in the light dark box for either sex. These results suggest that consumption of high fat diets are more powerful in affecting anxiety behavior compared to moderate alcohol consumption. Additionally, males and females respond behaviorally differently to high fat diets and have different alterations in feeding and drinking patterns compared to each other when alcohol is present.

Disclosures: R.R. Gelineau: None. N.L. Arruda: None. J.A. Hicks: None. I. De Pina Monteiro: None. A. Hatzidis: None. J.A. Seggio: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.23/CCC22

Topic: F.10. Food Intake and Energy Balance

Support: DK21397

Title: Nucleus tractus solitarius catecholamine neurons contribute to the intake suppressive effects of gastrointestinal satiation signals

Authors: *Z. ONG, H. S. WALD, X. S. DAVIS, H. J. GRILL;
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Abstract: Hindbrain nucleus tractus solitarius (NTS) catecholamine A2 neurons are implicated in food intake inhibitory control. A2 neurons, which express tyrosine hydroxylase (TH), are activated by gastrointestinal (GI) satiation signals, including cholecystokinin (CCK). Saporin toxin conjugated to target these neurons attenuates the intake suppressive effects of exogenous CCK. However, rather than inhibiting the excitability of A2 neurons, saporin conjugation lesions the A2 neurons making the functional effect more difficult to interpret. The present study therefore aimed to refine the procedures and selectively inhibit NTS A2 neurons using DREADDs (Designer Receptor Exclusively Activated by Designer Drugs) to test the hypothesis that NTS A2 neurons mediate the intake inhibitory effects of GI satiation signals. Male TH Cre transgenic rats received bilateral NTS injections of the Cre-dependent inhibitory DREADD, AAV-DIO-hSyn-hM4D(Gi)-mcherry and were trained to consume 12mL of liquid diet Ensure (preload) within 10min to physiologically stimulate endogenous GI signals. Rats were given intraperitoneal injections of 1mg/kg clozapine-N-oxide (CNO; a selective ligand for DREADD) or vehicle (veh) prior to a preload or no preload and subsequent chow intake determined at 30, 60, 90, 120min. Results reveal that preload significantly reduced subsequent chow intake ($p<0.05$ veh-preload vs veh-no preload) and CNO pretreatment attenuated the preload-induced intake suppression at 60min ($p<0.05$ veh-preload vs CNO-preload). Post mortem analysis of injection placements show that the attenuation of preload-induced intake suppression was specific to the inhibition of NTS A2 neurons, as the attenuation was not observed in rats with misplaced DREADD injections. Taken together, results provide support for the hypothesis that NTS A2 neurons participates in the intake inhibitory effects of GI satiation signals. Follow up experiments are underway to examine the contribution of A2 neurons in mediating the intake inhibitory effects of other GI signals, including CCK and glucagon-like peptide 1, to expand the range of effects explored and thereby provide a better understanding of the role of A2 neurons in food intake control. Supported by DK 21397

Disclosures: Z. Ong: None. H.S. Wald: None. X.S. Davis: None. H.J. Grill: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.24/CCC23

Topic: F.10. Food Intake and Energy Balance

Support: JSPS Young Scientist B (24790221)

JSPS Challenging Exploratory Research (26670453)

Naito Foundation

Title: Peripherally injected oxytocin activates vagal afferents to suppress food intake and ameliorates hyperphagic obesity in db/db mice

Authors: *Y. IWASAKI[1], Y. MAEJIMA[1], S. SUYAMA[1], M. YOSHIDA[2], M. KAKEI[2], T. YADA[1];
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Abstract: Oxytocin (Oxt), produced in the paraventricular nucleus and supraoptic nucleus of hypothalamus, regulates food intake. Previous reports have shown that not only central but also peripheral administration of Oxt suppresses feeding and ameliorates obesity. Peripheral oxytocin in circulation little enters the brain due to short half-life and tight restriction by the blood-brain barrier. Therefore, the route through which peripheral Oxt informs the brain is unclear. We investigated whether vagal afferents mediate the sensing and anorexigenic effect of peripherally injected Oxt in mice. First in *ex vivo* study, Oxt depolarized, evoked action potential firings and increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single vagal afferent neurons isolated from the nodose ganglion. The Oxt-induced $[Ca^{2+}]_i$ increases were inhibited by Oxt receptor antagonist. Incidence of Oxt-responses in nodose ganglion neurons was approximately 15%. These Oxt-responsive neurons also responded to anorexigenic hormone cholecystokinin and contained cocaine- and amphetamine-regulated transcript. Next in *in vivo* study, Intraperitoneal (ip) injection of Oxt decreased food intake and increased c-Fos expression in the nucleus tractus solitarius (NTS) of medulla, to which vagal afferents project. The feeding suppression and c-Fos expression in NTS were blunted by subdiaphragmatic vagotomy and capsaicin treatment. In obese diabetic *db/db* mice, leptin failed to but Oxt increased $[Ca^{2+}]_i$ in single vagal afferent neurons. Moreover, single injection or sub-chronic infusion of Oxt decreased feeding and body weight gain in *db/db* mice. These results demonstrate that peripheral Oxt injection suppresses feeding by activating vagal afferents, and that this “peripheral Oxt-vagal afferents-brain” axis is effective for treating hyperphagia and obesity.

Disclosures: Y. Iwasaki: None. Y. Maejima: None. S. Suyama: None. M. Yoshida: None. M. Kakei: None. T. Yada: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.25/CCC24

Topic: F.10. Food Intake and Energy Balance

Support: JSPS Challenging Exploratory Research (26670453)

Title: Na^+,K^+-ATPase in the hypothalamic arcuate nucleus senses lowering glucose to initiate feeding behavior

Authors: *T. YADA[1], H. KURITA[1], K. Y. XU[2], P. SANTOSO[1], Y. YANG[1], K. DEZAKI[1], M. NAKATA[1];
[1]Physiol., Jichi Med. Univ., Shimotsuke, Japan; [2]Univ. of Maryland Sch. of Med., Surgery, MD

Abstract: Reduction in the blood glucose levels has been considered one of the major metabolic signals that induce appetite under fasting conditions. The glucose-inhibited (GI) neurons in the hypothalamic arcuate nucleus (ARC) are activated by lowering glucose (LG) and suggested to be implicated in feeding. However, their glucose-sensing mechanism and physiological role remain to be determined. We here report that Na^+,K^+-ATPase (NKA) in ARC senses LG to trigger feeding behavior. LG reduced NKA's substrate ATP levels in isolated ARC slices. In ARC GI neurons confirmed by their cytosolic Ca^{2+} ($[Ca^{2+}]_i$) responses to LG, LG also decreased intracellular nicotinamide adenine dinucleotide phosphate ($[NAD(P)H]_i$) level, reflecting reduced ATP production, and increased $[Na^+]_i$, reflecting reduced NKA activity. Intracerebroventricular injection of NKA inhibitor ouabain activated agouti-related protein (AgRP) and neuropeptide Y (NPY) neurons in ARC, and evoked NPY-dependent food intake. Ouabain increased $[Ca^{2+}]_i$ in single NPY/AgRP neurons in ARC. By contrast, specific NKA activator SSA412 counteracted fasting-induced food intake and LG-induced $[Ca^{2+}]_i$ increases in ARC GI neurons. This study reveals novel glucose signalling in ARC that triggers feeding behaviour: LG suppresses NKA activity and thereby activates GI neurons and NPY/AgRP-dependent appetite. This study identifies ARC NKA as the sensor and converter of LG to key neuronal activity and feeding behavior.

Disclosures: T. Yada: None. H. Kurita: None. K.Y. Xu: None. P. Santoso: None. Y. Yang: None. K. Dezaki: None. M. Nakata: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.26/CCC25

Topic: F.10. Food Intake and Energy Balance

Support: Acknowledgement: This work is supported by the Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Title: Transcriptional alterations in a model of compulsive food taking or abstinence in the presence of contingent footshocks.

Authors: *M. T. MCCOY, N. TERRY, B. LADENHEIM, I. KRASNOVA, S. JAYANTHI, D. WALTHER, J. CADET;
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Abstract: Obesity has reached epidemic proportion worldwide and is a serious risk factor for increased morbidity and mortality. In addition, obesity is, in part, secondary to increased food consumption despite adverse medical consequences. The longstanding aspect of unregulated eating behaviors is probably associated with long-term molecular changes in brain regions that control food intake and/or are involved in regulating rewards. In the present study, male Sprague-Dawley rats were trained to self-administer (SA) palatable food for 9-h per day for 32 days. After this training period, the rats were exposed to contingent footshocks for a period of 18 days. Increasing shocks produced two groups: (1) one group of rats continued to press the lever for food despite footshocks (shock-resistant) and (2) another group that significantly reduce their lever pressing (shock-sensitive). We then used the Affymetrix GeneChip rat transcriptome 1.0 array platform to test for differential transcriptional changes in the nucleus accumbens of these rats. Genes with significant differences between resistant and sensitive rats belong to diverse functional classes including DNA Binding, Oxygen Transport, Signal Transduction, and Synaptic Plasticity. Genes that participate in Cellular Developmental Process, Response to Stress, Response to Oxidative Stress, and Regulation of Cell Death were also over-represented in the comparisons. Thus, this model of food SA with adverse consequences is beginning to provide some insight into the molecular mechanisms involved in compulsive food taking. These discoveries may help to develop approaches to test the role of specific genes in regulating food intake. Acknowledgement: This work is supported by the Department of Health and Human Services/ National Institutes of Health/ National Institute on Drug Abuse/ Intramural Research Program

Disclosures: M.T. McCoy: None. N. Terry: None. B. Ladenheim: None. I. Krasnova: None. S. Jayanthi: None. D. Walther: None. J. Cadet: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.27/CCC26

Topic: F.10. Food Intake and Energy Balance

Support: Research Development Fund, School of Medicine, USC.

South Carolina Clinical & Translational Research Institute (SCTR), NIH/NCATS Grant# UL1TR000062

Department of Veteran Affairs IO1 BX001804

Title: Regulation of feeding behavior: a new circuit from the raphe nuclei to the hypothalamus

Authors: *C. A. GRILLO[1], C. E. PETYAK[1], B. L. SOMERA[1], V. A. MACHT[1], J. L. WOODRUFF[1], J. R. FADEL[1], L. P. REAGAN[2];
[1]Dept Pharmacol, Physiol & Neurosci, Univ. of South Carolina Sch. of Med., Columbia, SC;
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Abstract: Feeding behavior is an essential activity for maintenance of life. Not surprisingly given this necessity, food intake has evolved to be under the control of multiple peripheral factors interacting with multiple neural circuits. One of those peripheral factors is the adipocyte-derived hormone leptin that is secreted in proportion to fat mass and regulates a broad spectrum of homeostatic functions. Leptin receptors (LepRs) are expressed on neurons of the CNS, mainly in several nuclei of the hypothalamus. The majority of the studies examining the effects of leptin on food intake have focused on the hypothalamus. However, recent reports have suggested that extra-hypothalamic LepRs also regulate food intake. One extra-hypothalamic region which has received recent interest is the raphe. Our studies show that leptin infused directly into the raphe of adult male rats inhibits food intake in a dose-dependent manner. In order to more specifically study the role of LepRs expressed in the raphe upon feeding behavior, we used an optogenetics approach. Infusing a lentivirus, we expressed the light sensitive channelrhodopsin (ChR2) under control of the LepR promoter in the raphe. Selective blue light activation of these neurons inhibited food intake compared to yellow light stimulation used as control. In order to test if this optogenetic approach affected the locomotor activity that could interfere with food intake, we performed an Open Field Test after the light stimulation of the raphe leptin-sensitive neurons, and no changes were observed. We hypothesized that food intake regulation observed depends on activation of raphe leptin-sensitive neurons that send projections to the hypothalamic nuclei. In order to test this hypothesis, we infused the lentivirus containing the ChR2 under the LepR promoter into the raphe and then stimulated with blue light the terminals that reach the arcuate nucleus in the hypothalamus. This terminal stimulation of raphe efferents to the arcuate nucleus significantly inhibited food intake. This later effect had a smaller magnitude than the stimulation of the neuronal bodies located in the raphe, indicating that the arcuate is likely not the only nucleus that receives raphe efferents. Collectively, these data demonstrate that activation of the LepRs expressed in the raphe nuclei inhibit food intake through a circuit that sends projections to the arcuate nucleus.

Disclosures: C.A. Grillo: None. C.E. Petyak: None. B.L. Somera: None. V.A. Macht: None. J.L. Woodruff: None. J.R. Fadel: None. L.P. Reagan: None.

Poster

256. Integration of Peripheral Signals for Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 256.28/DDD1

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant MH106330

Title: Regional effects of maternal high fat diet exposure on one carbon metabolism and methylation machinery

Authors: *S. E. MCKEE[1], S. ZHANG[2], L. CHEN[2], J. D. RABINOWITZ[2], T. M. REYES[3]; [1]Univ. of Pennsylvania, Cincinnati, OH; [2]Princeton Univ., Princeton, NJ; [3]Univ. of Cincinnati, Cincinnati, OH

Abstract: Offspring exposed to high fat diet in utero are at an increased risk for the development of metabolic and neurological disorders. To identify potential mechanisms linking early life diet and chronic disease, our lab uses a mouse model in which dams are fed a high fat diet throughout pregnancy and lactation. In adult offspring, we observe global DNA hypomethylation, gene-promoter hypomethylation and dysregulated DNA methyltransferase activity in various regions of the brain important in reward and executive function. These findings suggest that dysregulation in DNA methylation could be a mechanism by which offspring develop increased risk for disease. One carbon metabolism is the critical pathway donating one-carbon units for methylation reactions in the cell. It is well documented that nutrient intake and availability can alter enzymatic functioning and cycling of the one-carbon pathway, which can lead to altered epigenetic gene regulation. It remains undetermined whether perinatal exposure to high fat diet can alter one carbon metabolism and methylation machinery within the brain as a means to program adult disease. To determine this, male and female offspring from dams fed a high fat diet were sacrificed at weaning. Brain, liver and blood were collected to examine gene and protein expression of enzymes within the one carbon metabolism pathway and methylation machinery, activity of methyltranferases, and DNA methylation profiles in these offspring. It is expected that perinatal high fat diet exposure will perturb these systems and program offspring for adult disease.

Disclosures: S.E. McKee: None. S. Zhang: None. L. Chen: None. J.D. Rabinowitz: None. T.M. Reyes: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.01/DDD2

Topic: F.10. Food Intake and Energy Balance

Support: MRC grant MR/J013293/2

Graduate Research Scholarship, UCL

Wellcome Trust grant 106262/Z/14/Z

Wellcome Trust grant 106263/Z/14/Z

MRC grant MRC_MC_UU_12012/3

Title: Preproglucagon neurons in the caudal brainstem contribute to regulation of heart rate and blood pressure

Authors: *M. K. HOLT[1], J. E. RICHARDS[1], F. M. GRIBBLE[2], F. REIMANN[2], S. TRAPP[1]; [1]Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom; [2]Cambridge Univ., Cambridge, United Kingdom

Abstract: Glucagon-like peptide-1 (GLP-1) is an incretin and neuropeptide best known for its role in glucose homeostasis and appetite regulation. However, in the brain, additional GLP-1 mediated effects are emerging and therefore it may play a more complex role in homeostasis than first anticipated. Activation of brain GLP-1 receptors has been shown to increase energy expenditure, induce nausea and increase heart rate (HR). Within the brain, GLP-1 is produced by preproglucagon (PPG) neurons in the nucleus tractus solitarii (NTS). We have previously shown that PPG neurons project to autonomic control centres throughout the central nervous system, including sympathetic preganglionic neurons in the spinal cord, suggesting GLP-1 may modulate sympathetic activity.

Here we investigated whether HR and blood pressure (BP) are regulated by PPG neurons using implantable biotelemetry BP probes in freely behaving mice. NTS PPG neurons were selectively ablated by injecting a Cre-dependent adeno-associated virus encoding the diphtheria toxin subunit A (DTA) into male transgenic mice expressing Cre recombinase under the control of the glucagon promoter. Following baseline recordings of HR and BP mice were stereotaxically injected with either a DTA or control virus. Recordings of HR and BP were obtained for 24 hours at 4 and 6 weeks post injection.

Successful ablation of NTS PPG neurons was confirmed using immunohistochemistry, and in an additional cohort of mice by an antibody-based assay, which detects active GLP-1. Ablation of PPG neurons reduced active GLP-1 levels in brainstem by 60%, in hypothalamus by >80% and

in the mid thoracic region of the spinal cord by >60% compared to control animals.
Mice with ablated PPG neurons displayed a normal circadian rhythm demonstrating expected increases of HR and BP at the onset of the dark phase. We did not observe an overall change in either HR or BP when comparing post injection recordings to baseline recordings. Mice exhibited a biphasic distribution of HR and BP over 24 hours and HR and BP levels were found to be positively correlated with the activity level of the animal. We analysed HR and BP data from periods of inactivity and found that PPG-ablated mice demonstrated elevated HR (+34±14 bpm) and BP (+5.3±2 mmHg) 4 weeks after viral injection as compared to baseline resting HR and BP. In contrast, control mice exhibited a decrease in HR (-27±11 bpm) without a change in BP (-0.7±0.7 mmHg) when resting.
This study, the first to investigate the role of endogenous, central GLP-1 in cardiovascular control, suggests that NTS PPG neurons may contribute to HR and BP regulation.

Disclosures: M.K. Holt: None. J.E. Richards: None. F.M. Gribble: None. F. Reimann: None. S. Trapp: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.02/DDD3

Topic: F.10. Food Intake and Energy Balance

Support: National Research Foundation (2013M3C7A1056099) of South Korea

Title: Hypothalamic AMPK-induced autophagy increases food intake by regulating NPY and POMC expression

Authors: *T. OH;
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Abstract: Hypothalamic AMP-activated protein kinase (AMPK) plays important roles in the regulation of food intake by altering the expression of orexigenic or anorexigenic neuropeptides. However, little is known about the mechanisms of this regulation. Here, we report that hypothalamic AMPK modulates the expression of neuropeptide Y (NPY), an orexigenic neuropeptide, and pro-opiomelanocortin (POMC), an anorexigenic neuropeptide, by regulating autophagic activity in vitro and in vivo. In hypothalamic cell lines subjected to low glucose availability such as 2-deoxy-d-glucose (2DG)-induced glucoprivation or glucose deprivation, autophagy was induced via the activation of AMPK, which regulates ULK1 and mTOR complex 1 followed by increased NPY and decreased POMC expression. Pharmacological or genetic

inhibition of autophagy diminished the effect of AMPK on neuropeptide expression in hypothalamic cell lines. Moreover, AMPK knockdown in the arcuate nucleus of the hypothalamus decreased autophagic activity and changed NPY and POMC expression, leading to a reduction in food intake and body weight. AMPK knockdown abolished the orexigenic effects of intraperitoneal 2DG injection by decreasing autophagy and changing NPY and POMC expression in mice fed a high-fat diet. We suggest that the induction of autophagy is a possible mechanism of AMPK-mediated regulation of neuropeptide expression and control of feeding in response to low glucose availability.

Disclosures: T. Oh: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.03/DDD4

Topic: F.10. Food Intake and Energy Balance

Title: The hypothalamic corticotropin-releasing hormone system and its involvement in metabolism and energy homeostasis

Authors: *J. RICHTER[1], A. CHEN[1], W. WURST[2], J. M. DEUSSING[1];
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Abstract: The corticotropin-releasing hormone (CRH), family of neuropeptides and their receptors - type 1 (CRHR1) and type 2 (CRHR2) - play a major role in the adjustment of neuroendocrine, autonomic, and behavioral adaptations to stressors. We have revealed that CRH receptors are able to convey differential responses depending on their cellular context and the neuronal circuit they are located. CRH receptors and ligands are expressed throughout the brain and show a differential expression pattern in multiple hypothalamic nuclei, which are involved in the control of hunger and satiety. CRH is strongly expressed in the paraventricular nucleus (PVN), CRHR1 is highly expressed in the arcuate nucleus (ARC) while CRHR2 is strongly expressed in the ventromedial hypothalamus (VMH). There is increasing evidence, that CRH is involved in centrally- and peripherally-controlled metabolic function. And also members of the family of ligands of CRHR2, urocortins, are involved in the regulation of peripheral responses to homeostatic challenges and though, are modulating metabolism and energy balance. More recent studies suggest that urocortins play also a role in the modulation and control of metabolism in the central nervous system. However, until now, there is little knowledge about the pathways and the interaction partners through which CRH receptors mediate their effects on metabolism and

energy balance. To unravel the identity of CRHR-expressing neurons in the hypothalamus and to overcome the lack of valid CRHR antibodies, we used specific reporter mouse lines to visualize on the one hand CRHR1 and CRHR2 positive neurons and on the other hand nuclei-specific markers, e.g. agouti-related peptide (AgRP), proopiomelanocortin (POMC), leptin receptor (LR). We identified, that the majority of CRHR1 neurons in the ARC co-express AgRP and a distinct proportion of CRHR2 neurons is positive for LR. In further work, we aim to identify more specific markers co-expressed with CRHR2 and to unravel the neurotransmitter circuits of CRH receptors in the hypothalamic nuclei by in situ hybridization techniques and to learn more about the connectivities of the hypothalamic CRH system using viral-based tracing methods.

Disclosures: J. Richter: None. A. Chen: None. W. Wurst: None. J.M. Deussing: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.04/DDD5

Topic: F.10. Food Intake and Energy Balance

Support: MEXT/JSPS KAKENHI Grant 22687004

MEXT/JSPS KAKENHI Grant 23126517

MEXT/JSPS KAKENHI Grant 25126717

MEXT/JSPS KAKENHI Grant 26291066

MEXT/JSPS KAKENHI Grant 25440171

Title: The relationship between neuronal histamine and neurosecretory protein GM in the chick hypothalamus

Authors: *K. UKENA, K. SHIKANO, Y. BESSHO, E. IWAKOSHI-UKENA;
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Abstract: We recently identified two cDNA-encoded paralogous small secretory proteins: neurosecretory protein GL (NPGL) and neurosecretory protein GM (NPGM) in the chick hypothalamic infundibulum. We also detected homologous genes in the vertebrate genome. In a preliminary study, we found that NPGM-producing cells were present among the histaminergic neurons in the rat brain. Although it has been established that histamine is synthesized by the enzyme histidine decarboxylase (HDC) in mammals, the function of HDC and histaminergic

neurons in the avian brain have not yet been characterized. To study this aspect, we first cloned the HDC cDNA from the chicken hypothalamus. We found that the expressed protein possessed a high level of enzymatic activity. Quantitative RT-PCR analysis showed that the HDC mRNA was highly expressed in the hypothalamic infundibulum. In situ hybridization analysis revealed that the cells containing HDC mRNA were localized to the medial mammillary nucleus (MM) of the hypothalamic infundibulum. We subsequently raised a specific antibody against chick NPGM and examined the localization of NPGM-producing cells by immunohistochemical analysis. NPGM-like immunoreactive cells were distributed across the MM and the infundibular nucleus of the hypothalamus. In order to clarify the relationship between neuronal histamine and NPGM, we compared the localization of histaminergic neurons and NPGM-producing cells. We observed that histamine and NPGM were produced in the same neurons located in the MM. A quantitative PCR analysis showed that mRNA levels of HDC and NPGM in the hypothalamic infundibulum increased during fasting and decreased during post-hatching development. An intracerebroventricular injection of histamine or NPGM in chicks inhibited feeding behavior. These results suggest that histamine and NPGM, which are produced by the same neurons in the chicken brain, exhibit functional complementarity.

Disclosures: K. Ukena: None. K. Shikano: None. Y. Bessho: None. E. Iwakoshi-Ukena: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.05/DDD6

Topic: F.10. Food Intake and Energy Balance

Title: Deletion of melanin-concentrating hormone receptor 1 from the accumbens nucleus increases locomotor activity

Authors: *M. J. CHEE, S. E. FLAHERTY, III, P. PISSIOS, N. BRIANCON, J. S. FLIER, E. MARATOS-FLIER;
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Abstract: Melanin-concentrating hormone (MCH) is an orexigenic neuropeptide that is a critical regulator of energy homeostasis. Transgenic deletion of MCH or its receptor MCHR1 results in leanness by increasing energy expenditure and locomotor activity. Since MCHR1 expression is widespread, the function and contribution of different MCH-responsive neurons is poorly understood. We recently showed that a large proportion of MCHR1 cells are GABAergic, including those in the accumbens nucleus. We tested the role of GABAergic neurons for the effects of MCH on body weight, energy expenditure or locomotor activity by selectively deleting

MCHR1 from GABAergic neurons. We next deleted MCHR1 expression in distinct neuroanatomical regions to identify a candidate brain area that supports MCH locomotor actions. We generated the *MCHR1-flox* mouse and crossed it to the *vGAT-cre* mouse; the conditional knockout is designated *vGAT-MCHR1-KO*. In situ hybridization confirmed the absence of MCHR1 from GABAergic regions, most notably in the striatum and arcuate nucleus, but there is no loss of MCHR1 hybridization in glutamatergic regions, including the cerebral cortex, hippocampus and paraventricular hypothalamic nucleus. We next compared differences in body weight and locomotor activity of female *vGAT-MCHR1-KO* mice to their *vGAT-cre* controls. *vGAT-MCHR1-KO* mice were 11% leaner, with 20% less body fat, and had 70% greater total energy expenditure. However the most impressive difference was a 93% increase in total baseline ambulation. Furthermore, consistent with our previous report that increased dopaminergic tone in the accumbens nucleus underlies the hyperactivity of MCH-deficient mice, we found that *vGAT-MCHR1-KO* mice had an enhanced and prolonged response to GBR12909, a dopamine reuptake blocker. GBR12909 produced a two-fold increase in cumulative locomotor activity lasting more than 5 hours. To probe the role of the accumbens nucleus, where more than 95% of neurons are GABAergic, we stereotaxically injected an adeno-associated virus encoding cre recombinase-mCherry into the accumbens of *MCHR1-flox* mice. Deletion of MCHR1 expression from the accumbens nucleus increased total baseline locomotor activity by 82%. These findings show that MCH acts partly via GABAergic neurons to regulate body weight and energy expenditure. Furthermore, MCH signaling in GABAergic cells may inhibit dopamine transmission in the accumbens nucleus to regulate ambulatory activity.

Disclosures: M.J. Chee: None. S.E. Flaherty: None. P. Pissios: None. N. Briancon: None. J.S. Flier: None. E. Maratos-Flier: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.06/DDD7

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DA037566

Title: Nicotine and high fat diet differentially modulate body weight, behavior, and neuropeptide expression in female and male C57BL/6J mice

Authors: *C. A. CALARCO, S. LEE, M. R. PICCIOTTO;
Dept. of Mol. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Obesity is a major health problem in the US with greater than 30% prevalence in the adult population. The high rate of obesity is partially attributable to the popularity of high-fat, high-calorie foods in the Western diet. Despite the negative consequences of obesity such as heart disease and diabetes, many people find losing weight through lifestyle changes difficult. Nicotine exposure through tobacco use has been associated with lower body weight, and subsets of smokers report using nicotine to control appetite, or resisting quitting because of concerns about weight gain. However, many of the mechanisms by which chronic nicotine consumption suppresses weight gain remain unclear. In animal studies, nicotine is associated with reduced weight gain, reduced food consumption, and altered energy expenditure, but these findings vary based on the duration and the route of nicotine administration. Furthermore, sex differences in the effects of nicotine have been reported in rats but remain relatively unexplored. We used high-fat diet (HFD) in tandem with free access to nicotine to mimic the nicotine and food consumption patterns observed in humans, and designed our experiments to test the intake patterns, weight gain, and biochemical changes due to nicotine in male and female mice. Single-housed male and female C57BL/6J mice had *ad lib* access to nicotine via drinking water and either regular chow or 45% HFD for 30 days. As expected, HFD significantly increased body weight over the course of the experiment. However, this body weight gain was attenuated by nicotine in male, but not female, mice. HFD increased caloric intake only in male mice, and nicotine did not affect caloric intake in either diet condition. Male, but not female, mice exhibited less weight gain per calorie consumed when given access to nicotine, suggesting that metabolic and peripheral changes may contribute to the blunted weight gain associated with nicotine treatment and may vary by sex.
To examine the molecular and biochemical changes that accompany the observed changes in weight and feeding behavior, we investigated the expression level changes of multiple genes of interest in the hypothalamus and peripheral tissues. HFD and nicotine differentially regulated hypothalamic feeding peptides in the arcuate nucleus, including AgRP, NPY, and CART. We also examined expression of uncoupling proteins (UCPs) in both brown and white adipose tissue, as a proxy for metabolic function. Based on these data, we can conclude that the weight changes associated with nicotine and HFD consumption differ between male and female animals and that these differences may be due to altered peptide signaling in the hypothalamus.

Disclosures: C.A. Calarco: None. S. Lee: None. M.R. Picciotto: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.07/DDD8

Topic: F.10. Food Intake and Energy Balance

Support: Georgia State University, Department of Biology

Georgia State University, Brains and Behavior Program

Title: Neuropeptide-y directly inhibits ventral tegmental area dopamine neurons through g protein-coupled inwardly rectifying potassium channel currents

Authors: *K. STUHRMAN[1], A. G. ROSEBERRY[2];
[1]Neurosci. Inst., [2]Dept. of Biol., Georgia State Univ., Atlanta, GA

Abstract: The mesocorticolimbic dopamine system, the brain's reward system, regulates many different behaviors including food intake, food reward, and feeding related behaviors. There is substantial evidence that hypothalamioc feeding-related neuropeptides alter dopamine neuron activity to affect feeding. For example, neuropeptide Y (NPY), a strong orexigenic hypothalamic neuropeptide, decreases the activity of dopamine neurons in the ventral tegmental area (VTA) of the mesocorticolimbic DA system, and increases motivation for food when injected into the VTA. How NPY decreases the activity of dopamine neurons to mediate these effects is unknown, however. In these studies we have used whole-cell patch clamp electrophysiology in acute brain slices from mice to examine how NPY decreases VTA dopamine neuron activity. NPY activated an outward current in approximately fifty percent of dopamine neurons tested. The NPY current exhibited characteristics of a G protein-coupled inwardly rectifying potassium (GIRK) channel current, as it showed strong inward rectification, a reversal potential near that for potassium ions, and was blocked by extracellular barium. The NPY activated current was also dependent on intracellular calcium levels, as it was significantly smaller with low calcium buffering compared to high calcium buffering, which is similar to GIRK currents activated by dopamine and $GABA_B$ receptor agonists in VTA dopamine neurons. In addition to its direct effects on VTA dopamine neurons, NPY also slightly decreased the amplitude of excitatory glutamatergic post-synaptic currents onto VTA dopamine neurons. Overall we have shown that NPY reduces dopamine neuron activity both by directly activating an inhibitory GIRK current and by decreasing glutamatergic transmission onto VTA dopamine neurons. These studies provide an important advancement in our understanding of dopamine neuron activity and how it is controlled by NPY.

Disclosures: K. Stuhrman: None. A.G. Roseberry: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.08/DDD9

Topic: F.10. Food Intake and Energy Balance

Support: Texas Tech University Association of Biologists Grant-in-Aid

Obesity Research Cluster

Title: Tectal CRF R1 receptors modulate food intake

Authors: *C. PRATER, B. HARRIS, A. MERRILL, A. ALIYAS, K. ANDERSON, J. CARR; Biol., Texas Tech. Univ., Lubbock, TX

Abstract: The optic tectum (OT) and superior colliculus (SC) rapidly inhibit food intake when a visual threat is present. Previous work from our laboratory indicates that CRF, acting on CRF R1 receptors, may play a role in tectal inhibition of prey capture. Here we test the hypothesis that **tectal CRF neurons modulate food intake in juvenile *Xenopus laevis***. We tested five predictions: 1) Does tectal CRF injection decrease food intake? 2) Does a selective CRF R1 antagonist block CRF effects on feeding? 3) Does a selective CRF R1 antagonist block stressor-induced inhibition of feeding? 4) Does eliminating tectal cells expressing CRF R1 increase feeding? 5) Does food deprivation increase food intake and, if so, can this be reversed with CRF? *X. laevis* were administered oCRF alone or in combination with the selective CRF R1 antagonist NBI27914 or antagonist vehicle. Test agents were bilaterally injected into the tecta of juvenile frogs. CRF conjugated to the ribosomal toxin saporin (CRF-SAP) was administered 2 wk prior to testing to eliminate tectal cells expressing CRF R1. oCRF administered bilaterally into the tecta significantly reduced food intake compared to sham and vehicle injected juveniles. When frogs were injected with oCRF and antagonist vehicle, food intake was significantly reduced. When injected with both NBI27914 and oCRF, food intake was maintained at baseline levels. Frogs ate significantly less when exposed to a reactive stressor (ether vapors) and when pre-treated with antagonist vehicle prior to exposure. NBI27914 reversed stressor-induced inhibition of food intake. Neither CRF-SAP injection nor food deprivation (2 wk) significantly changed food intake. No significant differences in food intake were noted between males and females across all studies. Overall, we found support for questions 1-3 and conclude that activation of the tectal CRF R1 inhibits food intake in frogs. Furthermore, tectal CRF R1 receptors appear to be involved in the reduction of food intake that occurs in response to a reactive stressor. However, elimination of tectal CRF R1 neurons did not increase feeding suggesting that this system may be more important for stress-related vs. baseline feeding. This work was done in partial completion of requirements for the doctoral degree at Texas Tech University (C.P.)

Disclosures: C. Prater: None. B. Harris: None. A. Merrill: None. A. Aliyas: None. K. Anderson: None. J. Carr: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.09/DDD10

Topic: F.10. Food Intake and Energy Balance

Support: Grant-in-Aid for Exploratory Research (No.25670358) from Japan Society for the Promotion of Science (JSPS)

Title: Effects of FGF23/αKlotho on the NPY/AgRPergic system in the hypothalamus

Authors: *T. KOMORI, Y. MORIKAWA;
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Abstract: Food intake is regulated via neural circuits located in the hypothalamus. Previously, we have reported that fasting induces αKlotho, a gene associated with longevity, in the hypothalamic arcuate nucleus, especially in the neuropeptide Y (NPY)/agouti-related protein (AgRP)-expressing neurons (44th Annual Meeting of Society for Neuroscience 2014, abstract #11568). To gain insights into the functional roles of αKlotho in the hypothalamus, we analyzed αKlotho-deficient mice. As homozygous αKlotho-deficient mice have a short life span and show severe physical abnormalities like human premature aging, we used heterozygous αKlotho-deficient ($\alpha Klotho^{+/-}$) mice in the present study. The expression of αKlotho gene in the hypothalamus was reduced more than 40% in $\alpha Klotho^{+/-}$ mice compared to wild-type (WT) mice in the fed states. Fasting induced the expression of αKlotho in the hypothalamus of WT mice, but not in $\alpha Klotho^{+/-}$ mice. The body weights in $\alpha Klotho^{+/-}$ mice were similar to those in WT mice in both fed and fasted states. In addition, there was no change in the amount of daily food intake between WT and $\alpha Klotho^{+/-}$ mice. However, the amount of food intake after fasting was reduced in $\alpha Klotho^{+/-}$ mice compared to WT mice. In addition, the expressions of NPY and AgRP after fasting were lower in the hypothalamus of $\alpha Klotho^{+/-}$ mice than those of WT mice, respectively. It has been reported that αKlotho forms the receptor complex with a fibroblast growth factor receptor (FGFR) and acts as the functional receptor for FGF23. To investigate the effects of FGF23 on the expression of NPY and AgRP in the hypothalamus, we injected FGF23 intracerebroventricularly. Intracerebroventricular injection of FGF23 induced the expression of NPY and AgRP in the hypothalamus. These results suggest that FGF23/αKlotho is a novel regulator of the NPY/AgRPergic system in the hypothalamus.

Disclosures: T. Komori: None. Y. Morikawa: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.10/DDD11

Topic: F.10. Food Intake and Energy Balance

Support: Imperial JRF

MRC

Title: Optogenetic stimulation of MCH axons in the hippocampus: Effects on synaptic plasticity

Authors: *J. J. HARRIS[1], D. BURDAKOV[2];
[1]Imperial Col. London, London, United Kingdom; [2]Francis Crick Inst., London, United Kingdom

Abstract: Hypothalamic neurons containing the peptide, melanin concentrating hormone (MCH), may be important for memory consolidation, particularly during sleep. Studies have shown that disrupting the function of MCH neurons, either by deleting the neurons themselves (Le Bariller et al., 2015) or knocking out MCH receptors (Pachoud et al., 2010), impairs learning and disrupts synaptic plasticity in the hippocampus. Here we address the inverse question: does increasing MCH neuron function facilitate hippocampal plasticity? We injected cre-dependent channelrhodopsin into the lateral hypothalamus of MCH-cre mice. After 5-6 weeks, channelrhodopsin-expressing MCH axons were visible in hippocampal slices. In these slices, we illuminated the hippocampus with blue light (5 ms flashes delivered at 20 Hz for 30 second epochs), whilst electrically stimulating Schaffer collateral axons of CA3 with either a weak potentiating stimulus (100 Hz for 1 sec); a strong potentiating stimulus (100 Hz for 1 sec, four times); or a depressing stimulus (1 Hz for 900 sec). Changes in the slope of the field potential, measured in the stratum radiatum of CA1, were monitored. Optogenetic activation of MCH axons in the hippocampus altered the time-course of plasticity and augmented the effect of a weak potentiating stimulus, compared to a control condition, where blue light stimulation was identical but MCH axons did not express channelrhodopsin. These results align with the idea that MCH neurons, active during REM sleep, play a role in facilitating memory consolidation.

Disclosures: J.J. Harris: None. D. Burdakov: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.11/DDD12

Topic: F.10. Food Intake and Energy Balance

Support: National Science Center: „SONATA", UMO-201101/D/NZ4/03744;

Title: Hyperphagic obesity in adult Dicer CKO mice- the role of hypothalamic AgRP/NPY neurons.

Authors: *K. HAJDUKIEWICZ[1], W. KONOPKA[2];
[1]Neurobio. Ctr., Nencki Inst. of Exptl. Biol., Warsaw, Poland; [2]Nencki Inst. of Exptl. Biol. in Warsaw, Warsaw, Poland

Abstract: Obesity is a worldwide disease of complex etiology. The main appetite regulatory center is located within the brain, in hypothalamus. A putative mechanism responsible for the obesity phenotype involves microRNA interplay between feeding regulatory elements of the hypothalamic AgRP/NPY- and POMC-producing neurons. Dicer is a key enzyme in microRNA processing. In Dicer's absence, there is a pronounced lack of mature microRNAs and a disturbed regulation of translation. We want to observe how massive, spatially and temporally defined, loss of microRNAs impacts metabolism and obesity outcome. To achieve this goal, we injected rAAV-coding Cre recombinase under the AgRP specific promoter into the arcuate nucleus of mice with Cre-dependent Dicer sequence. Our preliminary data show that such administration leads to visible weight gain. This phenotypic effect is thought to be reflected by an imbalance between anorexygenic and orexigenic neuropeptide levels, as measured by the mass spectrometry approach. In addition, reporter constructs coding either mCherry, EYFP or ChR2-EYFP under the AgRP specific promoter were done and stereotacticly injected to arcuate nucleus. In most of the cases we could observe specific expression of reporter genes. This approach is an initial step to explore functional connectivity between AgRP/NPY-producing neurons and other brain structures in Dicer CKO mice.

Disclosures: **K. Hajdukiewicz:** Other; Nencki Institute of Experimental Biology in Warsaw,Ooland. **W. Konopka:** None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.12/DDD13

Topic: F.10. Food Intake and Energy Balance

Support: John Lee Pratt Foundation #436906

Title: Dietary macronutrient composition affects the influence of exogenous neuropeptide Y on food intake responses and hypothalamic gene expression in chicks

Authors: *B. MCCONN, E. R. GILBERT, M. A. CLINE;
Virginia Tech., Blacksburg, VA

Abstract: In chicks, central injection of neuropeptide Y (NPY) led to preferential consumption of a high fat diet containing lard as the main source of energy, although molecular mechanisms underlying these effects are unknown and it is unclear whether substituting the lard with a plant-based oil would yield similar effects. Thus the purpose of this study was to evaluate the effects of high protein, fat and carbohydrate diets on food intake responses, and hypothalamic mRNA abundance following intracerebroventricular (ICV) injection of 0, 0.2, or 2.0 nmol NPY at 4 days post hatch. Chicks were fed one of three isoenergetic (3,000 kcal metabolizable energy; ME/kg) and isonitrogenous (22% CP) corn and soybean meal-based diets ad libitum from hatch: 1) high carbohydrate [HC], 2) high protein [HP] (27% CP), or 3) high fat [HF] (30% ME derived from soybean oil). In Experiment 1, consumption of the HF diet led to a non-dose dependent similar magnitude of increased food intake after NPY injection, whereas chicks fed the HC and HP diets increased their food intake dose-dependently. These results, combined with our previous findings, show that a HF diet accentuates the potency of NPY in birds, regardless of the source of fat in the diet. In Experiment 2, total hypothalamic RNA was isolated from samples collected at 1 h post-NPY injection and used for measuring mRNA abundance of appetite-associated factors. There were main effects of diet on mRNA abundance of mesotocin (MT, avian homologue to oxytocin), agouti-related peptide (AgRP), corticotropin releasing factor receptor sub-type 1 (CRFR1), NPY, NPY receptor sub-types 1 and 2 (NPYR1 and 2, respectively), and melanocortin receptors 3 and 4 (MC3R and 4R, respectively). There was also a main effect of NPY treatment on NPY mRNA and also NPY treatment by diet interactions on expression of NPYR1 and MC4R mRNA. In conclusion, the potency of NPY-mediated stimulation of food intake at 4 days post hatch was augmented when chicks consumed a HF diet, and this coincided with changes in hypothalamic mRNA expression of key appetite-associated factors. These results have implications for better understanding the molecular basis of food craving and hording across species.

Disclosures: B. McConn: None. E.R. Gilbert: None. M.A. Cline: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.13/DDD14

Topic: F.10. Food Intake and Energy Balance

Support: FONDECYT REGULAR 1150274

Title: Des-Tyr DYN, a non opioid DYN peptide regulates physical activity, energy expenditure and hedonic food intake in the paraventricular hypothalamic nucleus.

Authors: *C. E. PEREZ-LEIGHTON[1], L. GAC[1], B. ALVAREZ[1], E. MORSELLI[2], M. HERNANDEZ[2], J. TESKE[3];
[1]Univ. Andres Bello, Santiago, Chile; [2]Facultad de Ciencias Biológicas, Pontificia Univ. Católica de Chile, Santiago, Chile; [3]Dept. of Nutritional Sci., Univ. of Arizona, Tucson, AZ

Abstract: The dynorphin (DYN) peptides are a family of neuropeptides that regulate different behaviors, including food intake and selection. DYN peptides can be opioid or non-opioids, depending on whether they act through opioid receptors. Opioid DYN peptides promote food intake through its actions at the hypothalamic paraventricular nucleus (PVN), an important site in the regulation of feeding behavior and physical activity. Our recent work has focused in the role of non-opioid peptide des-Tyr-DYN (DYN-A_{2-17}) in PVN in mice. We previously showed that injection of DYN-A_{2-17} in PVN simultaneously increases locomotor activity and food intake (Peptides 2016, 76:14-8). Here, we further characterize the effects of DYN-A_{2-17} in food intake and physical activity. DYN-A_{2-17} in PVN at different doses (0, 1, 3 nmol) increases physical activity, overall energy expenditure and physical activity-associated energy expenditure in the absence of food. We also show that a single dose of DYN-A_{2-17} (3 nmol) increases running wheel activity one hour post-injection. Next, we showed that DYN-A_{2-17} increases intracellular calcium in hypothalamic mice cell line, suggesting it acts as an excitatory neuropeptide in the hypothalamus. To further examine the role of DYN-A_{2-17} (0, 1, 3, nmol) in food intake, we evaluated its effects in palatable snack selection and intake compared to the opioid DYN peptide DYN-A_{1-13} (0, 1, 3 nmol) and orexin-A (0 0.2, 0.4 nmol), as the hypothalamic orexin/dynorphin (ox/dyn) neurons release both orexin and dynorphin (DYN) peptides in PVN and other brain sites. Mice were acclimated to short-term access (2 h) to four human palatable snacks and standard rodent chow. After establishing baseline preferences for snacks, mice were injected with each peptide at their different concentrations. Our data suggest only DYN-A_{1-13} increases overall food intake. Analyses of food selection suggest DYN-A_{1-13} and DYN-A_{2-17} increased snack of preferred snacks, but with higher potency of DYN-A_{1-13}, while orexin-A increased chow intake and decreased snack intake. Together, these experiments will improve our

understanding of the mechanisms by which the orexins, opioid and non opioid DYN peptides control energy balance.

Disclosures: C.E. Perez-Leighton: None. L. Gac: None. B. Alvarez: None. E. Morselli: None. M. Hernandez: None. J. Teske: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.14/EEE1

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant DA05010

Title: Role of enkephalin in dopamine d2-receptor expressing neurons in licking microstructure

Authors: *I. A. MENDEZ[1], H. A. LAM[1], S. N. LEE[1], J. BOULTER[1], S. B. OSTLUND[2], N. P. MURPHY[1], N. T. MAIDMENT[1];
[1]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; [2]Dept. of Anesthesiol. and Perioperative Care, UCI, Irvine, CA

Abstract: Opioid signaling is implicated in mediating the hedonic and motivational aspects of palatable foods. When licking for palatable solutions, global enkephalin knockout mice emit fewer bouts of licking than wildtypes, but similar bout lengths. Interestingly, the number of times a mouse engages in a new bout of licking is suggested to reflect motivational aspects of feeding behavior, while the length of the bout once engaged is determined primarily by the immediate hedonic impact of the food stimulus. *Objective:* In these studies, we investigated the role of endogenous enkephalin, specifically in neurons expressing dopamine D2 receptors, in licking microstructure. *Design:* D2 specific proenkephalin knockout mice (D2 PENK KO) and their wild-type littermates were trained to lick for 20% sucrose for 5 days in a lickometer. Subsequently, the effects of sucrose concentration (2% versus 20%) and hunger state (4 h versus 18 h food deprivation) were assessed. A second group of D2 PENK KO and wildtype mice were trained to lick for 20% sucrose for 7 days. Following training in these mice, the hypophagic effects of 5mg/kg naltrexone on licking were assessed. *Results:* Compared to wildtype mice, D2 PENK KO mice displayed fewer total licking bouts, but similar mean bout lengths, across sucrose concentrations and hunger states. In the second group of mice, naltrexone decreased total bout number in wildtype mice, but had no effect on mean bout length. No effects of naltrexone were observed in D2 PENK KO licking behavior. *Discussion:* Similar to previous observations in global PENK KO mice, D2 PENK KO mice emitted fewer bouts of licks, but similar licking

bout lengths. Naltrexone hypophagia was driven by decreases in the number of licking bouts, an effect that D2 PENK KO mice were insensitive to. Overall these findings suggests that enkephalin, specifically in D2 expressing neurons, plays an important role in establishing feeding patterns, and may therefore play a role in psychopathological conditions characterized by aberrant feeding behavior.

Disclosures: I.A. Mendez: None. H.A. Lam: None. S.N. Lee: None. J. Boulter: None. S.B. Ostlund: None. N.P. Murphy: None. N.T. Maidment: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.15/EEE2

Topic: F.10. Food Intake and Energy Balance

Support: Ministry of Science and Technology grant MOST 1032320B006005MY2

Title: Investigation of leptin mediated trafficking of K_{ATP} channels in the arcuate nucleus of high fat induced obese mice

Authors: J.-S. RUAN[1], *R.-C. HUANG[2], P.-C. CHEN[1];
[1]Physiol., Natl. Cheng Kung Univ., Tainan, Taiwan; [2]Chang Gung Univ., Tao-Yuan, Taiwan

Abstract: K_{ATP} channels are molecular sensors that couple cell metabolism to membrane excitability. Leptin is a hormone secreted primarily by white adipocytes and is sufficient to regulate body weight, feeding, energy expenditure and glucose metabolism. Within CNS, the hypothalamus is the main site for leptin action and high levels of the leptin receptor (ObRb) are expressed in several hypothalamic nuclei including arcuate nucleus (ARC).There are two populations of neurons in the ARC: neuropeptide Y (NPY) and agouti-related protein (AGRP) containing neurons, and proopiomelancortin (POMC) and cocaine-and amphetamine-regulated transcript (CART)-containing neurons. Neuronal K_{ATP} channels are widely expressed in the brain including POMC and AGRP neurons. Electrophysiological examination has shown that a sub-population, defined as glucose-responsive (GR) neurons. Glucose removal and leptin application hyperpolarize these neurons by activation of K_{ATP} channels in AGRP neurons and inhibition of GR neurons results in decreased transmission output and hence reduced food intake. Most obese patients suffer from a state called leptin resistance which is high circulating leptin concentrations cannot decrease energy intake. The mechanism of this leptin-resistance is still incompletely understood. Interestingly, leptin activates PI3K pathway to hyperpolarize AGRP neurons by opening K_{ATP} channels, but leptin engages PI3K signaling to control firing in POMC neurons.

We hypothesize that the surface K_{ATP} channel is the molecular target regulated by leptin to determine AGRP and POMC neuron activity and dysregulated surface expression of K_{ATP} channel may account for obese induced leptin resistance. Hence, we used hypothalamic N44 cell line and high fat diet induced obese mice to test the hypothesis. *In vitro*, we applied the saturated free fatty acid palmitate and different glucose concentration to mimic the condition of obese induced type II diabetes followed by the analysis of K_{ATP} channel trafficking using surface biotinylation and electrophysiological recording after leptin treatment. *In vivo*, we detected K_{ATP} channel activity in the POMC and NPY neurons of high fat induced obese mice by electrophysiological recording, imaging and biochemical assays. We hope to get a better understanding by how diet induced obese affects effect of leptin on K_{ATP} channel trafficking in arcuate nucleus.

Disclosures: J. Ruan: None. R. Huang: None. P. Chen: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.16/EEE3

Topic: F.10. Food Intake and Energy Balance

Support: CIHR RNL-132870

RDC 5404.1171.102

Title: Intrinsic and synaptic plasticity of appetite-promoting MCH neurons during growth, high-fat diet feeding, and food deprivation

Authors: *V. LINEHAN, M. HIRASAWA;
Div. of BioMedical Sciences, Fac. of Med., Mem. Univ. of Newfoundland, St John's, NL, Canada

Abstract: Melanin-concentrating hormone (MCH) neurons are one of the hypothalamic cells groups that promote food intake and weight gain. Therefore, these neurons may be critical for periods of pronounced weight gain such as development, rebound following food restriction, and obesity. To determine whether activation of MCH neurons accompanies these metabolic challenges, whole cell patch clamp recording was performed on MCH neurons from rat hypothalamic slices. Basal electrophysiological properties, miniature excitatory synaptic currents (mEPSCs), and paired pulse ratios were recorded. Two factors were tested: age (juvenile and adult) and dietary condition (high-fat diet (HF), 12-hour fasting, and standard chow fed controls).

We found that MCH neurons experience a decrease in excitability as animals grow. Specifically, compared to 4 week old rats (juvenile), MCH neurons of 7 and 14 week old rats (adult) had a hyperpolarized membrane potential, a lower firing frequency, and a higher firing threshold. Additionally, both the frequency and amplitude of mEPSCs decreased with age, suggesting pre- and postsynaptic changes. This was likely due to a reduction in synapse number and postsynaptic sensitivity as we found no age-dependent change in the paired pulse ratio, a measure of release probability. Activation of MCH neurons also occurs in adult rats that are fed a HF for 4-11 weeks, which induces significant weight gain. MCH neurons of HF fed rats were more excitable, which was due to downregulation of Na^+/K^+-ATPase. Moreover, there was an increase in the frequency of mEPSCs without a change in paired-pulse ratio, suggesting an increased number of excitatory synapses. Finally, activation of MCH neurons can also occur over shorter time scales, such as overnight fasting. However, unlike rats undergoing growth spurts or chronic HF feeding, fasting strengthens existing excitatory synaptic connections to these neurons without increasing their number. This was evident as an increase in mEPSC frequency and a decrease in paired-pulse ratio. Our study suggests that intrinsic and synaptic plasticity occurs in MCH neurons under different physiological states that involve an increased drive for food intake. Activation of MCH neurons through these mechanisms may be important for promoting positive energy balance to meet the physiological demands of growth, development, and weight gain.

Disclosures: V. Linehan: None. M. Hirasawa: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.17/EEE4

Topic: F.10. Food Intake and Energy Balance

Support: NARSAD Young Investigator Award

NIH Grant DK068400

AHA 11POST7430087

13POST16890000

Title: Differences between mice lacking neural Pomc enhancers 1 and 2 on food intake and body weight after peripheral leptin treatment.

Authors: *E. NA[1], D. D. LAM[2], E. YOKOSAWA[1], M. LOW[1];
[1]Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI; [2]Inst. of Neurogenomics, Helmholtz Ctr. Munich, Munich, Germany

Abstract: Past work from our laboratory has demonstrated that expression of the *Pomc* gene is under the regulatory control of two partially redundant enhancers, nPE1 and nPE2. Simultaneous deletion of these neural enhancers results in morbidly obese mice while deletion of either alone produces a mildly obese phenotype (Δ1 knockout) or no overt phenotype (Δ2 knockout). Here we show a dissociable effect of the anorexigenic hormone, leptin on Δ1 knockout and Δ2 knockout mice whereby Δ2 knockout mice are relatively unresponsive to peripheral leptin treatment with food intake being only mildly suppressed by leptin compared to WT and Δ1 knockout mice. We also show that Δ1 knockout mice are more sensitive to the anorectic effects of leptin with a substantial decrease in food intake and body weight after peripheral treatment compared to Δ2 knockout and WT mice, respectively. Interestingly, we do not show an effect of leptin treatment on *Pomc* mRNA expression in the arcuate nucleus of either the Δ1 or Δ2 knockout mice using *in situ* hybridization and qPCR. Collectively, these data suggest that these two neural enhancers have dichotomous effects on food intake and body weight as a result of exogenous leptin treatment and that deletion of nPE2 may render mice less sensitive to the anorectic effects of leptin despite relatively normal levels of *Pomc* mRNA expression.

Disclosures: E. Na: None. D.D. Lam: None. E. Yokosawa: None. M. Low: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.18/EEE5

Topic: F.10. Food Intake and Energy Balance

Title: Genetically anorexic and obese chickens differ in expression of hypothalamic genes involved in appetite and stress response mechanisms

Authors: *J. WANG, J. YI, E. R. GILBERT, P. B. SIEGEL, M. A. CLINE;
Dept. of Animal and Poultry Sci., Virginia Tech., Blacksburg, VA

Abstract: The Virginia lines of chickens were selected for either low (LWS) or high (HWS) body weight and are now comprised of individuals with different severities of anorexia and obesity, respectively. Differences exist between these lines in appetite-associated behaviors and hypothalamic gene expression that are accentuated by previous exposure to environmental stressors. In the current study, we determined the mRNA abundance of appetite-associated

neurotransmitters and receptors within four hypothalamic nuclei: the lateral hypothalamus (LH), paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), and arcuate nucleus (ARC), in stressed and non-stressed chicks. After hatch, chicks were stressed and hypothalamic nuclei were collected 5 days later. Anorexigenic factors were distributed differently between the two lines. The PVN and ARC were the main sites in HWS, whereas in LWS the PVN and LH were the major target nuclei with greater expression of genes that encode anorexigenic neuropeptides and receptors. Compared to HWS, LWS expressed more neuropeptide Y (NPY) in the ARC and NPY receptor 5 (NPYR5) in the PVN, LH and ARC. In both lines, stress increased corticotrophin-releasing factor (CRF) mRNA in the PVN, CRF receptor 2 (CRFR2) and growth hormone secretagogue receptor (GHSR) in the VMH, CRF receptor 2 (CRFR2) and urocortin 3 (UCN 3) in the LH, and leptin, CRF and CRFR2 in the ARC. There were several interactions between line and treatment. Specifically, there was greatest expression of CRF receptor 1 (CRFR1) and UCN 3 in the PVN of stressed LWS chicks then in all other treatment group combinations. These results suggest that hypothalamic gene expression of appetite-associated factors may contribute to differences in feeding behavior and stress responses between HWS and LWS and thus provide insights for understanding the molecular basis for differences in eating disorders.

Disclosures: J. Wang: None. J. Yi: None. E.R. Gilbert: None. P.B. Siegel: None. M.A. Cline: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.19/EEE6

Topic: F.10. Food Intake and Energy Balance

Support: NIH R01DK092587

NIH P20GM103629

NIH P30DK072476

NIH T32-DK064584

Title: Galanin neurons in the lateral hypothalamus modulate food reward and locomotor activity

Authors: E. QUALLS-CREEKMORE, S. YU, M. FRANCOIS, C. HUESING, C. MORRISON, H.-R. BERTHOUD, *H. MUNZBERG;
Pennington Biomed Res. Ctr., Baton Rouge, LA

Abstract: The lateral hypothalamus (LHA) is an important integrator of reward and appetitive behavior. Previous work in our lab has shown that LHA galanin neurons which co-express leptin receptors (LepRb) are implicated in food reward. Similarly, activation of LHA GABAergic neurons induces feeding behavior, and molecularly distinct populations of LHA GABA neurons may separately encode the consumption of food and the motivated behavior associated with food reward. Based on overall inhibitory central galanin actions, we hypothesized that LHA galanin neurons represent partially LHA GABAergic neurons. Thus, here we wanted to test if activation of LHA galanin neurons will modulate food reward behavior similarly to LHA GABA neurons. We examined the effect of chemogenetic activation of LHA galanin neurons on feeding behavior and metabolism with a cre-dependent adenoassociated virus for DREADD-Gq expression injected into the LHA of Galanin-cre mice. Our data show that chemogenetic activation of LHA galanin neurons increased food reward behaviors in an operant conditioning progressive ratio schedule. There was no change in consumption of palatable liquid diet (Ensure) in a free-access feeding task. We further verified chemogenetic activation in LHA GABA neurons in vGat-cre mice and successfully recapitulate increased food reward behavior. Also locomotor activity and energy expenditure was increased with activation of both LHA galanin and GABA neurons, suggesting that LHA galanin neurons represent a subset of GABA neurons that drive specifically food reward and locomotor activity. *Supported by R01DK092587, P20GM103629 (HM) P30DK072476 (SY, HM), T32-DK064584 (EQC)*

Disclosures: E. Qualls-Creekmore: None. S. Yu: None. M. Francois: None. C. Huesing: None. C. Morrison: None. H. Berthoud: None. H. Munzberg: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.20/EEE7

Topic: F.10. Food Intake and Energy Balance

Support: National Natural Science Foundation of China No.81300689

China Scholarship Council 201406280111

R37 DK042394

UL1RR024923

R01 DK100699

AHA 14SDG20370016

Title: PI3K is integral for the acute activity of leptin and insulin in arcuate AgRP neurons

Authors: *T. YAO[1,2], Y. GAO[1,3], J. SUN[1,4], Y. HUANG[1,4], T. LIU[1], K. WILLIAMS[1];
[1]Div. of Hypothalamic Res., UT Southwestern Med. Ctr., Dallas, TX; [2]Dept. of Physiol. and Pathophysiology, Xi'an Jiaotong Univ. Sch. of Med., Xi'an, China; [3]Inst. of Basic Med. Sci., Chinese Acad. of Med. Sci. and Peking Union Med. Col., Beijing, China; [4]Southern Med. Univ., Guangzhou, China

Abstract: Hypothalamic Agouti related protein (AgRP) neurons, which are key regulators of energy and glucose homeostasis, receive and integrate neural and humoral signals. The mechanisms by which the hormones insulin and leptin acutely modify the electrical activity of these neurons remains unclear. Here we show that loss of the PI3K catalytic subunits, p110α and p110β, in AgRP neurons abrogates the leptin- and insulin-induced inhibition of AgRP neurons. Moreover, concurrent disruption of p110α and p110β in AgRP neurons results in increased weight gain. The increased adiposity was concomitant with a hypometabolic phenotype: decreased energy expenditure independent of changes in food intake. Deficiency of p110α and p110β in AgRP neurons also impaired glucose homeostasis and insulin sensitivity. In summary, these data highlight the requirement of both p110α and p110β in AgRP neurons for the proper regulation of energy balance and glucose homeostasis.

Disclosures: T. Yao: None. Y. Gao: None. J. Sun: None. Y. Huang: None. T. Liu: None. K. Williams: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.21/EEE8

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant F30 DK107163-01

NIH Grant R01 DK-103808

Title: Lateral hypothalamic neurotensin neurons engage the mesolimbic dopamine system to regulate locomotor and ingestive behavior

Authors: *H. WOODWORTH, G. KURT, H. BATCHELOR, J. BROWN, R. BUGESCU, G. LEINNINGER;
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Abstract: The lateral hypothalamic area (LHA) acts in concert with dopamine (DA) neurons in the ventral tegmental area (VTA) to regulate the motivation to feed, drink and move. It remains unclear, however, how specific LHA neuronal populations modify ingestive and locomotor behaviors. We examined how LHA neurons expressing the neuropeptide neurotensin (Nts) engage the DA system to regulate behavior. LHA Nts neurons project to the VTA, where many DA neurons co-express neurotensin receptor 1 (NtsR1). Furthermore, these VTA NtsR1-DA neurons project to ventral striatal brain regions that regulate ingestive behavior and locomotor activity, such as the nucleus accumbens (NA). Activating the LHA Nts neuronal circuit using DREADD technology increases phospho-CREB in the NA, confirming that LHA Nts neurons functionally modulate the mesolimbic DA system. Next we examined the physiologic role of Nts signaling via this circuit, by activating LHA Nts neurons in wild-type (WT) mice and mice that lack NtsR1 (NtsR1KO mice). Activation of LHA Nts neurons increased locomotor activity and energy expenditure in both WT and NtsR1KO mice, which was blunted by a DA receptor 1 (DR1) antagonist. Activation of LHA Nts neurons also increased water intake in WT animals, but not NtsR1KO animals, indicating that NtsR1 is required for LHA Nts-induced drinking behavior. While activation of LHA Nts neurons did not alter chow intake in WT mice, it significantly increased food intake in both NtsR1KO mice and WT mice pre-treated with the selective NtsR1 antagonist, SR48692, indicating that NtsR1 is required to restrain feeding. Since the LHA Nts neuronal circuit increases energy expenditure without concomitant increase in feeding, we asked whether activation of the circuit could promote weight loss in obese mice. Chronic activation of LHA Nts neurons increased energy expenditure in diet-induced obese mice, but similar to mice with disrupted NtsR1 signaling, they also consumed more food and consequently did not lose weight. Together, these data reveal that LHA Nts neurons engage the mesolimbic DA system to modify feeding, drinking, and locomotor behaviors, and that intact signaling via NtsR1 is essential for maintaining appropriate ingestive behavior and body weight.

Disclosures: H. Woodworth: None. G. Kurt: None. H. Batchelor: None. J. Brown: None. R. Bugescu: None. G. Leinninger: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.22/EEE9

Topic: F.07. Autonomic Regulation

Support: NSF grant PLR-1341663

Title: Neuronal mechanisms involved in thermal tolerance of Antarctic fishes

Authors: *I. I. ISMAILOV[1], J. B. SCHARPING[2], I. E. ANDREEVA[1], M. J. FRIEDLANDER[1];
[1]Virginia Tech. Carilion Res. Inst., Roanoke, VA; [2]Virginia Tech. Carilion Sch. of Med., Roanoke, VA

Abstract: Antarctic teleosts *C. aceratus* (lacks hemoglobin, Hb-) and *N. coriiceps* (expresses hemoglobin, Hb+), both extremely stenothermic, are part of the suborder Notothenioidei that live on the Antarctic shelf at ~0°C. Hb- icefishes are less tolerant to acute increases in temperature (T°) than Hb+ animals, but the mechanisms of this difference are largely unknown. This study investigates for the first time the neural component of Notothenioid thermal tolerance by describing behavioral responses to rises in ambient T° as well as effects of local cerebellar and whole body warming on cerebellar Purkinje cells (PC) and the heart. Both Hb+ and Hb- species, freely swimming in a 700 L tank, responded to warming with a progressive increase in translational movement followed by a decline until loss of equilibrium occurred (average = 13.8±1.0°C in *C. aceratus* and 16.2±0.8°C in *N. coriiceps)*. Opercular beat frequency also exhibited a biphasic pattern, increasing with rises in T as little as 3°C and declining above 9°C in both species. Continuous bilateral pectoral fin movement without translation occurred in both species, at 6.5°C in *C. aceratus* (was also more pronounced) and at 10°C in *N. coriiceps*. Behaviors only characteristic of *N. coriiceps* began at ~6.5°C and included intermittent fin (anal, pelvic, dorsal, and caudal) "jerks", followed by body "jerks". Local step-wise warming of the corpus cerebelli of anesthetized fishes resulted in biphasic changes in spontaneous PC firing (increasing until ~17°C and then decreasing at higher T°, similar in both species) and elicited progressive bradycardia (greater in extent in *C. aceratus* and at lower T°, compared to *N. coriiceps*), reversible with cooling in both species. Irrigation of the gills of anesthetized animals with warmed water (while keeping the cerebellum chilled) led to progressive sinus tachycardia, followed by recurrent episodes of asystole in *N. coriiceps* and by a progressive bradycardia in *C. aceratus*, both leading to cardiac arrest with the onset not significantly different between Hb+ (18.5±2.0°C) and Hb- (19.7±1.8°C) species. In both fishes, tachycardia was accompanied by a decline in PC spiking. We conclude that 1) even slight warming leads to changes in motoric behavior in both species; 2) involuntary spastic activity in *N. coriiceps* at lower T° than in *C. aceratus* suggests that its nervous system is more susceptible to warming; 3) a central chemoreceptor zone may exist in the teleost cerebellum, modulating vagal tone on the heart; 4) the relationship between this zone and peripheral baro- and chemoreceptor modulation of cardiac and respiratory activity is yet to be determined. Supported by the NSF grant PLR-1341663.

Disclosures: I.I. Ismailov: None. J.B. Scharping: None. I.E. Andreeva: None. M.J. Friedlander: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.23/EEE10

Topic: F.07. Autonomic Regulation

Support: NIH Grant AR067858

Title: Trpm8-/- mice have impaired core temperature regulation and altered body composition in response to three-week cold exposure

Authors: *K. J. MOTYL, C. ROSEN;
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Abstract: TRPM8 (the transient receptor potential cation channel, subfamily M, member 8) is expressed in sensory neurons and is a detector of environmental cold temperature. TRPM8 activation can induce UCP-1 dependent thermogenesis, which has been shown to be positive for bone health. With age, however, brown adipose tissue function declines concomitantly with loss of bone. We wanted to test how TRPM8 was involved in the co-regulation of these two tissues during aging of both male and female mice. At 52 weeks of age, male C57BL6/J mice with a global deletion of TRPM8 (*Trpm8-/-*) have significantly reduced core temperature, total body mass, fat mass and fat-free mass compared to male *Trpm8*+/+. Although female *Trpm8-/-* mice have reduced body mass and fat mass, they do not have significantly altered lean mass or core temperature compared to *Trpm8*+/+ females. The absence of TRPM8 also significantly reduced total body bone mineral density (BMD) and content (BMC) male, but not female, *Trpm8-/-* mice. When challenged at 18°C and 4°C, male *Trpm8-/-* mouse body temperature decreased significantly compared to *Trpm8*+/+ mice at the same temperatures, while female mice had no genotype-specific alterations in body temperature. Both male and female, +/+ and *Trpm8-/-* mice lost bone after 3 weeks at 4°C, indicating TRPM8 was not required for cold temperature-induced bone loss. In conclusion, male *Trpm8-/-* mice, which have impaired temperature regulation at baseline and in response to cold, also have reduced BMD and growth during the first year of life. These findings are consistent with previous literature suggesting that functional brown adipose tissue promotes bone density.

Disclosures: K.J. Motyl: None. C. Rosen: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.24/EEE11

Topic: F.07. Autonomic Regulation

Title: Dorsal hypothalamic bombesin-like receptor-3 neurons regulate body temperature

Authors: *R. A. PINOL[1], S. H. ZAHLER[2], B. K. TAN[2], C. XIAO[2], O. GAVRILOVA[2], A. KRAVITZ[2], M. J. KRASHES[2], M. L. REITMAN[2];
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Abstract: Bombesin-like receptor 3 (Brs3) is an orphan G protein-coupled receptor expressed in several brain regions, including the dorsomedial hypothalamic region and paraventricular nucleus of the hypothalamus (PVH). Targeted deletion of Brs3 in mice causes obesity, with reduced energy expenditure and increased food intake. Brs3 agonists suppress food intake and stimulate brown adipose tissue (BAT) through sympathetic activation, increasing energy expenditure. The current aim is to delineate the circuitry through which Brs3 neurons regulate energy expenditure and body temperature (Tb). We measured the effect of local injections of Brs3 agonist MK-5046 on BAT temperature. Small volume, nucleus-targeted, MK-5046 injections in the dorsomedial hypothalamus (DMH), but not the PVH, raised BAT temperature. To assess the effect of acute activation of Brs3 populations in freely moving, conscious mice we expressed the excitatory DREADD hM3D(q) in Brs3-T2A-CreER^{T2} mice. Chemogenetic activation of DMH^{Brs3} neurons increased total energy expenditure and Tb, without an effect on physical activity or food intake. Since projections of dorsal hypothalamic area (DHA)/dorsal DMH (dDMH) neurons to the brain stem raphe pallidus (RPa) have been implicated in the regulation of Tb, we optogenetically probed if the DHA/dDMH^{Brs3} projections to RPa mediate thermogenesis. As expected, fiber terminal stimulation of the DHA/dDMH^{Brs3} projections increased Tb in freely moving, conscious mice. Immunohistochemically detected fos in DHA/dDMH^{Brs3} neurons increased from 4.1 ± 0.35 % in acutely warm-exposed to 24 ± 1.7 % in acutely cold-exposed animals. Accordingly, ablation (with a Cre-dependent diphtheria toxin A virus) of DMH^{Brs3} neurons in adulthood reduced the cold defense response compared to control mice (Cre-dependent GFP virus), but did not alter the stress-induced hyperthermic response. Body weight and food intake (chow or high-fat diet) were not affected by DMH^{Brs3} neuron ablation. These results suggest that the food intake suppression and thermogenic effects of Brs3 activation can be mediated by different subsets of Brs3-expressing neurons. Specifically, DHA/dDMH^{Brs3} neurons are able to increase energy expenditure and BAT temperature through projections to the RPa.

Disclosures: R.A. Pinol: None. S.H. Zahler: None. B.K. Tan: None. C. Xiao: None. O. Gavrilova: None. A. Kravitz: None. M.J. Krashes: None. M.L. Reitman: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.25/EEE12

Topic: F.07. Autonomic Regulation

Support: NIH Grant GM113894

Title: The insulin-like growth factor 1 receptor modulates core body temperature during calorie restriction

Authors: *R. CINTRON-COLON[1], M. SANCHEZ-ALAVEZ[1], W. NGUYEN[1], S. MORI[1], T. BARTFAI[2], M. HOLZENBERGER[3], B. CONTI[1];
[1]Chem. Physiol., The Scripps Res. Inst., La Jolla, CA; [2]Neurochemistry, Univ. of Stockholm, Stockholm, Sweden; [3]Inst. Natl. de la Santé et de la Recherche Médicale, Ctr. de Recherche UMR938, Hôpital Saint-Antoine, Paris, France

Abstract: In homeotherms, including rodents and humans, efficient thermoregulatory mechanisms maintain core body temperature within a narrow range against large differences in ambient temperature. However, when nutrients availability is limited, homeotherms can reduce their core body temperature. This response is regarded as an adaptive mechanism that decreases energy expenditure and it is believed to have evolved to prolong survival until food becomes available. Using genetic and pharmacological approaches in mice, we have found that the insulin-like growth factor 1 signaling pathway participates in the modulation of core body temperature during *ad libitum* feeding and likewise in calorie restriction in a gender-specific manner. Pharmacological blockade of the IGF-1R signaling induced hypothermia in male and female mice. In addition, central but not peripheral administration of recombinant IGF-1 prevented core body temperature reduction during calorie restriction. Similar results were obtained with mice carrying a hemizygous null mutation of the gene encoding the IGF-1 receptor, *Igf1r*$^{+/-}$, previously described to have reduced IGF-1R signaling and increased female lifespan. Compared to wild type littermates, female but not male *Igf1r*$^{+/-}$ mice had transient and circadian robust reduction of core body temperature when fed *ad libitum* and displayed an enhanced hypothermic response to calorie restriction. These findings indicate that IGF-1 receptors play a role in regulating the hypothermic response to calorie restriction and suggest that the mechanisms by which lowering IGF-1 signaling promotes longevity in homeotherms include core body temperature reduction.

Disclosures: R. Cintron-Colon: None. M. Sanchez-Alavez: None. W. Nguyen: None. S. Mori: None. T. Bartfai: None. M. Holzenberger: None. B. Conti: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.26/DP07 (Dynamic Poster)

Topic: F.07. Autonomic Regulation

Support: NIA Grant K99/ROO

Cancer Prevention and Research Institute of Texas (CPRIT)

Endowed Scholars Program

Texas Institute of Brain Injury Research

STARS

Title: Heterotypic signaling by neural HSF-1 specifies thermotolerance and age regulation

Authors: *I. J. GONZALEZ[1], P. M. DOUGLAS[2];
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Abstract: Heat Shock transcription Factor (*HSF-1*) orchestrates elaborate responses which enable animals to combat various cellular challenges ranging from acute injury to chronic stresses that can evolve into disease over time. During acute heat stress, *HSF-1* mediated induction of molecular chaperones buffers widespread protein misfolding and helps restore protein homeostasis in cells. Ectopic expression of *HSF-1* exclusively in the nervous system of *C. elegans* protects young worms against heat stress and extends lifespan. Under heat, *HSF-1* in the nervous system bolsters the magnitude of heat-induced chaperone expression and protects young worms. However, no chaperone induction is observed at permissive temperatures in long-lived worms expressing *HSF-1*. Instead, neuronal *HSF-1* signals to intestinal tissues and promotes the activation of the *FOXO* transcription factor, *DAF-16*, which mediates longevity assurance. Previous research has implicated the actin cytoskeleton as being an important target of *HSF-1* mediated increase in lifespan and thermotolerance. We identify a specific intestinal isoform of actin, ACT-5, as being an important target of *HSF-1* which provides an essential barrier to pathogenic invasion and ensures protection against stress and age. Furthermore, we seek to determine whether ectopic expression of *HSF-1* exclusively in the nervous system of *C.*

elegans can induce the protective effects of *HSF-1* in the intestine via bolstering of the actin cytoskeleton.

Disclosures: I.J. Gonzalez: None. **P.M. Douglas:** A. Employment/Salary (full or part-time): Part-time employment.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.27/EEE13

Topic: F.07. Autonomic Regulation

Support: Alberta Children's Hospital Research Institute

ACHRI-CIHR Training Program in Genetics, Child Development and Health and ACHRI

CIHR (MOP-77760)

Title: Activation of TRPV1 receptors in the vagus nerve exacerbates thermal hyperpnea in immature rats

Authors: *K. BARRETT[1], A. ROY[2], R. J. A. WILSON[3], M. H. SCANTLEBURY[4]; [1]Pediatrics, Alberta Children's Hosp. Res. Inst., [2]Physiol. and Pharmacol., [3]Physiol. and Pharmacology, Alberta Children's Hosp. Res. Inst., [4]Pediatrics, Clin. Neuroscience, Alberta Children's Hosp. Res. Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Rationale: Thermoregulation in mammals involves a complex interplay of autonomic and behavioral responses, including hyperthermia induced increases in breathing (thermal hyperpnea); immature animals are affected to a greater extent compared to adults. The vagus nerve has also been implicated in the thermal hyperpneic response. The mechanisms underlying this effect are incompletely understood, but may implicate the transient receptor potential vanilloid-1 (TRPV1) receptor which is a heat sensitive ionic channel. In this study we investigated the role of TRPV1 in thermal hyperpnea in immature rats.
Methods: Postnatal day (P) 10 rats were treated with the TRPV1 agonist, piperine, and the respiratory response to hyperthermia was assessed using head-out plethysmography in rats having the vagus nerve sectioned bilaterally or left intact. We also investigated the possibility of using DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology to specifically target TRPV1 expressing cells in the nodose ganglia of the vagus nerve to confirm their role in thermal hyperpnea. Confocal microscopy and neural recordings were used to assess

expression and functionality of the DREADD, respectively, in P15 rats that were injected with the construct at P1.
Results: Compared to vehicle-treated controls, pups injected i.p. with piperine exhibited an early, rapid and significantly greater increase in respiratory rate ($p<0.024$), tidal volume ($p<0.02$), minute ventilation ($p<0.042$) and rate of expired CO_2 ($p<0.02$). Pre-treatment with AMG-9810 (TRPV1 antagonist) significantly attenuated these effects of piperine ($p<0.019$). The exacerbation in the thermal hyperpneic response noted in rats treated i.p. with piperine was not observed in rats treated i.c.v. Vagotomy also reversed the effects of piperine on breathing in response to hyperthermia; tidal volume ($p=0.002$), minute ventilation ($p<0.01$) and rate of expired CO_2 ($p<0.01$). Regarding the DREADD experiments, we have successfully expressed the DREADD in the P15 rat nodose ganglia of the vagus nerve following injections of the construct at P1.
Conclusion: These results support that activation of TRPV1 receptors localized in the vagus nerve exacerbates the thermal hyperpneic response which may play a key role in thermoregulation. This may have important implications in certain pathophysiological conditions in which the TRPV1 receptor is sensitized, such as during inflammation.

Disclosures: K. Barrett: None. A. Roy: None. R.J.A. Wilson: None. M.H. Scantlebury: None.

Poster

257. Thermoregulation and Neuropeptide Regulators of Energy Balance

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 257.28/EEE14

Topic: F.07. Autonomic Regulation

Support: ERC-2011-StG-280565

Alexander von Humboldt Foundation/BMBF

CellNetworks Cluster of Excellence

Title: A TRP channel serves as a hypothalamic heat sensor that regulates thermal homeostasis

Authors: *H. WANG[1], K. SONG[2,1], G. KAMM[1], F. DE CASTRO REIS[3], P. HEPPENSTALL[3], H. WENDE[1], J. SIEMENS[1,4];
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Abstract: Body temperature homeostasis is critical for survival and well-being of all mammals. The hypothalamus comprises the principal higher-order brain center which regulates thermal homeostasis. The anterior part of hypothalamus, namely the preoptic area (POA), detects and regulates internal temperature via feedback mechanism. Previous electrophysiological recordings have identified a group of neurons, whose firing rates increases with a rise of local temperature. It has been proposed that body temperature is encoded by the firing rate of these warmth-sensitive neurons (WSNs).
Here, we have identified a TRP channel as a primary temperature sensor in WSNs. We demonstrate that the TRP channel mediates temperature-dependent calcium rise in neurons both in dispersed POA cultures and POA slice preparations. The temperature-sensitivity is largely abrogated in neuronal cultures obtained from global channel knockout brains.
To gain in vivo access to this group of neurons, we generated a Cre mouse line which labels the TRP channel-expressing cells with Cre recombinase. We injected cre-dependent DREADD-virus stereotactically into the POA of Cre mice to achieve spatial and temporal control of neuronal activity, specifically in the TRP channel-positive POA neurons.
Interestingly, chemogenetic activation or inhibition of the TRP channel-positive neurons bi-directionally modulates body temperature. To our surprise, chemogentic activation triggers reliable deep hypothermia of the mice, in part by peripheral vasodilation. In addition, this effect could be fully reproduced by only targeting an excitatory subpopulation of the TRP channel-positive neurons, which constitutes only 6% of the TRP+ neurons.
By tracing the outputs of these neurons, we have obtained evidence that neurons in the paraventricular nuclei of the hypothalamus (PVH) receive excitatory input from the TRP-expressing POA neurons and the PVH may potentially relay the hypothermic phenotype to downstream effector organs.
Taken together, we have identified a primary molecular warmth sensor of WSNs and address the importance of thermosensitive TRP channels in temperature interoception. Our work offers an entry point for the genetic dissection of hypothalamic thermoregulatory circuits.

Disclosures: H. Wang: None. K. Song: None. G. Kamm: None. F. de Castro Reis: None. P. Heppenstall: None. H. Wende: None. J. Siemens: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.01/FFF1

Topic: G.02. Motivation

Support: DA025634

Title: Effects of pharmacologically induced physiological need states on reward processing through intracranial self-stimulation.

Authors: *J. L. SEILER, S. M. CONWAY, M. F. ROITMAN;
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Abstract: Hunger potentiates goal-directed behavior for food and other rewards as well. The mechanisms by which hunger, and other physiological need states, act to motivate behavior remain incompletely understood. Recent work has demonstrated that hunger and thirst may motivate behavior through the generation of a negative affective state for which animals will actively work to alleviate. However, these conclusions are based on results from selective central manipulations to induce the need states and a very specific behavioral paradigm (e.g. real-time place preference). To test whether these results can generalize to other manipulations and behavioral paradigms, here, rats were implanted with stimulating electrodes positioned in the ventral tegmental area and an infusion cannula in the lateral ventricle. We then used intracranial self-stimulation (ICSS) to assess the affective state of rats after different pharmacological manipulations that induce feeding and drinking behavior. Rats were trained to lever press for brain stimulation reward (BSR) on a decreasing logarithmic scale of frequencies (termed 'pass') to determine the threshold at which BSR was no longer reinforcing. Training continued until rats maintained stable BSR threshold values for a minimum of 3 consecutive days. On test days, and after 3 passes, rats received central infusions of feeding- (ghrelin, neuropeptide Y (NPY), and 5-thio-d-glucose (5TG)) or drinking- (angiotensin II) inducing agents into the lateral ventricle followed immediately by an additional 6 passes. Data were then expressed as percent change in threshold relative to the average of the 3 pre-infusion passes. While NPY and 5TG caused increases in threshold (25.39 ± 11.16 and 29.43 ± 6.42 %, respectively), only ghrelin caused a significant increase (44.28 ± 11.9 %; $p<0.05$) relative to saline infusion (-1.34 ± 3.98 %). The direction of the effect is interpreted as an attenuation of BSR and is reminiscent of other pharmacological agents that induce negative affective states. Angiotensin II infusion had no effect on threshold (3.66 ± 6.2 %). These data are consistent with recent work suggesting that need states induce negative affect. However, different behavioral assays likely are differentially sensitive to changes in need states. Moreover, ICSS is sensitive to aspects of hunger but not thirst.

Disclosures: J.L. Seiler: None. S.M. Conway: None. M.F. Roitman: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.02/FFF2

Topic: G.02. Motivation

Support: AHA 16PRE2715005 (GNR)

Title: Mu-opioid receptor inhibition decreases voluntary wheel running in a dopamine-dependent manner rats in bred for high voluntary running

Authors: *G. RUEGSEGGER, F. BOOTH;
Univ. of Missouri, Columbia, MO

Abstract: The mesolimbic dopamine and opioid systems are postulated to influence the central control of physical activity motivation. To further understand the inherited neurobiological factors that contribute to physically active and inactive behaviors, we utilized selectively bred rats for high (HVR) or low (LVR) voluntary running behavior to examine: 1) inherent differences in mu-opioid receptor (Oprm1) expression and function in the nucleus accumbens (NAc), a brain region postulated to influence rewarding behaviors, 2) if wheel-running and food intake are differently influenced by intraperitoneal (i.p.) (5, 10, 20mg/kg) injection of the opioid receptor antagonist naltrexone in HVR and LVR rats, and 3) if dopamine is necessary for naltrexone-induced changes in running and feeding behavior in HVR rats. Experiment 1: Oprm1 mRNA and protein expression were increased in the NAc of HVR rats ($p< 0.05$). Application of the Oprm1 agonist D-Ala2, NMe-Phe4, Glyo5-enkephalin (DAMGO) to dissociated NAc neurons produced depolarizing responses in 44% (7/16) of HVR, and 25% (4/16) of LVR neurons. In depolarizing cells, the mean average response to DAMGO was greater in HVR (13.08 ± 2.50mV) than LVR neurons (4.90 ± 1.21mV, $p= 0.04$). Experiment 2: Naltrexone dose-dependently decreased wheel running and food intake in HVR, but not LVR, rats following i.p. ($p< 0.01$) injection. Experiment 3: i.p. naltrexone injection (20mg/kg) during the dark-cycle decreased Th mRNA in the ventral tegmental area (VTA) and Fos, Drd1, and Drd5 ($p< 0.05$) mRNAs in NAc shell of HVR, but not LVR, rats 2h post-injection. Experiment 4: lesion of dopaminergic neurons in the NAc with 6-hydroxydopamine (6-OHDA) ablated the decrease in running, but not food intake, observed in HVR rats following i.p. naltrexone administration. Collectively, these data suggest the higher levels of running observed in HVR rats, compared to LVR rats, are mediated, in part, by increased mesolimbic opioidergic signaling that requires downstream dopaminergic activity to influence voluntary running, but not food intake.

Disclosures: G. Ruegsegger: None. F. Booth: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.03/FFF3

Topic: G.02. Motivation

Support: FRM grant

AMIDEX grant

Title: Optogenetic modulation of the subthalamic nucleus impairs the motivation for food in rats.

Authors: *A. TIRAN-CAPPELLO[1], Y. PELLOUX[2], C. MONTANARI[1], M. DEGOULET[1], C. BAUNEZ[1];
[1]CNRS - Inst. De Neurosciences De La Timone, Marseille, France; [2]NIH, Natl. Inst. on Drug Abuse Intramural research program, Baltimore, MD

Abstract: Among the basal ganglia, the subthalamic nucleus (STN) is a well-known structure in the treatment of Parkinson's disease as it represents the major target for application of deep brain stimulation (DBS). Over the past few years, the increasing evidence that STN is critical for limbic and associative processes, lead to investigate its relevance as a potential target for the treatment of addiction and compulsive disorders. It has been established that the inactivation of the STN by various methods (lesion and high-frequency DBS) enhances the motivation for food and reduces the motivation for cocaine (for review Pelloux and Baunez, 2013). These opposite properties regarding natural reward and drugs of abuse appear to be a unique feature of the STN. In order to further characterize its role in the modulation of reward and motivation, we used optogenetic tools to modulate the activity of the rat STN. We injected either a fast kinetic Channelrhodopsin (ChetaTC) or an inhibitory Archaerhodopsin in the STN and tested whether light stimulation could alter food motivation in a similar manner to what was observed with electric DBS. For this purpose, we subjected the rats to FR5 and PR schedules, using sweet food pellets as reinforcer, to monitor their willingness to work despite a low or increasing amount of effort, respectively.
Interestingly, optogenetic stimulation using ChetaTC and electrical stimulation at high frequencies induce opposite behavioral responses, suggesting optogenetic stimulation enhances neuronal activity within the STN in contrast to electrical DBS.

Disclosures: A. Tiran-Cappello: None. Y. Pelloux: None. C. Montanari: None. M. Degoulet: None. C. Baunez: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.04/FFF4

Topic: G.02. Motivation

Support: Intramural Research Program, National Institute on Drug Abuse

Start-up funds provided by Loyola University Chicago

Title: Optogenetic excitation in the ventral tegmental area of glutamatergic or cholinergic inputs from the laterodorsal tegmental area drives reward

Authors: *S. STEIDL[1], H. WANG[2], M. ORDONEZ[1], A. CHAKRABORTI[2], S. ZHANG[2], M. MORALES[2];
[1]Psychology, Loyola Univ. Chicago, Chicago, IL; [2]Neural Networks Section, Integrative Neurosci. Res. Br., Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Converging evidence shows that dopamine neurons of the ventral tegmental area (VTA) receive both cholinergic and glutamatergic inputs from the laterodorsal tegmental nucleus (LDTg). By combining retrograde tract tracing, immunolabeling and in situ hybridization, we found that a higher proportion of LDTg-glutamatergic than LDTg-cholinergic neurons innervate the VTA in rats. We then compared the behavioral effects of VTA light stimulation of fibers from either LDTg-cholinergic or LDTg-glutamatergic neurons, using a 3-chamber place preference apparatus. For these studies, channelrhodopsin-2 (ChR2) was expressed in cholinergic neurons in the LDTg of ChAT::Cre mice (ChAT-ChR2 mice) or in glutamatergic neurons in the LDTg of VGluT2::Cre mice (VGluT2-ChR2 mice). ChAT-ChR2 mice, whose entry into a light-paired chamber resulted in VTA light stimulation of LDTg-cholinergic axons for the duration of their chamber stay, spent more time in this chamber. On a subsequent test day ChAT-ChR2 mice showed a conditioned place preference for the light-paired chamber in the absence of light. VGluT2-ChR2 mice, whose entry into a light-paired chamber resulted in VTA light stimulation of LDTg-glutamatergic axons for the duration of their chamber stay, entered this chamber significantly more times than a light-unpaired chamber, but remained in the light-paired chamber for short time periods and did not show a conditioned place preference. When entry into the light-paired chamber resulted in a single train of VTA light stimulation, VGluT2-ChR2 mice also entered the light-paired chamber significantly more times than the light-unpaired chamber, but spent approximately equal amounts of time in the two chambers. We suggest that excitation of LDTg-glutamatergic inputs to the VTA may be more important for the reinforcement of initial chamber entry while excitation of LDTg-cholinergic inputs to the VTA may be more important for the rewarding effects of chamber stays. We suggest that LDTg-cholinergic and LDTg-glutamatergic inputs to the VTA each contribute to the net rewarding effects of exciting LDTg axons in the VTA.

Disclosures: S. Steidl: None. H. Wang: None. M. Ordonez: None. A. Chakraborti: None. S. Zhang: None. M. Morales: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.05/FFF5

Topic: G.02. Motivation

Support: Intramural Funding

Title: Persistent conditioned place preference to rewarding aggression experience in male CD-1 mice

Authors: *C. HEINS[1], S. GOLDEN[1], S. RUSSO[2], Y. SHAHAM[1];
[1]Natl. Inst. on Drug Abuse, NIH, Baltimore, MD; [2]Dept. of Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York City, NY

Abstract: We recently developed a conditioned place preference (CPP) procedure, commonly used to study the rewarding effects of abused drugs, to demonstrate that dominant CD-1 male mice form CPP to a context previously associated with defeating subordinate C57 mice. Here we further characterized this aggression reward phenomenon. In Exp. 1, we screened CD-1 mice (10 sessions) that displayed a spectrum of unconditioned aggressive behavior toward younger subordinate C57 intruder mice. We then trained the CD-1 mice in the CPP procedure where one context was intruder-paired while the other context was not. We tested for aggression CPP 1 day after training. In Exp. 2, we used a similar procedure and tested the CD-1 mice for aggression CPP 1 day and 18 days after training. In Exp. 3, we trained the CD-1 mice tested in Exp. 2 to lever-press for palatable food and tested them for footshock punishment-induced suppression of food-reinforced responding. We found robust and persistent aggression CPP in CD-1 mice that either immediately attacked the C57 mice during all screening sessions (aggressive phenotype, ~50% of the sample) or mice that gradually developed the aggressive behavior during the screening phase (variable aggressive phenotype, ~25%). In contrast, CD-1 mice that did not attack the C57 mice during the screening days (non-aggressive phenotype, ~25%) developed conditioned aversion to the context previously paired with the C57 mouse. We also found that the aggressive phenotype did not predict resistance to punishment. Results demonstrate that like experience with abused drugs, aggression experience is highly rewarding and persistent.

Disclosures: C. Heins: None. S. Golden: None. S. Russo: None. Y. Shaham: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.06/FFF6

Topic: G.02. Motivation

Support: NIGMS 1FI2GM117583-01

Title: Addiction-like aggressive behavior in CD1 mice

Authors: *S. A. GOLDEN, R. C. HEINS, D.-T. LIN, Y. SHAHAM;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Background: Two core features of drug addiction are high motivation to seek the drug and high relapse rates during abstinence. These features have been studied for many years using rodent models of drug self-administration and relapse. Based on evidence in humans that some aggressive individuals are highly motivated to seek aggressive encounters, and that violent offenders show high relapse rates, we studied whether these 'addiction-like' features are observed in mouse models of aggression self-administration and relapse.
Methods: In Exp. 1, we trained adult male retired breeder CD-1 mice to lever-press to gain access to attack subordinate male C57BL6/J intruder mice on an ascending 2^{nd} order reinforcement schedule (1^{st} order lever: FR2, FR5, FR10, FR20 for 2 days each; 2^{nd} order lever: FR1) using a 15-block design (3 min ITI), and then tested them on a progressive ratio reinforcement schedule. In Exp. 2, we trained CD-1 mice for aggression self-administration on an FR1 reinforcement schedule for 9 days using a 20-block design. We then tested them for relapse to aggression seeking under extinction conditions (30-min tests) after 1 or 14 abstinence days.
Results: In Exp. 1, CD1 mice readily acquired operant aggression self-administration on the 2^{nd} order schedule and maintained aggression self-administration under the progressive ratio schedule. In Exp. 2, the mice were highly motivated to seek aggression reward in the relapse tests after 1 and 14 abstinence days.
Conclusion: Aggressive CD-1 mice show addiction-like behavior characterized by high motivation to perform an operant response to attack another mouse and persistent relapse vulnerability during abstinence.

Disclosures: S.A. Golden: None. R.C. Heins: None. D. Lin: None. Y. Shaham: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.07/FFF7

Topic: G.02. Motivation

Support: CONACyT 239403

Title: Restricted access to palatable food induces neuronal plasticity and behavioral changes similar to addiction

Authors: *G. MUÑOZ ESCOBAR, SR[1], S. D. GRACIA GONZALEZ[2], C. ESCOBAR BRIONES[2];
[1]Anatomía, Facultad de Medicina, Univ. Nacional Autonoma De México, Distrito Federal, Mexico; [2]Dept. de Anatomia, Univ. Nacional Autonoma de México, México, Mexico

Abstract: Restricted access to palatable food (PF) in satiated rats induces anticipatory activity, binge eating and increase of neuronal activity in corticolimbic areas, suggesting an addiction-like behavior. The aim of the present study was to determine whether PF access needs to be restricted in order to generate the addiction-like behavior and to identify behavioral and neuronal changes that provide evidence of an addiction process. To asses this hypothesis we compared: in rats with free access to regular chow and water: 1. Control group (CTRL)-no access to PF, 2. restricted daily access to PF (5 gr chocolate) with a fixed schedule (CH-ENTR), 3. PF (5 gr chocolate) delivered daily with a random schedule (CH-RDA), 4. PF ad libitum (CH-ADLIB): After 4 weeks we found that CH-ENT rats developed anticipatory activity and entrained body temperature, exhibiting a significant decrease of temperature in anticipation to chocolate access, similar to the changes observed in animal models of drug addiction. Such entraining effects were not observed in CH-RDA rats. In a test of effort, all groups of rats were exposed during 5 min to a sealed wire-mesh box containing a piece of chocolate and interaction with the box was monitored, during base line all rats were exposed to an empty box. We observed that both groups under restricted access to PF (CH-ENTR/CH-RDA) showed increased effort behaviors in order to obtain chocolate, indicating that restriction is enough to produce an increase in the motivation and effort to obtain PF. After 21 days of chocolate withdrawal, CH-ENTR and CH-RDA rats exhibited a binge episode, when allowed access to a bar of chocolate. Daily restricted access induced accumulation of αFosB protein and increased GFAP protein in the nucleus Accumbens, Basolateral amygdala and Prefrontal cortex. The magnitude of plastic changes correlated with the intensity of binge eating and effort behaviors to obtain chocolate. We conclude that daily restricted access to PF promotes the development of neuronal plasticity and behavioral changes associated with addiction-like behavior to food. Acknowledgments. This study was supported by

CONACyT 239403. Geovanni Muñoz Escobar received a scholarship by SNI Programa de apoyo a ayudantes de investigación nivel III o emérito.

Disclosures: G. Muñoz Escobar: None. S.D. Gracia Gonzalez: None. C. Escobar Briones: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.08/FFF8

Topic: G.02. Motivation

Support: MJ Murdock Charitable Trust

Title: Amygdalar, accumbal and ventral tegmental ghrelin and endocannabinoid signaling in alcohol reward

Authors: H. M. BAUMGARTNER, L. J. ZALLAR, S. ABTAHI, E. E. GARLING, *P. J. CURRIE;
Dept. Psychology, Reed Col., Portland, OR

Abstract: Extensive evidence now implicates the gastric peptide ghrelin in the regulation of homoestatic and energetic circuits of the hypothalamus. More recent work has investigated the role of ghrelin in alcohol reward. Indeed we have previously reported that systemic and ventral tegmental (VTA) ghrelin treatment significantly increases alcohol intake whereas hypothalamic injections do not reliably stimulate consumption. In the present report we confirm that both VTA and accumbal ghrelin microinjections increase alcohol intake at 2 and 6 h post-administration. In this paradigm rats were gradually habituated to 8% alcohol, and maintained at 8%, over the course of 12 weeks. In subsequent testing we investigated the effects of ghrelin, in separate groups of rats, with guide cannula aimed at either the central nucleus of the amygdala (CeA), the basolateral amygdala (BLA), the nucleus accumbens (NAcc), or the VTA. These groups of rats had limited prior alcohol exposure of no more than 4 weeks before treatment began. In all treatment groups, ghrelin did not stimulate alcohol consumption whereas food intake was increased in the VTA after ghrelin injection. Further, the CB1 inverse agonist, AM251, alone decreased alcohol and food consumption after injection into the CeA whereas VTA AM251 attenuated ghrelin's action. Overall our findings indicate that ghrelin is most effective in altering alcohol intake in Sprague Dawley rats with chronic prior alcohol exposure, and with stabilized alcohol intakes, whereas the peptide's role in early alcohol exposure does not appear to be robust. Additionally, our work provides some support for CeA endocannabinoid signaling in alcohol consummatory behavior.

Disclosures: H.M. Baumgartner: None. L.J. Zallar: None. S. Abtahi: None. E.E. Garling: None. P.J. Currie: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.09/FFF9

Topic: G.02. Motivation

Support: NIH Grant R01 MH 085642 to Stephen Gammie

NIMH Grant R01 MH 080225 to Lauren Riters

Title: Neurochemical phenotype of cannabinoid CB1 receptors in the ventral tegmental area of mice

Authors: *C. ZHAO[1], L. RITERS[1,2], S. GAMMIE[1,2];
[1]Zoology, Univ. of Wisconsin-Madison, Madison, WI; [2]Neurosci. Training Program, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Cannabinoid CB1 receptors modulate motivation, reward, and addiction-related behaviors via interacting with dopaminergic and GABAergic signaling pathways in the ventral tegmental area (VTA). However, neuroanatomical evidence in support of an intimate functional crosstalk between CB1 receptors and dopaminergic as well as GABAergic systems in the VTA remains fairly limited. In trying to define the expression of CB1 receptors and clarify the phenotype of CB1-containing neurons in the VTA of postpartum female mice, we used confocal microscopy and a double immunofluorescence labeling with tyramide signal amplification (TSA). Our results revealed that intensely labeled CB1-immunoreactive neurons were abundantly expressed throughout VTA, with the immunoreactivity predominantly located in the neuronal cell bodies. VTA CB1-immunoreactive neurons co-displayed tyrosine hydroxylase (TH, a marker for dopaminergic neurons) immunoreactivity within single cells and the levels of coexpression of CB1 and TH varied depending on the subdivision examined. Specifically, paranigral nucleus (PN, 70.5%) and parabrachial pigmented nucleus (PBP, 65.5%) displayed high rates of colocalization, while the lowest percentage of colocalization existed in the rostral linear raphe nucleus (RLi, 2.3%). As identified by markers for GABAergic neurons, the vast majority of CB1-expressing cells (82.8-88.4%) were GABA-immunoreactive in the VTA, regardless of the subdivisions tested. Further analysis with calcium-binding proteins as markers for GABAergic interneurons, demonstrated that a portion of CB1-expressing cells were local GABAergic interneurons as they displayed a distinct pattern of coexpression with calbindin D-

28K and calretinin. In conclusion, these data provide a neuroanatomical basis for the functional links for how endocannabinoid signaling interacts with both dopaminergic and GABAergic systems in the modulation of reward-related behaviors in the VTA.

Disclosures: C. Zhao: None. L. Riters: None. S. Gammie: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.10/FFF10

Topic: G.02. Motivation

Support: National Institute on Drug Abuse Intramural Research Program

Title: Glutamatergic and GABAergic projections from the Lateral Preoptic Area to the Lateral Habenula differentially drive motivated behavior.

Authors: *D. J. BARKER, J. MIRANDA BARRIENTOS, D. ROOT, H. WANG, S. ZHANG, M. MORALES;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The Lateral Habenula (LHb) has been identified as a brain region that plays important roles in addiction and depression. A primary input to the LHb comes from the Lateral Preoptic Area (LPO). Here, we characterized the anatomical and functional network between the LPO and the LHb. To identify specific LPO neurons targeting the LHb, the retrograde tracer fluorogold was injected into the LHb (1% via iontophoresis). Next, we established the phenotype of the fluorogold-tagged neurons by *in situ* hybridization detection of transcripts encoding either glutamic acid decarboxylase 65/67 mRNA (GAD, a marker of GABAergic neurons) or vesicular glutamate transporter 2 mRNA (VGluT2, a marker of glutamate neurons). Surprisingly, we found that within the total population of fluorogold-positive neurons only 15.9 ± 3.2% expressed GAD mRNA, and as many as 74.7 ± 3.2% expressed VGluT2 mRNA. To establish the functional role of LPO inputs to the LHb, we transfected glutamatergic or GABAergic neurons of the LPO with channelrhodopsin-2 and stimulated terminals in the LHb. *In vitro* stimulation terminals during whole cell recordings support anatomical data, showing weak inhibitory currents and stronger excitatory currents. Ongoing *in vivo* stimulation suggests that glutamatergic and GABAergic neurons both elicit motivated behavior, but of opposing valence. These findings provide evidence for a major excitatory projection and minor inhibitory projection from the lateral preoptic area to the lateral habenula that had been previously uncharacterized. Based on our current understanding of the habenula, this suggests that the LPO-LHb glutamatergic

pathway may be important for numerous psychopathologies including depression or the aversive effects of psychostimulants. Future work will focus on the participation of the LPO-LHb glutamatergic pathway in ongoing behavior in order to specifically investigate its participation in psychiatric illness.

Disclosures: D.J. Barker: None. J. Miranda Barrientos: None. D. Root: None. H. Wang: None. S. Zhang: None. M. Morales: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.11/FFF11

Topic: G.02. Motivation

Support: NIDA-IRP

Title: The lateral habenula has vesicles that accumulate either GABA or glutamate

Authors: *S. ZHANG[1], D. H. ROOT[2], D. J. BARKER[2], M. MORALES[2];
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Abstract: The laberal habenula (LHb) is involved in reward, aversion, addiction and depression through descending interactions with several structures, including the ventral tegmental area (VTA). Our recent studies have revealed that within the LHb a single axon terminal from VTA neurons co-transmit glutamate and GABA via a previously unrecognized combinatorial synaptic architecture. In this combinatorial synaptic architecture single axon terminals co-express vesicular glutamate transporter 2 (VGluT2) and vesicular GABA transporter (VGaT), establish both asymmetric and symmetric synapses, express AMPA receptors postsynaptic to asymmetric synapses, and express GABA-A receptors postsynaptic to symmetric synapses (Root et al., 2014). The spatial segregation of asymmetric and symmetric synapses within single mesohabenular axon terminals suggests that glutamate and GABA are released at different synapses and from distinct synaptic vesicles. However, it remains to be untested if dual glutamatergic-GABAergic neurons store glutamate (via VGluT2) and GABA (via VGaT) in the same vesicular populations. To determine whether glutamate and GABA are stored and released from the same or from a different pool of vesicles, we purified synaptic vesicles from the LHb of Sprague-Dawley rats (n = 60). By dual immunolabeling of these isolated synaptic vesicles and their analysis under electron microscopy, we detected two distinct pools of vesicles, a pool of vesicles containing VGluT2-immunolabeling and another pool containing VGaT-

immunolabeling. We found that from a total of 2,815 immunolabeled vesicles 58.92 ± 0.97% expressed VGluT2, 38.92 ± 0.81% expressed VGaT, and a very small proportion (2.15 ± 0.29%) appeared to co-label for VGluT2 and VGaT. The lack of co-existence of VGluT2 and VGaT at the vesicular level was next confirmed by the lack of co-immunoprecipitation of VGluT2 and VGaT from the isolated vesicles. Consistent with our ultrastructural findings, protein preparations of vesicles immunoprecipitated with antibodies to VGluT2 showed vesicular immunodetection of both VGluT2 and the vesicular marker synaptophysin, but not VGaT. Moreover, protein preparations of vesicles immunoprecipitated with antibodies to VGaT showed vesicular immunodetection of both VGaT and synaptophysin, but not VGluT2. Therefore, we conclude that within the LHb, accumulation of glutamate by VGluT2 occurs in vesicles different from those that accumulate GABA by VGaT.

Disclosures: S. Zhang: None. D.H. Root: None. D.J. Barker: None. M. Morales: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.12/FFF12

Topic: G.02. Motivation

Support: NIH R01 DA019921

Title: Corticosterone in the ventral hippocampus differentially affects accumbal dopamine release in drug-naive and amphetamine-withdrawn rats

Authors: *B. BRAY, M. A. WEBER, G. FORSTER;
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Abstract: Amphetamine withdrawal is associated with dysphoria and hypersensitivity to stress in humans and rats that can prompt relapse. This effect may be mediated by enhanced stress-induced corticosterone in the ventral hippocampus (vHipp), as is observed in rats. vHipp corticosterone can induce glutamate release and the vHipp sends glutamatergic projections to the nucleus accumbens shell (NAcS) to enhance dopamine output and reward salience. To directly test whether vHipp corticosterone can enhance accumbal dopamine output, a stress-relevant concentration of corticosterone (0.28 ng/µl) was infused into the vHipp of anesthetized adult male Sprague-Dawley drug-naive rats and in rats during amphetamine withdrawal. Resulting NAcS dopamine output was measured by *in vivo* chronoamperometry. In drug-naive rats, vHipp corticosterone *enhances* accumbal dopamine output, peaking 55 min post infusion. This suggests a novel mechanism by which stress exposure could enhance reward value to promote goal-

oriented behavior. Corticosterone activation of glucocorticoid receptors (GRs) is thought to be excitatory in the vHipp. Preliminary findings suggest the dopamine increase can be blocked by the GR antagonist mifepristone, implicating GRs as the underlying mechanism for the corticosterone-induced accumbal dopamine output. In amphetamine withdrawal, vHipp corticosterone immediately *reduces* accumbal dopamine output, peaking 20 min and then 80 min post-infusion. This suggests stress-induced corticosterone in the vHipp could contribute to dysphoric states that prompt drug-seeking and relapse during withdrawal. Furthermore, findings support an opponent-process theory of addiction, in which blunted dopamine reward responses and enhanced corticosterone stress responses contribute to negative reinforcement of drug-taking. GR expression is reduced in the vHipp during amphetamine withdrawal, but vHipp mineralocorticoid receptor (MR) expression is not altered. Thus, corticosterone activation of vHipp MRs may mediate the reduction in accumbal dopamine output during withdrawal, which is currently being tested. Overall, these findings suggest the neural corticosterone system could play an important role in driving positive stress coping mechanisms in healthy conditions, with dysregulation of this system potentially contributing to relapse during amphetamine withdrawal.

Disclosures: B. Bray: None. M.A. Weber: None. G. Forster: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.13/FFF13

Topic: G.02. Motivation

Support: New Zealand Marsden Fund

Title: Physical effort discounts ACC-VTA moment-by-moment estimates of future reward

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Abstract: A recent value-state theory of dopaminergic signalling suggests that activity in dopaminergic brain areas represents a temporally discounted, moment-by-moment estimate of future reward such that the signal ‘ramps up’ as the temporal distance to an expected reward decreases. We asked if changes in the response requirements to attain a fixed reward would alter the activity in brain areas that may signal this value-state. We hypothesized (i) that an increase in the physical effort required to attain a fixed reward will lead to reduced activity and (ii) that a decrease in the physical effort required to attain a fixed reward will lead to increases in activity.

We examined anterior cingulate cortex (ACC) and ventral tegmental area (VTA) local field potentials in freely-moving rats as they performed a cue-guided effort task in which a visual cue signalled either a non-effortful or an effortful upcoming trial. In the effortful condition, rats had to climb over a 30cm barrier to obtain a fixed reward. VTA theta (4-12 Hz) power decreased immediately prior to the barrier ($p < .005$), potentially indicating a discounting of the instantaneous value of the upcoming reward. In contrast, ACC and VTA theta power increased abruptly ($p = 5.8 \times 10\text{-}5$ and $p < .005$, respectively) when the rat entered the barrier-containing region of the maze on trials when the barrier was absent, potentially indicating an increase in the instantaneous reward value. Additionally, cross-frequency coupling of VTA gamma (30-80 Hz) amplitude to the phase of ACC theta varied consistently between the two task conditions: coupling was weaker when the barrier was present and greater when the barrier was absent ($p < 0.001$). Interestingly, elevations in power and coupling persisted in an extinction condition in which the barrier was never present but condition cues remained ($p < 0.001$). In contrast, neither ACC and VTA theta responses in the reward region were affected by the effort condition suggesting that the appraisal of the reward, as it is consumed, is unaffected by changing response requirements. We found no significant relationship between running speeds and theta modulations. Our results supported both of the hypotheses, which, suggests that ACC and VTA activity reflects an instantaneous value state: the value of the reward as it is perceived at that moment, which is discounted by physical effort.

Disclosures: T.W. Elston: None. D.K. Bilkey: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.14/FFF14

Topic: G.02. Motivation

Title: Effects of memantine administration on progressive ratio schedule performance of rats after binge eating induction

Authors: *W. ZEPEDA-RUIZ[1], D. N. VELAZQUEZ-MARTINEZ[2];
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Abstract: Binge eating is an eating disorder characterized by the overconsumption of food in brief periods, with high motivation to obtain and consume palatable food despite negative consequences. According to the Incentive Motivation Theory subjects with this disorder present alterations in the wanting component, implying they assign a larger incentive value to palatable

food than subjects without the disorder. Since there are similarities of binge eating to addictive behaviors, some drugs employed in the study of addictions reduce the symptoms of the disorder (e.g. induced a decrease in the size or frequency of binges episodes); one of these drugs is memantine (an uncompetitive N-methyl-D-aspartate (NMDA) antagonist). It has been reported that intracranial administration of memantine decreased food seeking behavior, reduced the compulsive-like eating of palatable food and decreased responding for palatable food. Although memantine reduced the consumption of palatable food it is not clear if this decrement was associated to changes in the incentive value of food. The objective was to evaluate the intracranial administration of memantine on the performance of the progressive ratio schedule using the Progressive Ratio Model proposed by Bradshaw et al. (Psychopharmacology, 2012, 222: 549-564); this model provides four parameters; k and T_0 are associated to post-reinforcement pause and δ to the motor component, but α reflects the specific activation (incentive value). When binge eating was inducted, there was an increase in the specific activation parameter with no changes in the motor parameter. After memantine administration, values of the parameter α (associated to incentive value) decreased. Our results are in accordance to those studies that reported alterations in the wanting component.

Disclosures: W. Zepeda-Ruiz: None. D.N. Velazquez-Martinez: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.15/FFF15

Topic: G.02. Motivation

Support: NIH DA016285-4

Title: Examination of the persistence of the anti-craving effects of acute environmental enrichment in rats

Authors: J. HYDE, E. GLUECK, K. NORTH, D. GINDER, O. DEMCHENKO, J. SULC, K. JIGANTI, *J. W. GRIMM;
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Abstract: Background: Exposure to acute (17h) environmental enrichment (EE) reduces sucrose taking and seeking in rats. Our study examined the persistence of this effect. Following 10 days of sucrose self-administration, male rats responded for either sucrose or a tone + light sucrose-paired cue for one week following acute EE. The first day of testing began either immediately following EE, or one day later after rats had been returned to their regular, single-housed

condition (home cage). This “delayed” condition allowed us to identify whether the anti-craving effect of EE would last beyond the immediate switch from the EE condition to the operant conditioning chamber. Methods: Male Long-Evans rats underwent 10 days (2h/day) of sucrose self-administration before entering acute EE. EE consisted of 3 rats housed in a large, multi-level cage with novel stimuli (toys). After EE (or remaining in the home cage as Controls), rats underwent one week of 1h/day test sessions, responding for either sucrose or the sucrose-paired cue. The persistence of the effects of EE on these measures of sucrose craving was examined by having some rats return to the home cage for one day following EE, prior to the first day of testing (delayed condition). Results: EE-exposed rats responded for significantly less sucrose the two days following EE, compared to Controls. This effect was verified in the delayed condition where responding for sucrose was less than Controls even though EE rats had spent 24h in their home cage prior to testing in the operant conditioning chamber. This persistent effect of EE was not observed in rats responding for the sucrose-paired cue. Specifically, although rats responded significantly less for the sucrose-paired cue compared to Controls immediately following EE, there was no difference between EE and Control rats in the delayed condition. Discussion: Our data indicate that the anti-craving effect of acute EE persists for at least 2 days beyond the immediate transition from EE to the self-administration context. However, this persisting effect only occurs with rats responding for sucrose itself, as opposed to responding for a sucrose-paired cue. We have previously hypothesized that the anti-craving effect of EE is due to rats perceiving the reinforcing effects of sucrose and the sucrose-paired cue as being reduced in the self-administration context following EE. For rats responding for the sucrose-paired cue this negative contrast appears to need to occur immediately prior to testing. Further research is required to evaluate this hypothesis in more detail, as well as to determine how the persisting effect with sucrose self-administration is mediated.

Disclosures: J. Hyde: None. E. Glueck: None. K. North: None. D. Ginder: None. O. Demchenko: None. J. Sulc: None. K. Jiganti: None. J.W. Grimm: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.16/FFF16

Topic: G.02. Motivation

Support: University of Missouri Research Board

Title: Sex determines effect of exercise on diet preference: role of opioid reward system and microbiome diversity

Authors: *J. R. LEE[1,2], J. MUCKERMAN[1], A. WRIGHT[1], D. DAVIS[3], A. ERICSSON[3], C. HAGAN[3], T. CHILDS[4], F. BOOTH[4], M. WILL[5];
[2]Interdisciplinary Neurosci. Program, [3]Dept. of Vet. Pathobiology, [4]Dept. of Biomed. Sci., [5]Psychological Sci., [1]Univ. of Missouri, Columbia, MO

Abstract: Previous studies suggest an interaction between physical activity and diet preference. However, this relationship has not been well characterized for potential sex differences that may occur. In order to examine the influence of sex on the relationship of physical activity and diet selection, a long-term diet preference test was employed. At 49 days of age, 20 male and 20 female Wistar rats were divided into either sedentary or wheel running conditions for a one week acclimation period. Standard lab chow was then replaced with three unique diets for a four week diet preference test. A high-fat, high-sucrose, and high-corn starch diet were presented simultaneously in the home cage. Sedentary or wheel running conditions remained throughout the duration on the experiment. Body weight, running distance, and intake of each diet were measured daily. At the conclusion of the four week diet diet preference test, animals were sacrificed and microbiome samples and brain tissue from 3 brain regions was collected. Fecal samples were collected before and after the diet preference phase to determine microbiome diversity. Results indicate sex dependent interactions between exercise and both behavioral and physiological measures. Females in both wheel and sedentary conditions preferred the high-fat diet. Males in the sedentary group also preferred the high-fat diet, while the males in the wheel condition consumed significantly less high-fat diet than the other groups and equally preferred all three diets. The influence of physical activity on physiological measurements also revealed sex differences. A significant sex by exercise condition interaction was observed for mRNA expression in both the mu opioid receptor, OPRM1, and preproenkephalin, PENK in the ventral striatum and neuropeptide Y, NPY in the hypothalamus. Characterization of gut microbiota diversity during the pre-diet and post-diet samples for all groups suggest a sex-dependent interaction between physical activity and diet preference. The significant sex differences in response to exercise expressed by both behavioral and physiological measures suggest a potential motivational or metabolic difference between males and females and highlight a necessity for further exploration between male and female response to exercise.

Disclosures: J.R. Lee: None. J. Muckerman: None. A. Wright: None. D. Davis: None. A. Ericsson: None. C. Hagan: None. T. Childs: None. F. Booth: None. M. Will: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.17/FFF17

Topic: G.02. Motivation

Support: NIH/NINDS R01 NS025529

CHDI Foundation Grant A-5552

Title: Striosomes may control dopamine activity via disynaptic projections to macaque lateral habenula

Authors: *S. HONG, S. AMEMORI, K.-I. AMEMORI, A. M. GRAYBIEL;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: The lateral habenula (LHb) modulates motivation and behavior via its influence on the dopamine (DA) and serotonin (5-HT) systems (e.g., Hong et al., 2011). In non-human primates, the LHb receives a major input from the border neurons in the globus pallidus internal segment (GPb; Hong et al., 2008). A leading hypothesis is that the GPb cells might receive input from neurons in the striosomal compartment of the striatum (Graybiel and Ragsdale, 1978), given evidence in rodents (Rajakumar et al. 1993). Definitive evidence of such a circuit in the primate brain is still missing. To fill this major gap, in two rhesus monkeys, we placed microelectrodes in the striatum for microstimulation and placed a second microelectrode in the LHb to record the responses to striatal microstimulation. We mapped different microstimulation sites in the striatum (to which we applied 3 pulses, 33 μA each, at 300 Hz), and determined whether the microstimulation affected multi-unit activity in the LHb, indicating functional connectivity. We found that, at some sites, stimulation of the striatum induced significant inhibitions or excitations of LHb activity. These effective striatal sites were distributed unevenly along the depth of the stimulating electrode's track; sometimes a brisk excitement or inhibition was induced in the LHb, but at other depths, no LHb response occurred. We made electrolytic marking lesions when we found an effective site, and the brains of the two monkeys were then processed for histological examination using KChIP1 (to demonstrate striosomes), glial fibrillary acidic protein (GFAP, to allow detection of the penetration track) and Prussian blue (to detect iron deposition at marking lesions). Our analyses demonstrated that 9 out of the total of 11 effective sites were in or immediately bordering striosomes, one was in the matrix compartment, and one was in a fiber bundle. The rather long orthodromic latency of ~20 ms for LHb responses suggested disynaptic connectivity, contrasting with the ~5 ms latency for GPb cell stimulation. Our results strongly suggest that neurons in the striosomal compartment of the striatum controls LHb activity via its connection to the GPb. This work, still in progress, has the potential to identify in non-human primates a crucial pathway for the control of mood and motivation.

Disclosures: S. Hong: None. S. Amemori: None. K. Amemori: None. A.M. Graybiel: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.18/FFF18

Topic: G.02. Motivation

Support: MEXT KAKENHI 23120007

Title: The striatal striosome compartment encodes the value of sensory stimulus

Authors: *T. YOSHIZAWA, M. ITO, K. DOYA;
Okinawa Inst. of Sci. and Technol., Onna-Son, Okinawa, Japan

Abstract: The striatum consists of the striosome (patch) and matrix compartments. Although the striatum is involved in value-based learning, the specific roles of these compartments has not been clear because the mosaic structure makes it difficult to identify the compartments during electrode recording. We previously hypothesized that the striosome compartment represents the state value, whereas the matrix represents the action value in reinforcement learning (Doya, 2000). In this study, to test whether the striosome neurons represent value information or not, we conducted endoscopic deep-brain *in vivo* calcium imaging (nVistaHD, Inscopix) of transgenic mice with selective GCaMP6s expression in the striosome neurons (Gerfen et al., 2013).
Mice were classically conditioned with four odor cues predicting reward (water 2 or 4 μl), aversive stimuli (air puff), or none. Each trial began with a conditional stimulus (CS; odor, 2 sec.), followed by a delay period (0.5 sec.) and an unconditional stimulus (water/air puff/none). Within the first 7 days (100 trials/day), licking behavior was formed during the CS and delay period for the odor stimuli associated with rewards. This result indicates that mice predicted upcoming reward from odor stimuli. We recorded the activity of the same striosome neurons over 15 days and compared the activities in early learning stage with late learning stage. In the late stage (the odor-reward associations were well learned), some neurons showed activities correlated with the reward amounts during the CS and delay periods, whereas some other neurons showed air-puff predictive activities. To confirm that these activities encode the value information, we applied a regression analysis. The activities of 67% of striosome neurons significantly correlated with the reward values predicted by the different odors. This proportion was significantly larger than in the early stage (39%, $p=0.0145$). The absolute value of regression coefficients was also larger than in the early stage (Median; 0.020 vs. 0.043, $p=0.0073$). While 43% of neurons showed significantly correlated activities with the presentation of the odor associated with air puff in the late stage, there were no significant differences in the proportion and regression coefficients between the early and late stages.
The existence of stirosome neurons coding value information was revealed for the first time using selective optical recording. The result suggests that one of the roles of striosome neurons is

to associate sensory states with predicted reward, in consistence with our hypothesis about value coding in the striosome compartment.

Disclosures: T. Yoshizawa: None. M. Ito: None. K. Doya: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.19/FFF19

Topic: G.02. Motivation

Title: A within-subjects operant model to examine strength theory and ego depletion in rats: effects of glucose administration

Authors: *A. L. LASKE, J. E. MEYERS-MANOR, 55105, E. P. WIERTELAK;
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Abstract: Ego depletion research has viewed self-control as a limited mental resource that may be used up. According to proponents of this theory, also referred to as Strength Theory, when this resource is in short supply, self-control may become impaired. The prefrontal cortex is believed to house circuitry that acts as a central executive cognitive system, responsible for organizing the processes that enable self-control. Glucose, as the main source of energy for brain functioning, thus figures strongly in replenishing executive processes. Evidence suggests that replenishing glucose may reverse ego depletion in humans and dogs. The current study was designed to test the possibility of glucose reversal of ego depletion in rats, in order to shed light on the mechanisms of glucose utilization in the brain and investigate further the possibility of ego depletion in modulating self-control.Using an operant conditioning model, eight female Sprague-Dawley rats completed two weeks of baseline training. Baseline training involved shaping the rats to two levers: a long-delay lever and a short-delay lever. Self-control was measured by the percentage of time that subjects chose the long-delay lever over the short-delay lever. A within subject designed was used for testing. White noise bursts were blasted for 10 seconds per minute for 20 minutes in order to establish ego-depletion. Administration of 0.5 mL of glucose was used for depletion reversal.Results indicate that all subjects showed a decrement in their responding compared to baseline following the white noise bursts. This decrement appeared to be reversed by glucose administration following ego depletion. Analysis revealed that subjects in the glucose condition had significantly higher percentages of long-delay reward presses than those rats tested in the water only condition. The current study provides evidence that glucose plays a crucial role in reversing ego depletion as defined here, and improving self-control in rats. This study may help inform research examining the possibility of ego depletion

and glucose in humans, here specifically, the controversy surrounding putative mechanisms of glucose utilization in this effect.

Disclosures: A.L. Laske: None. J.E. Meyers-Manor: None. E.P. Wiertelak: None.

Poster

258. Reward Mechanisms

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 258.20/FFF20

Topic: G.02. Motivation

Support: T32 DA007262

Title: Fos expression after exposure to an effort discounting procedure

Authors: *E. E. HANSON[1], S. H. MITCHELL[2];
[1]Neurosci., [2]Behavorial Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Objective: Apathy is a hallmark of numerous psychiatric disorders, and is thought to impede the development of more healthful behavior patterns. However, our understanding of the processes regulating instances of effort-aversion is incomplete. This project addressed this research gap by measuring markers of neuronal activity to determine the contribution of different brain areas to the decision to work for a food reward. Methods: 48 Long-Evans rats participated. Rats were divided into four groups: a food-restricted experimental group; a food-restricted, yoked-control group; a food-restricted control group; and an unrestricted food control group. Rats in the food-restricted experimental group were trained on an effort-discounting task based upon a modified adjusting amount procedure (Richards et al. 1997, J Exp Anal Behav 67: 353). Rats chose between 150-µL of sucrose solution available following completion of an effortful response, and a smaller sucrose reward available following a negligible-effort response. Rats were tested on five effort levels (0.01, 0.15, 0.35, 0.6, 0.9 Ns), varied daily, where the effort was determined from the force integrated over time. Food-restricted, yoked-control rats were placed in the operant chambers and received sucrose solution on a schedule that was yoked to animals within the food-restricted experimental group. Food-restricted and unrestricted control rats were placed in the operant chambers but did not complete the task. Behavioral data were used to characterize individual differences in the willingness to engage in effortful responding by constructing effort discounting curves and calculating the area under the curve. Prior to being euthanized on their last session, rats experienced 5-10 sessions at the 0.6 Ns effort condition. Brain tissue was then stained for cFos, a habituating marker of neural activation, and FosB, another member of the Fos family of transcription factors. Numbers of Fos-positive cells were

counted visually, and compared between groups for each region. Results: For the trained rats, the subjective value of the 150-μL reward decreased hyperbolically as a function of increasing effort. When rats were tested at a single effort prior to their last session, the subjective value of the 150-μL sucrose was stable across sessions. Preliminary cell-counting suggests an enrichment of cFos-positive cells in the anterior cingulate cortex and accumbens, for food-restricted, experimental animals over controls. Conclusions: These results are consistent with the view that the striatum is involved with the choice to perform effort for a food reward, rather than the value of the reinforcer itself.

Disclosures: E.E. Hanson: None. S.H. Mitchell: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.01/FFF21

Topic: G.02. Motivation

Support: NSERC 402642

Title: Role of the prelimbic prefrontal cortex in operant responding to appetitive, aversive and conflicting cues

Authors: *L. M. HAMEL, B. CAVDAROGLU, R. ITO;
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Abstract: The prelimbic prefrontal cortex (PFC) has been associated with the learning and expression of motivated behaviour elicited by reward and fear-related cues. However, the specific effects of previous manipulations in this region have varied, likely based in part on the nature of the cues, response demands, and outcome contingencies. The current study aimed to elucidate the role of the prelimbic PFC in a set of experiments where analogous tasks of appetitive and aversive cued responding were performed under extinction and non-extinction conditions. The task additionally introduced an opportunity for responding under conditions of conflict where both positively and negatively valenced signals are presented simultaneously. Rats were trained to perform an operant task wherein different discrete auditory and light stimuli were associated with either positively valenced (sucrose pellet reward), negatively valenced (foot-shock) or neutral outcomes (no programmed consequences) upon lever pressing on a variable ratio schedule. Prior to behavioural testing, animals were intracerebrally infused in the prelimbic prefrontal cortex with a combination of the $GABA_A$ and $GABA_B$ receptor agonists muscimol/baclofen or a saline vehicle control in a within-subjects design. During a test under

extinction conditions, the cues were presented in alternating trials signifying either the positive or negative contingencies, or with a superimposition of both the positively and negatively valenced conflicting cues. The same test of cue-induced responding was additionally performed under non-extinction conditions where the animals received either the reward, foot-shock, or a combination thereof. The results indicated that under extinction conditions, inactivated animals performed fewer lever presses in response to the reward cue, as instantiated through a faster rate of extinction. Under non-extinction conditions, no difference was observed between groups in responding for reward; however the inactivated animals pressed more than controls for the negative outcome, and additionally responded more in the conflicting valenced situation, suggesting a diminished aversion. The results reveal contrasting effects of prelimbic PFC inactivation under extinction and non-extinction conditions in cued operant responding, and support a role for this region in the evaluation and integration of cues representing positive and negative valence.

Disclosures: L.M. Hamel: None. B. Cavdaroglu: None. R. Ito: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.02/FFF22

Topic: G.02. Motivation

Support: NSERC Grant 402642

Title: Cocaine pre-exposure enhances negative incentive motivation in a novel mixed-valence approach-avoidance task

Authors: *D. NGUYEN, Y. NESARAJAH, S. ERB, R. ITO;
Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Approach and avoidance processes are critical components of drug addiction, wherein the addict is compelled by both desire and avoidance for the drug. Evidence suggest that prior exposure to drugs such as cocaine induces long-lasting alterations in brain systems that are fundamentally involved in processing reward and aversion. Indeed, work in our laboratory have previously shown that cocaine pre-exposure dysregulates approach-avoidance conflict resolution in behaving rats tested in paradigms of motivational conflict, biasing decision-making towards appetitive sucrose reward seeking over passive avoidance of foot shock punishment. However, to more completely model the aesthetics of addiction where the addict must actively seek out the drug in order to avoid a negative state of withdrawal, there must be an investigation of avoidance

motivation wherein avoidance of an aversive stimulus requires an active instrumental response. The effects of cocaine pre-exposure on active avoidance processing in animals under a state of motivational conflict is currently unknown. In the present study, male Long Evans rats were subjected to a cocaine sensitization regimen (15-30mg/kg, IP, 7d), prior to beginning of behavioral training. Rats were then trained in a novel modified shuttle box paradigm consisting of two separate but conjoined arms. Rats were trained to lever press for sucrose reward in the presence of a light CS under a specified schedule of reinforcement. Rats were then trained in the other arm to lever press to actively avoid impending foot shock punishment upon presentation of a tone CS. Following successful conditioning, rats were subjected to a forced-choice conflict test in which the reward and avoidance CS were presented simultaneously. Here, over multiple trials, rats could choose to respond on the reward lever for sucrose reinforcement with the consequence of unavoidable and inescapable foot shock, or to respond on the avoidance lever to actively avoid foot shock with the consequence of not receiving sucrose reward. Our preliminary data reveal that cocaine pre-exposed rats prefer the active avoidance response over sucrose reward, suggesting a drug-induced shift in balance of motivational processing.

Disclosures: D. Nguyen: None. Y. Nesarajah: None. S. Erb: None. R. Ito: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.03/FFF23

Topic: G.02. Motivation

Support: NSERC Grant 402642

Title: Differential effects of ventral hippocampal CA1 and CA3 inactivation on learned approach-avoidance decision making in rats

Authors: *A. SCHUMACHER, F. VILLARUEL, R. ITO;
Psychology, Univ. of Toronto, Scarborough, ON, Canada

Abstract: The simultaneous presence of stimuli with opposing valences evokes an approach-avoidance conflict, which can result in the emission of maladaptive responses. The hippocampus is thought to be involved in the resolution of such conflict by exaggerating the value of negative outcomes and increasing the tendency to avoid. Previous work from our laboratory has implicated the ventral, but not dorsal hippocampus in mediating decision making under high approach-avoidance conflict. The present study sought to further investigate the role of different subfields within the ventral hippocampus (vHPC) in learned approach-avoidance conflict. Male

Long Evans rats were trained to associate different non-spatial cues with positive, negative and neutral outcomes in three arms of a radial maze. Following successful cue valence acquisition, rats underwent transient inactivation of the CA1 or CA3 subfields of the vHPC using a cocktail of $GABA_A$ and $GABA_B$ receptor agonists, immediately before a conflict test in which they were presented with a superimposition of positive and negative cues in one arm (conflict cue), and neutral cue in another arm. In addition, all rats underwent tests of anxiety and novelty preference. We found that inactivation of the CA1 subfield led to marked avoidance of the conflict cue, while inactivation of the CA3 subfield resulted in the opposite pattern of behavior, with significant preference for the conflict cue. This was despite the fact that both CA1- and CA3-inactivated rats showed reduced anxiety and impaired novelty preference compared to control rats. Thus, our findings suggest that the CA1 and CA3 region of the vHPC subserve distinct and opposing behaviors in the face of approach-avoidance conflict.

Disclosures: A. Schumacher: None. F. Villaruel: None. R. Ito: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.04/FFF24

Topic: G.02. Motivation

Support: NSERC Discovery Grant 402642

Title: Repeated ketamine exposure impairs the acquisition of negative incentive salience in rats

Authors: *R. ITO, A. SCHUMACHER;
Psychology, Univ. of Toronto, Scarborough, ON, Canada

Abstract: Repeated exposure to sub-anesthetic doses of ketamine in rats has previously been shown to induce cognitive deficits, as well as behavioural changes akin to the positive and negative symptoms of schizophrenia, giving much face validity to the use of ketamine administration as a pharmacological model of schizophrenia. However, close examination of the preclinical literature reveals that there is, at present, no standard dosing regimen for subchronic ketamine administration, throwing into question the translational value of previous findings. Indeed, previous work from our laboratory have shown that two widely used regimens of repeated ketamine administration can induce differential alterations in novelty processing, sucrose preference and working memory, giving rise to the notion that different dosing regimens may elicit differential neural and neurochemical adaptations. This study therefore sought to further characterize the behavioral and neurochemical effects of two different ketamine pre-

treatment regimens, focusing on the acquisition of positive and negative incentive learning. Male Long Evans rats received either 5, or 14 intra-peritoneal injections of 30mg/kg ketamine or saline across 5 or 7 days respectively. After a withdrawal period of 10 days, rats were trained to associate different visuo-tactile cues with different outcomes (positive, negative and neutral) in three arms of a radial maze, and the rate of learning assessed with conditioned cue preference/avoidance tests. Rats then underwent a locomotor test involving the administration of a challenge dose of amphetamine (AMPH). One day after the locomotor test, neurochemical analyses of the striatum were conducted on the brains extracted from a subset of animals from each treatment group. We found that the low-dose (cumulative) ketamine pre-treatment regimen elicited selective impairment in the acquisition of conditioned cue avoidance, but not conditioned cue preference. In contrast, the high-dose ketamine pre-treatment led to a transient enhancement in conditioned cue preference, compared to the saline pre-treated group performance. Furthermore, the low-dose ketamine pre-treated rats showed *enhanced* locomotor activity following an AMPH challenge, indicative of locomotor sensitizaton, while the high-dose ketamine pre-treated rats showed *decreased* locomotor activity, compared to saline rats. These data demonstrate that different regimens of repeated ketamine administration can induce differential effects on incentive learning, which may depend on dissociable adaptations occurring in the midbrain dopamine systems.

Disclosures: R. Ito: None. A. Schumacher: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.05/FFF25

Topic: G.02. Motivation

Support: NSERC 402642

Title: Opposing roles of infralimbic and prelimbic cortices in contextual biconditional discrimination memory retrieval

Authors: *S. RIAZ, D. KHAN, P. PUVEENDRAKUMARAN, R. ITO;
Univ. of Toronto, Scarborough, ON, Canada

Abstract: The medial prefrontal cortex (mPFC) has been widely implicated in the contextual control of appetitive and aversive conditioning. Furthermore, the two subdomains within the mPFC, the infralimbic (IL) and prelimbic (PL), have been shown to differentially control context-dependent behaviour. Thus, while activity in the PL cortex has been shown to promote

the expression of conditioned fear and drug seeking behavior, IL activation has been associated with the extinction and inhibition of conditioned fear and drug seeking. However, the potential roles of the PL and IL subregions in contextually driven appetitively motivated behaviours with natural rewards remain underexplored. The present study sought to further examine the functional dichotomy of the mPFC in contextual control over appetitively motivated behaviour, employing a contextual biconditional discrimination (CBD) task in combination with temporary pharmacological inactivation of the PL and IL. To this end, adult male Long Evans rats received CBD training involving the sequential presentation of two distinct auditory stimuli (X and Y) in two different contexts (A and B; differentiated by size and odor). Rats were trained to nose poke in response to the presentation of one stimulus (e.g. X+) for the delivery of sucrose reward, and to withhold a nose poke response to the presentation of the second stimulus (e.g. Y-) in a context-specific manner (e.g. AX+, AY-; BX-, BY+). Following successful acquisition, rats received an intracerebral microinjection of a cocktail of GABAR agonists (Muscimol/Baclofen) or saline into the PL or the IL cortices, prior to undergoing a CBD training session and a CBD extinction test. While PL inactivation resulted in robust impairment in CBD memory retrieval, preliminary data suggest that IL inactivation enhanced discrimination memory. Taken together, these data indicate that the PL and IL have functionally distinct, and potentially opposite roles in the processing of appetitively motivated contextual memories.

Disclosures: S. Riaz: None. D. Khan: None. P. Puveendrakumaran: None. R. Ito: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.06/FFF26

Topic: G.02. Motivation

Support: NSF 1121147 to ML

Klarman Family Foundation to ML

Title: Chemogenetic perturbations of the medial prefrontal cortex and the control of consummatory behavior

Authors: *T. K. SWANSON[1], L. M. AMARANTE[2], M. C. CLASEN[2], I. TERWINDT[2], M. I. KONAKLIEVA[2], R. KUSKOVSKY[2], M. LAUBACH[2];
[1]Biol., [2]American Univ., Washington, DC

Abstract: Previous studies from our lab have established that the medial prefrontal cortex (mPFC) contains neurons that vary with the initiation of feeding bouts (Horst and Laubach, 2013) and that reversible inactivations of the rostral part of this region lead to fragmented licking in incentive contrast procedures (Parent et al., 2015). Here, we examined the role of this cortical region with a special focus on its connections with lateral hypothalamic regions known to be involved in feeding and homeostasis. Several experiments were conducted using chemogenetics (using the glutamate targeting CaMKIIa promoter) and pharmacological manipulations of the mPFC. First, bidirectional perturbations using the CNO-based Gq and Salvinorin B-based KOR DREADDs had opposing effects in a progressive ratio licking task, with the Gq DREADD increasing breakpoints and the KOR DREADD decreasing breakpoints. These effects were not observed in control rats that were tested with CNO and Salvinorin-B but without the active chemogenetic AAVs infused into the mPFC. Second, a group of rats was tested with the CNO-based Gi DREADD during a continuous access procedure (5% sucrose or maltodextrin). Some of these animals were implanted with guide cannula to compare effects of CNO and muscimol. Inactivation of the mPFC altered measures of consumption such as the rate of intake and duration of licking bouts. CNO and musicmol were comparable on this simple intake behavior, suggesting that effects of muscimol act on glutamate projection neurons within the mPFC. Analysis of anterograde projections (using antibodies against reporter proteins such as HA and co-infused biotinylated dextrans) from these animals is ongoing and will reveal if variability across rats is associated with different projections from the mPFC to feeding centers in the hypothalamus and reward centers in places such as the nucleus accumbens. Third, retrograde tracing (using cholera toxin subunit B) was done in the area of the lateral hypothalamus, and reveals convergent projections from the mPFC as well as the agranular insular cortex, orbitofrontal cortex, ventral striatum, and ventrolateral periacqueductal gray. Together, these studies suggest that a distributed network of brain area involving the rostral mPFC and the lateral hypothalamus mediates control over consummatory behavior.

Disclosures: T.K. Swanson: None. L.M. Amarante: None. M.C. Clasen: None. I. Terwindt: None. M.I. Konaklieva: None. R. Kuskovsky: None. M. Laubach: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.07/GGG1

Topic: G.02. Motivation

Support: NSF 1121147 to ML

Klarman Family Foundation to ML

NSF GRFP to LMA

Title: Functional interactions among medial, orbital, and motor cortices in the control of consummatory actions

Authors: *L. M. AMARANTE[1], M. S. CAETANO[2], N. K. HORST[3], M. LAUBACH[1]; [1]American Univ., Washington, DC; [2]UFABC, Sao Paolo, Brazil; [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Previous studies from our lab have shown that incentive contrast effects with liquid sucrose rewards are sensitive to both reversible inactivation (Parent et al., 2015a) and modulations of cholinergic and ghrelinergic signaling (Parent et al., 2015b) of the rat rostral medial prefrontal cortex (mPFC), i.e. rostral prelimbic and medial orbital areas. Multi-electrode recordings were made across three cortical areas (orbital, medial, and motor) as rats performed in a licking procedure. Time-frequency analysis (newtimf.m from EEGLab) of field potentials and population spike activity recorded in mPFC, and in the orbitofrontal and oral motor cortices, revealed enhanced event-related spectral power and inter-trial coherence (ITC) of phase angles at the licking frequency were synchronized to individual licks as well as the initiation and termination of licking bouts. ITC was enhanced around individual licks and when rats initiated bouts for the higher value fluid. Circular correlation analysis found no clear effect of bout duration or inter-bout interval on ITC. To probe the role of ongoing phasic activity in the control of licking behavior, electrical and optogenetic (C1V1) stimulation was delivered to sites in the medial frontal and oral motor cortices. Lick-entrained stimulation lead to immediate cessations in licking. These findings suggest that lick-entrained neuronal activity selectively encodes the relative value of ingested solutions and controls ongoing consummatory actions. As our recordings were made across multiple frontal areas, we examined functional interactions among the areas using dimensionality reduction methods (e.g. fastICA, Fourier ICA, milca) as well as an algorithm for calculating mutual information in time-series data (Kraskov et al., 2004). We found consistent evidence for a clustering of recording sites by cortical region. Data from each rat showed three broadly mapped independent components that were strictly partitioned across the cortical areas. Based on these findings, we applied an algorithm for directional coherence analysis (Neurospec 2.1) and found evidence for directional influences of frontal agranular cortex (in a region known to contain the oral motor cortex) driving signals in the medial and orbital cortices at two frequencies (8-12 Hz and ~20 Hz). The 20 Hz component was suppressed during periods of active licking. Orbital fields also exhibited directional influences on the medial fields that were broadly distributed around the licking frequency. Our findings suggest that the oral motor cortex coordinates rhythmic activity in the medial and orbital frontal cortices to enable monitoring of reward value.

Disclosures: L.M. Amarante: None. M.S. Caetano: None. N.K. Horst: None. M. Laubach: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.08/GGG2

Topic: G.02. Motivation

Title: Cellular resolution calcium imaging and optogenetic excitation reveal a role for IL to NAc projection neurons in encoding of spatial information during cocaine-seeking

Authors: *C. M. CAMERON, J. PILLOW, I. B. WITTEN;
Psychology, Princeton Univ., Princeton, NJ

Abstract: A major obstacle in the treatment of cocaine addiction is the high propensity of addicts to relapse even after extended drug-free periods (withdrawal). Previous work has implicated the glutamatergic projection from the infralimbic cortex (IL) to the nucleus accumbens (NAc) in drug-seeking following cocaine self-administration and withdrawal. However, it is not clear what information is carried in this projection to support drug-seeking behavior. It is also unknown whether activity in this circuit functions to modulate ongoing behavior, or if it provides feedback to shape future behavior. In order to better understand what information is being encoded by IL→NAc projecting neurons during extinction of cocaine-seeking, we combined cellular resolution calcium imaging with a retrograde Cre virus and a Cre-dependent GCaMP to record projection-defined neurons in awake and behaving rats after 1 (n=125 neurons, 5 rats; E1) or 15 (n=61 neurons, 4 rats; E15) days of cocaine withdrawal. Preliminary analysis indicates that a small portion of IL→NAc neurons (23% E1, 22% E15) display time-locked activity to active lever responding, with most of these neurons increasing firing after the lever press. However, a much larger portion (46% E1, 80% E15) of neurons exhibited significant spatial tuning within the cocaine-associated context. We next used optogenetics to activate the same projection in rats. Channelrhodopsin (n=13) or YFP (n=4) was expressed in the IL under a CaMKII promoter and optical fibers implanted in the NAc, allowing for selective optogentic control of IL→NAc projection neurons. Laser stimulation was delivered on a subset of trials during a single extinction session after cocaine self-administration training and withdrawal. On the stimulation trial, activity in this projection significantly decreased active lever presses (t_{12}=3.3; $p<0.01$) and increased inactive lever presses (t_{12}=3.8; $p<0.01$), while stimulation had no significant effect on behavior on the next trial (t_{12}=1.4; $p>0.05$, active lever; t_{12}=0.90; $p>0.05$, inactive lever). Laser stimulation had no effect on lever pressing in control animals (t_3=0.5; $p>0.05$, active lever; t_3=0.0; $p>0.05$, inactive lever). These data suggest that manipulation of the IL→NAc projection predominantly influences discrimination between the active and inactive lever on the current trial, rather than providing feedback to update subsequent drug-seeking. Taken together with the imaging data, this suggests that the IL→NAc projection

may be responsible for providing spatial information to the NAc that shapes ongoing drug-seeking behavior.

Disclosures: C.M. Cameron: None. J. Pillow: None. I.B. Witten: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.09/GGG3

Topic: G.02. Motivation

Support: Social Science Matrix, UC Berkeley

Title: Toward a Greater Moderation and efficient markets: Neuroeconomics-based financial-system regulation may reduce bubble-crash effects of brain risk-seeking and -avoiding networks

Authors: *J. L. HARACZ;
Dept. Psychol. Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Objective: Neuroimaging studies of subjects exposed to lab asset-price bubbles yielded results that were interpreted on the basis of "irrational exuberance" (Smith et al., 2014), "theory of mind" (De Martino et al., 2013), and having a "future time perspective" (Ogawa et al., 2014). However, academic scholars, financial-system regulators, and journalists often have emphasized excessive financial risk taking to explain asset-price bubbles. Clinicians report risky behaviors in association with substance-related or behavioral addictions (e.g., gambling disorder and internet gaming disorder). Therefore, a systematic literature review will focus on the neurobiology of addiction and risky behavior to develop a hypothesis about neural networks potentially involved in asset trading underlying financial-market bubbles.
Methods: The review included neuroimaging studies of risky decision-making tasks (e.g., the Balloon Analog Risk Task [BART] and the Columbia Card Task [CCT]) and substance-related or behavioral addictions.
Results: Healthy subjects' BART decisions were predicted by the balance of fMRI activations in a risk-avoiding cortical-control network and a risk-seeking network (Helfinstein et al., 2014). Resting-state fMRI studies of functional connectivity revealed that, compared to control subjects, individuals with heroin addiction (Xie et al., 2014; Zhai et al., 2015; Zou et al., 2015) or internet gaming disorder (Dong et al., 2015) show greater connectivity between regions in the reward valuation circuit (e.g., nucleus accumbens, ventromedial prefrontal cortex, and amygdala), but less connectivity in the frontoparietal cognitive-control network. Across all subjects, functional connectivity in the valuation and control networks was inversely correlated (Xie et al., 2014;

Dong et al., 2015). Healthy subjects' near-infrared spectroscopy (NIRS)-recorded prefrontal cortical activity was higher in the "cold" deliberative version of the CCT compared to the "hot" affective CCT version (Holper & Murphy, 2014).
Conclusions: If financial risk taking underlies asset-price bubbles, then the above pattern of results is consistent with the hypothesis that asset trading-related lateral neocortical activity is higher during non-bubble periods of markets, so low trade-related lateral neocortical activity may be a NIRS-detectable biomarker of bubbles. Lowered economic volatility of the "Great Moderation" may have ended with the recent global financial crisis (Rudebusch, 2010), but neuroeconomics-based financial-system regulation may become useful for reducing asset-price volatility, thereby enhancing market efficiency.

Disclosures: J.L. Haracz: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.10/GGG4

Topic: G.02. Motivation

Support: UMB: Anatomy and Neurobiology

NIDA:IRP-IPA

Title: Disconnection of basolateral amygdala-insular cortex interferes with goal-tracking but not sign-tracking.

Authors: *H. M. NASSER, E. N. LESSER, D. S. LAFFERTY, D. J. CALU;
Univ. of Maryland Dept. of Anat. and N, Baltimore, MD

Abstract: The Pavloivan lever autoshaping procedure reliably distinguishes sign- and goal-tracking behaviors in rats. In this procedure a lever is inserted and retracted, after which a food reward is delivered into a food cup. Rats are not required to press the lever or approach the food cup, but sign-tracking rats approach the lever, while goal-tracking rats approach a food cup. Prior work indicates that sign-trackers show heightened motivation for a food-predictive lever-cue and subsequently show greater cue-induced drug relapse than goal-trackers. Prior work demonstrates that disconnecting basolateral amygdala (BLA) and nucleus accumbens impairs sign- but not goal-tracking. Here we examine the role of reversibly disconnecting BLA from insular cortex (IC) on the expression of sign- and goal-tracking behaviors. We trained rats in lever autoshaping for four days to determine their tracking tendency. On alternating test days we injected vehicle or

GABA agonist cocktail, baclofen and muscimol, unilaterally into the BLA and contralaterally into IC prior to reinforced lever autoshaping sessions. Disconnection of BLA and IC reduced goal- but not sign-tracking behaviors. These findings suggest that the BLA-IC pathway is necessary for supporting food-cup directed behaviors in goal-trackers but not lever-directed behavior of sign-trackers. This finding together with prior work, suggests that sign- and goal-trackers rely on dissociable BLA pathways to drive distinct conditioned responses during lever-autoshaping.

Disclosures: H.M. Nasser: None. E.N. Lesser: None. D.S. Lafferty: None. D.J. Calu: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.11/GGG5

Topic: G.02. Motivation

Support: Lieber Institute for Brain Development

Title: Dynamic causal modeling of human motivation neural circuitry

Authors: *A. KOHLI[1], D. N. BLITZER[2], D. R. WEINBERGER[1], C. F. ZINK[1];
[1]Clin. Div., Lieber Inst. For Brain Develop., Baltimore, MD; [2]Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: Motivation dysfunction is a prevalent and debilitating symptom of several mental disorders; therefore, determining how motivation is represented in the human brain is important to eventually combat motivation deficits. The goal of the current study was to use Dynamic Causal Modeling (DCM) of fMRI data - a method to model effective connectivity between activated brain regions and the impact of task conditions on intrinsic connections - to determine how motivation neural circuitry is connected and how motivation modulates the connectivity pattern. A modified version of the Monetary Incentive Delay task, designed to isolate neural activity of motivation from that of reward, was presented to healthy subjects (n = 105) under fMRI. A whole brain group level ANOVA ($p < 0.05$, FWE-corrected, $k > 50$, n = 105) revealed five significant brain regions, all in which the motivation cues evoked greater activation than neutral cues (Motivation Cues > Neutral Cues): ventral striatum (vStr), midbrain, pre-motor area (PMA), supplementary motor area (SMA), and visual cortex. In order to determine how these regions communicate in the context of motivationally salient stimuli, a DCM analysis was conducted. Average time series were extracted from volumes of interest (VOIs) defined as activated voxels ($p < 0.05$, uncorrected) within a 10 mm sphere centered at the peak voxels in

each of the 5 aforementioned brain regions for each individual. Subjects who did not have activated voxels in all VOIs were excluded from the DCM analysis (final n = 60). An initial DCM was entered into the model space with motivation cues serving as driving input into the model at visual cortex and 15 possible intrinsic connections between brain regions (based on known anatomical circuitry). Connectivity parameters were estimated to determine connection strength. The parameters corresponding to 5 intrinsic connections were significant ($p < 0.05$): visual->midbrain, midbrain->PMA, midbrain->SMA, midbrain->vStr, PMA->SMA. We interrogated the influence of motivation on these specific connections by allowing the cue associated with highest level of motivation to modulate each connection. The resulting estimated modulation parameters showed significant ($p < 0.05$) high motivation modulation of the visual->midbrain, midbrain->vStr, and midbrain->PMA connections. Our results provide a model of how motivation is represented in the human brain not only in terms of which brain regions are involved, but also how they are connected in the context of motivation processes, and how evoked-motivation strengthens the connectivity.

Disclosures: A. Kohli: None. D.N. Blitzer: None. D.R. Weinberger: None. C.F. Zink: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.12/GGG6

Topic: G.02. Motivation

Support: SFRH/BD/51992/2012

SFRH/BD/98675/2013

SFRH/BD/90374/2012

SFRH/BD/89936/2012

IF/00883/2013

Portuguese North Regional Operational Program (ON.2 – O Novo Norte)

National Strategic Reference Framework (QREN)

Title: Activation of D1 and D2 expressing neurons in the nucleus accumbens enhances motivation

Authors: *C. SOARES-CUNHA[1,2], B. COIMBRA[2], A. DAVID-PEREIRA[2], S. BORGES[2], L. PINTO[2], P. COSTA[2], N. SOUSA[2], A. J. RODRIGUES[2];
[1]Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal; [2]Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sciences, Univ. of Minho and ICVS/3B's - PT Government Associate Lab., Braga/Guimaraes, Portugal

Abstract: The nucleus accumbens (NAc) plays an important role in reward processing and reinforcement. Up to 95% of NAc neurons are GABAergic medium spiny neurons (MSNs) that are typically segregated into two distinct subtypes - those expressing dopamine D1-like receptors and those expressing dopamine D2-like receptors. While striatal D1-MSNs have been classically associated with positive reinforcement and reward, D2-MSNs have been associated with negative reinforcement and aversion.
In this work we demonstrate that activation of both D1- and D2-MSNs in the NAc are positively correlated with behavioural performance in motivation-dependent paradigms, namely the Pavlovian-to-instrumental transfer and progressive ratio schedule of reinforcement. In addition, we show that brief optogenetic activation of NAc D1- and D2-MSNs during reward-predictive cues strongly boosts motivation to work for food in mice, indicating that both neuronal subtypes can contribute in the same direction in the modulation of motivation.
We further extended these results by selectively manipulating D2 neurons in rats: brief optogenetic activation/inhibition of NAc D2-MSNs during reward-predictive cues strongly enhanced/diminished motivation. *In vivo* electrophysiological recordings of downstream regions namely the ventral pallidum and ventral tegmental area showed that the manipulation of D2 neurons was pathway-specific.
Our results suggest that the classic view of D1/D2 functional antagonism in the NAc does not hold true for all dimensions of reward-related behaviours, and that D2-MSNs may play a more prominent *pro-motivation* role than originally anticipated.

Disclosures: C. Soares-Cunha: None. B. Coimbra: None. A. David-Pereira: None. S. Borges: None. L. Pinto: None. P. Costa: None. N. Sousa: None. A.J. Rodrigues: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.13/GGG7

Topic: G.02. Motivation

Support: NIH Grant R01MH106972

NIH Grant R01DA029035

Title: Effects of cholinergic receptors blokade in the orbitofrontal cortex on Pavlovian-to-instrumental transfer task

Authors: *Y. CUI[1], A. T. LIU[1], K. WASSUM[2], S. B. OSTLUND[1];
[1]UCI, Irvine, CA; [2]Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Conditioned stimuli are important guides for behavioral actions. Previous studies have shown that post-training orbitofrontal cortex (OFC) lesions disrupt the use of cue-triggered outcome expectations on action selection, assayed using the outcome-specific Pavlovian-to-instrumental transfer (PIT) task. However, it is not yet known how local neuromodulatory systems in the OFC contribute to this aspect of reward seeking. We were interested in the possibility that OFC acetylcholine signaling might play a role, given recent findings that systemic antagonism of either nicotinic or muscarinic acetylcholine receptors block the expression of specific PIT. Rats were trained to nose-poke to receive two different rewards that were predicted by two different auditory stimuli, which was done to establish a pair of unique stimulus-outcome associations (S1-O1 and S2-O2). Subsequently, the rats were trained to perform two different lever-press actions (left and right), each earning a different reward (A1-O1 and A2-O2). The impact of pavlovian stimuli on instrumental performance was then examined. The two different auditory stimuli were periodically presented while rats were free to choose between the two lever-press actions. Before testing, rats were given bilateral intra-OFC infusions of vehicle, mecamylamine (nicotinic acetylcholine receptor antagonist), or scopolamine (muscarinic acetylcholine receptor antagonist) to temporarily block cholinergic signaling in this region. When rats were tested on vehicle, the stimuli were effective in selectively elevating performance of whichever action was trained with the same outcome as that cue (e.g., S1-A1). We found that blocking cholinergic receptors either with mecamylamine or scopolamine dramatically reduced this effect. Importantly, reinforced lever pressing was not affected either drug treatment, indicating that the disruption of cue-motivated behavior was not the result of gross motor impairment. These results indicate that acetylcholine signaling at both nicotinic and muscarinic receptors in the OFC plays an important role in the use of cue-evoked reward expectations for instrumental action selection.

Disclosures: Y. Cui: None. A.T. Liu: None. K. Wassum: None. S.B. Ostlund: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.14/GGG8

Topic: G.02. Motivation

Support: NIDA R01: R01-DA-038599

Early Stage Training in the Neurosciences Training Grant T32 NS076401

Title: Time-course expression and locomotor effects of chemogenetic modulation of prelimbic projections to the paraventricular nucleus of the thalamus in the rat

Authors: *S. A. LOPEZ[1], I. R. COVELO[2], M. KOMAIHA[3], S. M. FERGUSON[5], S. B. FLAGEL[4];
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Abstract: The paraventricular nucleus of the thalamus (PVT), a midline structure ventral to the third ventricle, is well-positioned to act as an interface between limbic and motor circuits, integrating information about hunger, memory, pain and arousal. Recently, the PVT has been implicated in mediating motivated and emotional behaviors, including food- and drug-seeking. To better understand the role of the PVT in cue-motivated behaviors, we utilize novel chemogenetic technology that allows us to specifically manipulate PVT circuitry in behaving animals. To do this, we use a dual vector approach to selectively express designer receptors exclusively activated by designer drugs (DREADD) in afferents from the prelimbic cortext (PrL) to the PVT in rats. Specifically, a Cre-dependent-flip-excision AAV viral vector expressing silencing (G_i-) or activating (G_q-based) DREADDs is infused into the PrL, while a retrograde CAV-Cre viral vector is infused into the PVT. This technique allows us to transiently modulate (silence/activate) the functional connectivity of the PrL-PVT circuit. However, given that this is a relatively new technology, determining an optimal methodological protocol is needed. The current study aims to identify the appropriate amount of time required from transfection of the DREADD viruses to the time of maximal receptor expression levels, taking into account differences between G_i and G_q- coupled DREADDs. Since DREADD receptor activation relies on the otherwise inert chemical ligand, Clozapine-N-oxide (CNO), those rats that have a longer "incubation" time for DREADD expression, and presumably more receptors, may exhibit different CNO-induced behaviors than those rats with shorter "incubation" times. Thus, the effects of CNO (3 mg/kg) vs. vehicle (6% dimethyl sulfoxide in sterile water) on locomotor activity were tested for both G_q and G_i experimental groups following a 2-, 4- and 6-week "incubation" time. Our results suggest that maximum levels of DREADD expression occur at 6 weeks following transfection. Similarly, CNO effects on locomotor activity become evident after 4 weeks. These data identify the optimal time period for DREADD expression using the dual vector approach, enhancing our ability to examine the role of specific brain circuits in motivated behaviors.

Disclosures: S.A. Lopez: None. I.R. Covelo: None. M. Komaiha: None. S.M. Ferguson: None. S.B. Flagel: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.15/GGG9

Topic: G.02. Motivation

Support: Intramural Research Program of NIDA/NIH

Title: Anterior insular cortex inhibition attenuates optogenetic self-stimulation of the medial prefrontal cortex

Authors: *A. F. PIERCE, S. IKEMOTO;
Behavioral Neurosci., Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: The medial prefrontal cortex (mPFC) is well known to be an integral part of reward circuitry through its connections to the ventral striatum. However, the extent to which the mPFC's connections play a role in reward processes remains unclear. The mPFC and anterior insular cortex have reciprocal connectivity, and the insular cortex has recently been implicated in reward and affective states related to drug abuse. In humans, damage to the insula decreased desire to smoke in previously addicted cigarette smokers. In rats, the blockade of insular D1 receptors resulted in decreased nicotine self-administration. Recently our lab has found that rats will learn to respond vigorously for optogenetic stimulation in the mPFC in an operant task. In addition, this optogenetic stimulation of mPFC in an fMRI machine elicits a phasic BOLD response in the anterior insula. Therefore, we hypothesize that the projection from the mPFC to the anterior insular cortex plays a role in the rewarding effects of mPFC self-stimulation, and inhibition of insula will cause a decrease in mPFC self-stimulation. To test this hypothesis, C57/BL/6j mice received a unilateral injection of AAV encoding for channelrhodopsin with an optic fiber implant in the mPFC and bilateral injections of AAV encoding for inhibitory DREADDs in the insular cortex. Mice learned to lever press for optogenetic stimulation over a 5 day training period and on test days mice received intraperitoneal injections of Clozapine N-oxide or vehicle, before a self-stimulation session. Our initial data suggest that inhibition of insular cortex by DREADDs significantly reduces lever pressing reinforced by mPFC stimulation. Therefore, we tentatively suggest that the anterior insular cortex plays an important role in reward initiated from the mPFC, and the interaction of the anterior insular cortex with the mPFC may be involved in illnesses associated with the mPFC such as drug addiction and depression.

Disclosures: A.F. Pierce: None. S. Ikemoto: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.16/GGG10

Topic: G.02. Motivation

Support: DARPA Contract N66001-10- C-2008

Title: Decoding reward, punishment, and motivation in the primary sensory and motor cortices of non-human primates

Authors: *J. P. HESSBURG[1], A. TARIGOPPULA[1], D. B. MCNIEL[1], J. S. CHOI[1], J. T. FRANCIS[2,1];
[1]Physiol. and Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Biomed. Engin., Cullen Col. of Engineering, Univ. of Houston, Houston, TX

Abstract: Signals of reward, punishment, and motivation have been recorded in a number of areas in the brain (Bissonnette et al. 2013, Roesch et al. 2004), including a reward signal in the primary motor cortex (M1)(Marsh et al. 2015). This work is continuing this investigation in the hand and arm regions of M1 and the primary sensory cortex (S1). Two non-human primates (NHP) were trained to complete a gripping task on a virtual robotic arm, where the animal gripped and held a given level of force for a specified period of time. Prior to each trial, visual cues were displayed to inform the NHP if the trial would result in a juice reward if completed successfully, a punishment consisting of a five-second timeout if completed unsuccessfully (e.g. if the applied force was too great or released too early), or no reward or punishment, where the task would move immediately to the next trial. Subsets of trials with no cues and with catch trials, where a cue was presented but no reward or punishment given, were included to investigate reward and punishment prediction and error. Neural data were recorded from the hand and arm regions of M1 and S1, and spike sorted to isolate individual units. Time intervals around the cue presentation and reward or punishment delivery were analyzed and used as input into machine learning classifiers to investigate how the value of reward and punishment were represented in these regions, and how the interplay between the two was represented as motivation. We hypothesize that in addition to reward and punishment, motivation is represented in M1 and S1. Investigating the intricacies of these signals in M1 and S1 will allow future brain-machine interfaces (BMI) to capture the breadth of these signals in one or two brain regions that also contain sensorimotor information, rather than requiring multiple implants in multiple regions. These data will be useful in creating algorithms for more robust and nuanced BMI control, taking greater advantage of the range of information available in these regions for better neural control of robotic prostheses.

Disclosures: J.P. Hessburg: None. A. Tarigoppula: None. D.B. McNiel: None. J.S. Choi: None. J.T. Francis: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.17/GGG11

Topic: G.02. Motivation

Support: This work was supported by NIDA/NIH IRP

Title: The supramammillary-septum glutamate pathway modulates affective processes

Authors: *A. KESNER, R. SHIN, S. IKEMOTO;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: The supramammillary nucleus (SuM) is a posterior hypothalamic nucleus that provides dense projections to the septo-hippocampal system. Past research on SuM and septal systems has primarily focused on their role in hippocampal theta rhythm activity, as well as in stress and aversion. However, classically, the septum is implicated in reward as it was the first brain nuclei identified as rewarding when electrically stimulated. Likewise, our lab has shown that pharmacological excitation of SuM neurons induces reward. Thus, we feel these closely linked brain regions are understudied in terms of their roles in reward processes and affect. We first found that rats self-administered the excitatory glutamate agonist, AMPA, into the septal area. Since the SuM provides dense glutamatergic input to the septal area, we designed experiments to investigate whether the SuM mediates reward via its connection to the septal area. We confirmed general cell body stimulation of SuM is rewarding using a *human synapsin-1* mediated *channelrhodopsin-2* (ChR2) in wild-type (C57/BL7) mice. Mice with ChR2 and optic fibers in SuM quickly learned to respond on a lever reinforced by photostimulation and switch responding when lever assignments are reversed. Mice do not reliably self-stimulate when optic fibers are placed in areas adjacent to SuM - the mammillary bodies or ventral tegmental area. Next, using a Cre-dependent ChR2 and vGlut2-Cre, vGat-Cre or Th-Cre mice, we show this rewarding excitation of SuM neurons is likely mediated by glutamatergic neurons, but not dopaminergic or GABAergic neurons. Then using optogenetic terminal-stimulation we dissect which glutamatergic projections from SuM mediate self-stimulation behavior. Mice learned to respond for the stimulation of SuM glutamatergic neurons terminating in the lateral septum, but not terminals in the paraventricular thalamic nucleus, ventral subiculum, or basal forebrain. Currently we are conducting complementary experiments to study the effect of inhibition of SuM-septal glutamate neurons in order to establish a causal role for these neurons in motivation

processes. We conclude that stimulation of septal cell bodies, whether via direct administration of AMPA, or optogenetic elicited release of glutamate from supramammillary neurons, is rewarding. This data speaks to current controversy in the field, as the septum's involvement in reward related processes has recently been called into question. Our results implicate this highly conserved midline system in modulating positive affective processes, warranting future research into its role in psychiatric disorders such as anxiety and addiction.

Disclosures: A. Kesner: None. R. Shin: None. S. Ikemoto: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.18/GGG12

Topic: G.02. Motivation

Support: Human Brain Project, European Union Seventh Framework Programme (FP7/2007-2013), no. 604102

Title: Depicting the neural architecture constituting human motivational decision making: from single-cell to whole brain activation.

Authors: *T. GAZIT[1], T. GONEN[2], G. GUREVITCH[1,5], H. YAMIN[1], M. NEUFELD[3,5], S. KIPERVASSER[3,5], F. FAHOUM[3], I. FRIED[4,5,6], T. HENDLER[1,5];
[1]Ctr. for Brain Functions, [2]Neurosurg., [3]Neurol., [4]Functional Neurosurg., Tel Aviv Med. Ctr., Tel Aviv-Yafo, Israel; [5]Tel Aviv Univ., Tel Aviv, Israel; [6]UCLA, Los Angeles, CA

Abstract: Motivation is a key aspect in human decision making, shaped by the interaction between environmental reinforcing cues and our specific goals. Reinforcements signal both incentive (reward/punishment) and hedonic (appetitive/aversive) accounts, with their integration often raising a conflict evident in pathological symptoms such as diminished or excessive approach in depression and addiction, and avoidance in eating disorders. Depicting the neural signature of motivational dimensions could advance domain-based brain diagnosis and treatment. **We aimed to illuminate the neural dynamics underlying motivational decision making by deconstructing it into stages; incentive cue, decision (to approach or avoid), action (approach or avoid) and response to outcome (reward or punishment).**
Method: Using an interactive computer game enabled dynamic modelling of high- and low goal-conflict. Intracranial recordings in 7 epilepsy patients provided high-resolution multilevel neural measurements; single-neuron activity (120 neurons) and intracranial EEG (iEEG) in mesio-temporal and medial prefrontal cortex (mPFC). In addition whole brain fMRI activation was

obtained in 50 healthy volunteers.
Results: A cascade of neural activations was observed using intracranial recordings: increased neural firing and gamma power immediately following the incentive cue appearance in the amygdala, hippocampus and mPFC; with increased synchronization among these regions, possibly representing integrative effort to comprehend the stimulus. Subsequently, increased theta in the hippocampus marked the decision phase, more so under high goal conflict; and gamma decreased in the amygdala during the action phase, possibly marking action facilitation. Finally, in response to punishment outcome, amygdala and dorso-mPFC showed higher neural activity (both single cell firing and local iEEG) compared to the hippocampus and ventro-mPFC, and the opposite trend was observed in response to reward outcome. fMRI revealed distributed network activation corresponding to either cues of high or low conflict, or outcome type.
Discussion: This is the first multi-scale neurobehavioral portraying of motivational decision makingin humans. Our findings may guide neural based interventions corresponding to pathological conditions involving specific motivational stages.

Disclosures: T. Gazit: None. T. Gonen: None. G. Gurevitch: None. H. Yamin: None. M. Neufeld: None. S. Kipervasser: None. F. Fahoum: None. I. Fried: None. T. Hendler: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.19/GGG13

Topic: G.02. Motivation

Support: NIH Grant F32MH103931

NIH Grant R01MH04557

NIH Grant P50MH086404

Title: How do cortical hubs in the striatum impact on downstream basal ganglia circuitry?

Authors: *S. R. HEILBRONNER[1], M. A. A. MEYER[2], S. N. HABER[2];
[1]Pharmacol. and Physiol., [2]Univ. of Rochester, Rochester, NY

Abstract: The frontal cortico-basal ganglia network plays a central role in action selection, decision-making, associative learning, and motivation. At the level of the cortico-striatal projection, terminal fields from functionally diverse cortical regions overlap in the striatum, allowing for information integration. These areas of convergence ("hubs") are critical to our understanding of the integrative functions of the basal ganglia. Here, our aim was to follow these

converging connections from the frontal cortex through downstream basal ganglia regions, beginning with the striato-pallidal projection, and continuing with the pallidal-thalamic projection.
Our first goal was to understand the overall topography of the striato-pallidal projection based on prefrontal cortical (PFC)-striatal projections. Using these results, we analyzed the data to understand how information from cortico-striatal hubs is conveyed to the pallidum. Specifically, we asked whether the pallidum had cortical hub-like features. Our second goal was to determine whether striatal hubs project particularly broadly within the pallidum. Finally, we wanted to extend our understanding of indirect PFC representations from the pallidum to the thalamus.
We injected anterograde or bidirectional tracers into specific subregions of the striatum in macaques. These subregions were chosen on the basis of their combinations of frontal cortical inputs, which we have mapped previously. We outlined the dense terminal fields in the pallidum, then rendered our injection sites and terminal fields in a 3-D standard macaque brain for comparison and visualization purposes. Finally, we analyzed the striato-pallidal projections according to location and combination of frontal cortical inputs. We performed a similar process for the pallidal-thalamic projection.
Striatal projections to both the GPe/VP and the GPi adhered to a strong topography, such that the location of the striatal injection can explain up to 90% of the variation in resulting pallidal terminal fields. Furthermore, different striatal subregions projected to different regions of the pallidum, with relatively little overlap. However, when examining the (indirect) influence of the cortex on the pallidum, there was substantial overlap, with multiple cortical regions projecting to the same pallidal areas. This could largely be attributed to the impressive convergence of cortical input to the striatum. Finally, we did not find any evidence that those areas of the striatum receiving inputs from a particularly large number of cortical regions (hubs) projected more broadly within the pallidum than other striatal areas.

Disclosures: S.R. Heilbronner: None. M.A.A. Meyer: None. S.N. Haber: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.20/GGG14

Topic: G.02. Motivation

Support: NIH F32 AA022832

NIH R01 MH102638

Title: Extended amygdala neuropeptide circuits for bi-valent hyperarousal states

Authors: *W. J. GIARDINO, D. J. CHRISTOFFEL, L. DE LECEA;
Psychiatry, Stanford Univ., Stanford, CA

Abstract: Appropriate levels of arousal (wakefulness) are necessary for survival, but maladaptive *hyperarousal* is a hallmark of compulsive reward-seeking and pathological anxiety. Hypocretin (Hcrt) neuropeptides are master regulators of arousal that are generated exclusively in the lateral hypothalamus (LH), and LH neurons exhibit major reciprocal connectivity with a region of the extended amygdala known as the bed nucleus of the stria terminalis (BNST). The arousal-related neuropeptides cholecystokinin (CCK) and corticotropin-releasing factor (CRF) are strongly expressed in the BNST, and produce excitatory activity in LH-Hcrt neurons. We expressed AAV-DJ-DIO constructs containing eYFP-tagged ChR2 (or eYFP-only) in the BNST of *CCK*-IRES-Cre and *CRF*-IRES-Cre mice, and implanted fiberoptic cannulae over the BNST. Relative to controls, photostimulation of *CCK*-BNST neurons produced appetitive responding (real-time place preference at 5Hz and 10Hz), and photostimulation of *CRF*-BNST neurons generated avoidance behavior (real-time place aversion at 5Hz and 10Hz). We validated these stimulation parameters with whole-cell patch-clamp recordings in acute BNST slices, using 10ms blue light pulses to evoke single action potentials at frequencies up to 20Hz with high fidelity. Consistent with results from optogenetic studies, bulk chemogenetic excitation of *CCK*- and *CRF*-BNST neurons with hM3Dq designer receptors produced distinct behavioral effects. Specifically, *CCK*-BNST activation enhanced perseverative behavior, while *CRF*-BNST activation strictly generated anxiety-like behavior. Chemogenetic silencing of *CCK*-BNST neurons expressing hM4Di designer receptors revealed a necessary role for this cell group in appropriate approach/avoidance responding toward bi-valent social stimuli. Detailed anatomical characterizations of the BNST identified preferential expression of CCK in dorso*medial* and posterior (principal nucleus) subregions vs. preferential expression of CRF in dorso*lateral* and anterior subregions. Whole-brain mapping of efferents from BNST cell bodies revealed divergent output patterns, namely *CCK*-specific major projections to medial habenula and ventral premammillary nucleus. Similarly, mapping of afferent inputs using cell type-specific rabies retrograde tracing identified signature components of *CCK*- vs. *CRF*-BNST networks. In summary, we generated functional atlases of previously-uncharacterized neurocircuits emanating from distinct cell groups within the extended amygdala, highlighting additional mechanisms for bi-directional emotional control of heightened arousal.

Disclosures: W.J. Giardino: None. D.J. Christoffel: None. L. de Lecea: None.

Poster

259. Motivation Neurocircuitry: Cortex, Extended Amygdala, and Striatum

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 259.21/GGG15

Topic: G.02. Motivation

Support: Intramural Research Program, National Institutes of Health

Brain and Behavior Research Foundation

Title: Gustatory responses in macaque monkeys revealed with fMRI

Authors: *P. M. KASKAN[1], A. M. DEAN[1], M. A. NICHOLAS[1], A. R. MITZ[2], L. G. UNGERLEIDER[3], E. A. MURRAY[1];
[1]Section on Neurobio. of Learning and Memory, Lab. of Neuropsychology, [2]Lab. of Neuropsychology, [3]Section on Neurocircuitry, Lab. of Brain and Cognition, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Tastes and their associated values drive food consumption and influence choice behavior. Because macaque monkeys are often used to understand the neural mechanisms underlying reward-guided decision making and value learning, we characterized the gustatory system in macaques with fMRI while measuring licking to control for oral movements, and to assess monkeys' preferences. Though anatomical data indicate that taste information from the ventroposteromedial nucleus of the thalamus (VPMpc) goes to area G (gustatory) in macaques (Pritchard et al., 1986; Ogawa, 1994), physiological responses to taste appear to be more widespread in cortex, including somatosensory cortex (Ito and Ogawa, 1994; Ifuku et al., 2006) and areas within orbitofrontal cortex (Rolls et al., 1990; Lara et al., 2009). To identify taste-responsive cortex in macaques, we delivered small quantities (0.1ml) of sucrose, citric acid, or distilled water in random order without any predictive cues (e.g., visual stimuli) while using event-related fMRI. In addition, we used an MRI-compatible lick sensor to measure monkeys' licking during scans. fMRI signals associated with licking in the absence of fluid delivery were used to mask responses to fluid delivery/receipt and associated licking. Licking in the absence of fluid delivery and fluid receipt times for each tastant were incorporated into a general linear model to analyze fMRI data. By contrasting BOLD responses to either sweet or sour tastes to those from distilled water, and masking with BOLD signals associated with licking in the absence of fluid delivery, we identified taste responses in G, 12o, 13b and somatosensory cortex area 3b in three macaques. Areas activated by fluid delivery in general - as defined by a contrast of all tastes relative to baseline - included area 14, the ventral striatum, the ventral pallidum, the basal nucleus of amygdala, and the perirhinal cortex. Our findings of gustatory responses in G, 12o, 13b and somatosensory cortex area 3b are in agreement with single unit neurophysiological recordings in macaques. Whole brain fMRI in combination with quantification of licking behavior and monkeys' choice preferences for different tastes will be used to identify brain regions that signal value.

Disclosures: P.M. Kaskan: None. A.M. Dean: None. M.A. Nicholas: None. A.R. Mitz: None. L.G. Ungerleider: None. E.A. Murray: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.01/GGG16

Topic: G.02. Motivation

Title: Illuminating aggression circuitry in *Drosophila*

Authors: *M. P. WOHL[1,3], K. ASAHINA[2];
[1]MNL-KA, [2]Salk Inst., La Jolla, CA; [3]UCSD, La Jolla, CA

Abstract: The neural circuits underlying the motivation for, or the likelihood of, performing a behavior have been more difficult to dissect than those underlying sensory and motor systems. For instance, aggression encompasses a set of behaviors for which the likelihood of deployment can have serious consequences, and yet little is known about how neural circuits contribute. The relatively simple nervous system of the fruit fly, *Drosophila melanogaster*, is a powerful model for elucidating the neural basis of behavior, especially given the plethora of genetic tools that allow for the dissection of neural circuits at the single-neuron level. A subset of male specific neurons, called aSP-g neurons, were discovered that express the neuropeptide tachykinin, that when activated, significantly increase a male fly's level of aggression only when paired with other males. The neuropeptide tachykinin, as well as one of its receptors, TakR86C, are directly involved with the increased, context dependent, aggression observed during aSP-g stimulation. We hypothesize that a subset of downstream neurons are likely to express TakR86C. We targeted neurons which express the tachykinin receptor TakR86c with the creation of a CRISPr/CAS9 reagent that selectively drives the expression of fluorescent proteins in this population. We found that TakR86C-expressing neurons are numerous and widespread however overlap with aSP-g neurons in some areas. We further refined our search of downstream elements by labeling presynaptic sites of aSP-g neurons using GFP tagged synaptotagmin, which identified a ring-shaped neural arbor in the superior medial protocerebrum. Induction of photoactivateable GFP encircling the "ring structure" revealed TakR86C-expressing neurons in close contact with aSP-g presynaptic zones. Calclium imaging confirmed that TakR86C neurites in this region respond to aSP-g optogenetic activation. We conducted a search of available enhancer trap lines for labeling of processes in the area of interest. Lines that overlap with TakR86C expressing neurons will be tested for functional connection with aSP-g neurons. With these techniques, we hope to identify one or more populations of neurons downstream of aSP-g neurons, the properties of which may aid in our understanding of the transformation from motivation to behavior.

Disclosures: M.P. Wohl: None. K. Asahina: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.02/GGG17

Topic: G.02. Motivation

Support: Japan Society for the Promotion of Science

Naito Foundation

Title: Identification of negative regulators for aggressive behaviors in *Drosophila*

Authors: *K. ISHII, K. ASAHINA;
Mol. Neurobio. Lab., The Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: Aggressive behaviors are generally important for animals to gain social benefits over other individuals. On the other hand, continuous excess excessive aggression is energetically costly and may override sacrifice other behaviors with social and biological advantages. For this reason, animals have evolved sophisticated systems to balance the aggressive state with “accelerators” and “brakes” to modulate the behavioral outcome. Recent studies have focused mainly on the “accelerator” side of neuromodulatory systems that activate aggression, whereas the “brake” systems for suppressing the aggressive state are relatively less understood.
To address this issue, we set up a genome-wide RNAi behavioral screen to identify genes that suppress aggressive behaviors, using *Drosophila melanogastor* as a genetically tractable model. We selected genes with predicted expression in neurons, and expression ofpan-neuronally expressed each UAS-RNAi construct was driven by the pan-neuronal elav-gal4. Among over 1,200 RNAi mutants, we found 11 strains reproducibly showing increased aggression in group-housed males, depending on the presence of the gal4 element. The candidate genes included intracellular signaling enzymes, neuropeptides, gustatory receptors, autophagy-related genes, and others. We further constructedare constructing gene-targeted deletion mutants using the CRISPR/Cas9 system, and confirmedin order to confirm the phenotypes of increased aggression for several candidate genes. We have already confirmed that several genes involved in intracellular signaling cascades are indeed necessary to keep aggression from escalating. We are applying the CRISPR/Cas9 genome -editing technique to create a “knock-in” alleles that allows visualization and manipulation of neurons expressing these genes.
Our study is the first to genetically identify a comprehensive set of negative regulators in aggressive social behaviors. Through further characterization of each candidate gene, regarding the expression patterns and relationship with known positive regulators/neural circuits, Through further characterization of each candidate gene, regarding the expression patterns and relationship with known positive regulators/neural circuits, we expect to gain a systematic understanding of the neuromodulatory systems for animal aggression.

Disclosures: K. Ishii: None. K. Asahina: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.03/GGG18

Topic: G.02. Motivation

Title: Genetic dissection of aggressive behavior in the BXD recombinant-inbred mouse strains

Authors: *W. E. CRUSIO[1], M.-P. ALGEO[1], B. BONHEUR[1], L. LU[2], R. W. WILLIAMS[2], A. DELPRATO[3,4];
[1]Univ. Bordeaux and CNRS, Bordeaux (Pessac), France; [2]Dept. of Genetics, Genomics and Informatics, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]BioScience Project, Wakefield, MA; [4]Inst. de Neurosciences Cognitives et Intégratives d'Aquitaine, Univ. of Bordeaux and CNRS, Pessac, France

Abstract: Intermale aggression is a complex social behavior that is likely regulated by multiple genes. In this study the BXD recombinant inbred mouse strains (RIS) were used to map quantitative trait loci (QTLs) underlying behaviors associated with intermale aggression. The BXD RIS have been used in a wide range of behavioral and metabolic studies but to the best of our knowledge, this is the first study in which aggressive behavior is tested. Four hundred and fifty-seven males from 55 strains (including the parentals) were observed at an age of 13 +/- 1 week in a resident-intruder test following 10 days of isolation. Attack latency was measured directly within a 10 minute time period and the test was repeated 24 hours later. The variables analyzed were the percentage of attacking males in a given strain (on days 1 and 2, and both days combined), as well as the attack latency on days 1 and 2. On day 1, 29% of the mice attacked, which increased to 37% on day 2. Strain differences were highly significant for all variables measured, indicating significant heritability. From these data, we identified suggestive QTLs on mouse chromosomes 7, 11, 12, and 13 for both attack and latency variables. Additional strains will need to be tested to obtain significant results and increased resolution for interval mapping. mRNA expression data and phenotype correlation analyses show significant relationships with the amygdala and striatum, and with fear and anxiety. Given the fact that only a relatively small proportion of animals attacked and that most strains showed very low proportions of attacking males, the BXDs may not be very well suited to localize QTLs involved in the regulation of aggression.

Disclosures: W.E. Crusio: None. M. Algeo: None. B. Bonheur: None. L. Lu: None. R.W. Williams: None. A. Delprato: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.04/GGG19

Topic: G.02. Motivation

Support: UT Austin

Title: Social dominance status predicts vicarious fear learning from conspecifics in male laboratory rats

Authors: *C. E. JONES[1,2], M.-H. MONFILS[1];
[1]Psychology, Univ. of Texas at Austin Dept. of Psychology, Austin, TX; [2]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Acquiring information about stimuli that predict danger, through either direct experience or inference from a social context, is crucial for individuals' ability to generate appropriate behaviors in response to threats. Utilizing a modified demonstrator-observer paradigm (fear conditioning by proxy) that allows for free interaction between subjects, we show that social dominance hierarchy, and the interactive social behaviors of caged rats, is predictive of the potency of social fear transmission, with subordinate rats displaying increased fear responses after interacting with a fear conditioned dominant rat during fear retrieval. We found that 1) the majority of standard laboratory cages had an easily identifiable social hierarchy, 2) subordinate rats learned to freeze more when observing and interacting with a more dominant animal, and 3) play fighting indices of the social relationship correlated with the occurrence of ultrasonic vocalizations during social transmission sessions, freezing displayed after exposure to a fearful conspecific, and the neuroendocrine (corticosterone) response to a vicariously conditioned stimulus.

Disclosures: C.E. Jones: None. M. Monfils: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.05/GGG20

Topic: G.02. Motivation

Support: NIMH Grant R15MH102807

Title: Involvement of ventral pallidal vasopressin in the sex-specific regulation of opposite-sex preference in rats

Authors: *B. DIBENEDICTIS, C. J. SMITH, H. K. CHEUNG, E. R. NUSSBAUM, A. H. VEENEMA;
Psychology Dept., Boston Col., Chestnut Hill, MA

Abstract: The ventral pallidum (VP) is a critical node of the mesocorticolimbic 'reward' circuit, and is a component of the 'social decision-making network.' Arginine vasopressin (AVP) is a neuropeptide implicated in the regulation of many social behaviors, and AVP signaling via the V1A receptor (V1AR) within the VP is necessary for the expression of socially motivated affiliative behaviors in male prairie voles. Here, we aimed to characterize the AVP system in the VP and subsequently tested the functional significance of the VP-AVP system in sociosexual motivation in male and female rats. We found that male and female rats expressed equivalent levels of V1AR binding in the VP. However, males had nearly twice the density of AVP fibers in the VP compared to females. These findings suggest that males may experience enhanced AVP signaling in the VP. Given this sex difference, we next blocked AVP signaling in the VP by antagonizing VP-V1ARs in male and female rats and tested subjects' preference to investigate an unfamiliar male or unfamiliar estrous female confined to corrals located on opposite ends of a three-chamber apparatus. Under vehicle conditions, males showed a robust preference to investigate an opposite-sex over same-sex conspecific. However, VP-V1AR antagonism significantly reduced males' innate preference to investigate the estrous female. Compared to males, vehicle-treated estrous female subjects displayed only a modest preference for the opposite-sex. Interestingly, VP-V1AR antagonism resulted in a significant increase in females' preference to investigate the opposite sex. Importantly, all subjects could reliably discriminate between male and female stimulus rats regardless of drug treatment, suggesting a change in motivational state rather than a perceptual impairment. These results provide a novel functional link between a sex difference in ventral pallidal AVP fiber density and the sex-specific regulation of a sexually motivated behavior necessary for reproductive success.

Disclosures: B. Dibenedictis: None. C.J. Smith: None. H.K. Cheung: None. E.R. Nussbaum: None. A.H. Veenema: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.06/GGG21

Topic: G.02. Motivation

Support: NIMH R01MH102456

Title: Lateral septum vasopressin system interacts with nucleus accumbens and prefrontal cortex to regulate social play in sex-specific ways

Authors: *N. NASCIMENTO, G. S. RO, C. J. REPPUCCI, R. BREDEWOLD, A. H. VEENEMA;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Social play is a highly rewarding and motivated behavior, which peaks at the juvenile period and facilitates the development of social skills needed throughout the lifespan. We recently showed that the AVP system in the lateral septum (LS) modulates social play behavior in juvenile rats in sex-specific ways. Specifically, blockade of the AVP V1a receptor (V1aR) in the LS increased social play in males but decreased social play in females. Here, we aimed to determine the neural pathway by which the LS-AVP system modulates social play behavior in sex-specific ways. To study this, single-housed juvenile male and female rats were exposed in their home cage to an age- and sex-matched unfamiliar juvenile for 10 min ("play") or placed similarly without the introduction of a play partner ("no play"). In addition, all rats were given either a vehicle or a V1aR antagonist injection into the LS 20 min prior to the play or no play session. Rats were killed 80 min after the play test, and brain tissue was processed using immunohistochemistry to detect the expression of c-Fos, an early marker of neuronal activation. Preliminary results show a higher number of c-Fos positive cells in vehicle-treated females exposed to play compared to their male counterparts in the anterior shell of the nucleus accumbens (NAcc) and in the anterior prelimbic and infralimbic divisions of the prefrontal cortex (PFC). Interestingly, V1a receptor antagonist-treated rats did not show this sex difference. Together, this suggests that social play is associated with sex differences in neuronal activation in NAcc and PFC subregions and that these sex differences are eliminated by LS-V1aR blockade. Because the LS projects directly to the NAcc, future studies will investigate the causal involvement of the anterior shell of the NAcc in the sex-specific regulation of social play by the LS-AVP system.

Disclosures: N. Nascimento: None. G.S. Ro: None. C.J. Reppucci: None. R. Bredewold: None. A.H. Veenema: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.07/GGG22

Topic: G.02. Motivation

Support: NIMH R01MH102456

Title: Involvement of dopamine and noradrenaline in he sex-specific regulation of social play by vasopressin

Authors: *R. BREDEWOLD[1], N. F. NASCIMENTO[2], A. H. VEENEMA[2];
[1]Dept. of Psychology, [2]Pychology, Boston Col., Chestnut Hill, MA

Abstract: Social play is an affiliative and rewarding behavior displayed by nearly all mammals and peaks during the juvenile period. We recently showed that arginine vasopressin (AVP) acting via the V1a receptor (V1aR) within the lateral septum (LS) regulates social play in opposite directions in male and female juvenile rats. The LS receives dopaminergic input from the ventral tegmental area and adrenergic input from the locus coeruleus. Therefore, we sought to determine whether AVP interacts with dopamine (DA) and/or noradrenaline (NE) to regulate social play behavior in sex-specific ways. Using retrodialysis combined with microdialysis in awake and freely moving juvenile rats, we found that exposure to social play increased DA release in the LS of females, but not of males. Interestingly, V1aR blockade in the LS abolished this sex difference in DA release during social play. In contrast to DA release, exposure to social play did not alter NE release in the LS of either sex. However, V1aR blockade in the LS caused an increase in NE release in the LS of females but not of males. These findings suggest that the sex-specific regulation of social play by the LS-AVP system involves differential monoaminergic neurotransmission in the LS of male and female juvenile rats. Using pharmacological manipulations, we currently determine the causal involvement of DA and NE released in the LS in the sex-specific regulation of social play by AVP.

Disclosures: R. Bredewold: None. N.F. Nascimento: None. A.H. Veenema: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.08/GGG23

Topic: G.02. Motivation

Support: NSF IOS-0923301

Title: Role of arginine-vasopressin (AVP), serotonin (5-HT) and galanin (GAL) within the anterior hypothalamus (AH) on aggression and social communication in male and female hamsters

Authors: *N. HARDCASTLE, T. E. LARKIN, J. I. TERRANOVA, Z. SONG, A. NORVELLE, H. E. ALBERS;
Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Aggression and social communication are necessary to establish and maintain social heirarchies. In hamsters, AVP, 5-HT and GAL are involved in the regulation of aggression and a form of social communication called flank marking. In male hamsters, AVP injected into the AH stimulates aggression and flanking marking. In females, AVP injected into the AH inhibits aggression and stimulates flank marking. The aim of this study was to determine if there is a sex difference in the effects of GAL on aggression and whether GAL or 5-HT influence AVP-induced flank marking. Socially isolated hamsters implanted with a guide cannula aimed at the AH were handled daily and estrous cycles were monitored in females. Hamsters were injected with 9.0 µM AVP alone or in combination with 1 mM of the 5-HT1a agonist 8-OH-DPAT (DPAT) or 100 µM GAL. The number of flank marks were recorded during a 5 min test. There was a sex by drug interaction ($p < 0.01$). GAL and DPAT reduced AVP-induced flank marking in males (AVP: 55.3 ± 3.0; AVP/DPAT: 26.44 ± 5.81; AVP/GAL 8.0 ± 3.87) and females (AVP: 51.7 ± 7.3; AVP/DPAT: 31.2 ± 5.8; AVP/GAL 34.7 ± 6.1). Males injected with GAL/AVP flank marked significantly less than females injected with GAL/AVP. The effect of GAL on aggression was recorded in a resident-intruder test after microinjections of 0, 10, 100, or 1000 µM GAL. Although there was a significant main effect of sex (Male: 47.6 ± 9.6; Female: 94.6 ± 14.8) GAL was not found to alter the expression of aggression in either males or females. These data suggest that GAL does not modulate the expression of aggression but that both GAL and 5-HT influence the expression of AVP-induced social communication by their actions in the AH.

Disclosures: N. Hardcastle: None. T.E. Larkin: None. J.I. Terranova: None. Z. Song: None. A. Norvelle: None. H.E. Albers: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.09/GGG24

Topic: G.02. Motivation

Support: NIMH R01MH102456 to AHV

Title: Socially rewarding behavior recruits orexin/hypocretin neurons in juvenile male and female rats

Authors: *C. J. REPPUCCI, C. K. GERGELY, N. F. NASCIMENTO, G. S. RO, R. BREDEWOLD, A. H. VEENEMA;
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Abstract: Orexins/hypocretins (ORX) are neuropeptides whose central synthesis is restricted to neurons in the lateral hypothalamic area (LHA) and adjacent dorsomedial and posterior hypothalamic nuclei. The ORX system has been shown to modulate many reward-driven and motivated behaviors, but its role in socially rewarding behavior is largely unknown. Social play is a highly rewarding motivated behavior predominately displayed by juveniles, and expressed by nearly all mammalian species. Here we examined whether social play recruits ORX neurons in juvenile male and female rats. Single-housed juveniles were exposed, in their home cage, to an age- and sex-matched unfamiliar juvenile for 10 min ("Play" condition) or received similar handling but no partner ("No Play" condition). Rats were sacrificed 80 min after the test, and brain tissue was later processed using fluorescent immunohistochemistry methods for combined detection of the immediate early gene product Fos and identification of ORX-positive neurons in the LHA. Preliminary analysis showed that social play increased recruitment of ORX neurons in the juxtadorsomedial LHA (LHAjd); a greater percentage of ORX neurons were double-labeled for Fos in the Play compared to the No Play condition. Interestingly, this effect was stronger in males compared to females. These findings suggest a novel role for LHAjd ORX neurons in socially rewarding behavior at the juvenile age. The LHAjd is interconnected with regions that comprise the social behavior and mesocorticolimbic reward networks, and ORX fibers innervate many of these regions. Thus, this neuronal population is well-positioned to coordinate the expression of socially rewarding behavior. Future work will examine recruitment of specific ORX-dependent pathways during social play, and assess whether pharmacological manipulations of the ORX system alter expression of social play in males and females.

Disclosures: C.J. Reppucci: None. C.K. Gergely: None. N.F. Nascimento: None. G.S. Ro: None. R. Bredewold: None. A.H. Veenema: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.10/GGG25

Topic: G.02. Motivation

Support: NSF IOS-0923301

Title: Novel behavioral test for social motivation in syrian hamsters

Authors: *J. BORLAND, E. SONG, K. GRANTHAM, L. AIANI, A. NORVELLE, K. FRANTZ, H. E. ALBERS;
Neurosci. Inst., Georgia State Univ. Neurosci. Inst., Atlanta, GA

Abstract: Social interaction is a powerful motivator that is observed in every animal species. A well-validated behavioral test that investigates the rewarding properties of social interaction in rodents is the conditioned social preference (CSP) test. Yet the validity of CSP as a pure measure of social reward is limited because CSP has a significant memory component. In this study we developed an approach that allows animals to choose either to engage or not engage in social interaction and is independent of social memory. A three-chamber arena (two small, one large) with a one-way, entry-only door separating the two small chambers from the main chamber was constructed. Hamsters were placed into the main chamber, and then allowed to choose to enter a small chamber with a conspecific, an empty chamber, or neither. 20 seconds after choice, hamsters were placed immediately back in the main chamber by an experimenter. Social motivation was operationally defined as the number of door entries into a chamber containing a conspecific during a 10-minute test. The amount of force required to open the door was varied with weights to provide an additional measure of social motivation. Male and female Syrian hamsters had significantly ($p=0.009$) more entries into the social chamber (14.7 +/-4.58 entries) than the non-social chamber (7.5 +/-0.58 entries), with impact of door weights remaining to be determined. These data demonstrate that male and female Syrian hamsters are motivated to engage in same-sex conspecific social interaction. Supported by NSF IOS-0923301

Disclosures: J. Borland: None. E. Song: None. K. Grantham: None. L. Aiani: None. A. Norvelle: None. K. Frantz: None. H.E. Albers: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.11/GGG26

Topic: G.02. Motivation

Title: Dissecting prefrontal circuitry that supports social investigation.

Authors: *M. MURUGAN[1], J. P. TALIAFERRO[1], M. PARK[2], H. JANG[2], I. B. WITTEN[1]; [1]Princeton Neurosci. Inst., [2]Princeton Univ., Princeton, NJ

Abstract: Although appropriate social interactions are crucial for the survival and well-being of all mammals (including humans), very little is known about the neural substrates that support such behaviors. A major obstacle that has prevented more progress in this area is the intricacy of the underlying neural circuitry, as a large number of interconnected brain regions are involved in mammalian social interactions. Several lines of evidence implicate the prelimbic cortex (PL) as important to social behavior. However, the PL projects to a number of downstream regions and it remains unclear as to which, if any, of these projections are essential for regulating social behavior. In this study we focused our attention on three anatomical subpopulations of PL neurons: PL projections to the nucleus accumbens (NAc), the basolateral amygdala (BLA) and the ventral tegmental area (VTA. First, to measure how much overlap existed between these three subpopulations of PL neurons we used dual color retrograde tracing with cholera-B-toxins (488, 555, 647nm). We found that these three subpopulations were largely distinct with little overlap (< than 10%; % overlap - PL→NAc vs PL→BLA : 8.14, PL→NAc vs PL→VTA : 3.98, PL→BLA vs PL→VTA : 0.26). To test if manipulating the activity of these different subpopulations had an effect on social investigation, we injected ChR2 bilaterally into PL and stimulated PL afferents in the NAc, BLA or VTA bilaterally while animals were engaged in a social assay. We found that optogenetic activation of PL→NAc neurons (ChR2 n=10; YFP n=8, p =0.00386) but not the PL→BLA (ChR2 n=9, YFP n=8, p = 0.8873) or the PL→VTA (ChR2 n=11, YFP n=8, p=0.9215) neurons disrupted social investigation. Additionally, no effect was observed on the investigation of the novel objects. Furthermore, to characterize the neural dynamics of this population during social interactions we monitored the activity of the PL→ NAc using cellular-resolution imaging in freely moving mice in a modified version of the three chamber social assay. Interestingly, we observed that a significant subset of these neurons (16/131 neurons) responded in the location of the social target mouse even when the location of the social target was altered. By combining cellular resolution imaging, viral intersectional strategies and optogenetics, this study provides insight into the neural circuits that underlie social behavior, pointing to an important role for the prelimbic cortical projection to the nucleus accumbens in mediating social investigation.

Disclosures: M. Murugan: None. J.P. Taliaferro: None. M. Park: None. H. Jang: None. I.B. Witten: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.12/HHH1

Topic: G.02. Motivation

Support: NSF GRFP 2012138127

NSF IOS1253386

Title: Involvement of mu opioid receptors in the regulation of juvenile social novelty seeking behavior: Brain region specific effects and modulation by social separation

Authors: *C. J. SMITH, A. M. RATNASEELAN, S. LI, A. H. VEENEMA;
Boston Col., Chestnut Hill, MA

Abstract: The drive to approach and explore novel conspecifics is inherent to social animals and may promote optimal social functioning. Juvenile animals seek out interactions with novel peers more frequently and find these interactions to be more rewarding than their adult counterparts. We have previously shown that juvenile rats spend more time interacting with a novel conspecific than a cage mate (sex and age matched). However, the underlying neural mechanisms have yet to be determined. Given their role in the regulation of rewarding juvenile social behaviors, we hypothesized that μ-opioid receptors (MORs) might play an important role in the facilitation of juvenile social novelty-seeking behavior. Using the MOR antagonist CTAP, we show that central (intracerebroventricular) MOR blockade reduces social novelty-seeking behavior in juvenile male rats. This effect appears to be specific to the opioid system as oxytocin, vasopressin V1a, or dopamine D2 receptor blockade had no effect. Moreover, this effect appears to be brain region-specific as local MOR antagonism in the anterior nucleus accumbens, but not the basolateral amygdala, reduces social novelty-seeking, despite the fact that both brain regions display dense MOR binding in juvenile rats. Finally, an acute period of social separation (3 hours) reduces juvenile social novelty-seeking behavior, an effect that can be rescued by central MOR agonism (using DAMGO). Taken together, these results demonstrate that MOR activation facilitates juvenile social novelty-seeking behavior by acting on the nucleus accumbens and restores juvenile social novelty-seeking behavior following social separation. This research was supported by NSF GRFP 2012138127 to CJS and NSF IOS1253386 to AHV.

Disclosures: C.J. Smith: None. A.M. Ratnaseelan: None. S. Li: None. A.H. Veenema: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.13/HHH2

Topic: G.02. Motivation

Support: National Institute on Drug Abuse Grant R01DA022628 (LJMJV)

Organization for Scientific Research (NWO) Veni grant 91611052 (VT)

Marie Curie Career Reintegration Grant PCIG09-GA-2011-293589 (VT)

Title: Role of opioids and endocannabinoids on the expression and motivational properties of social play behaviour in rats

Authors: *M. ACHTERBERG[1], M. M. H. VAN SWIETEN[2], D. J. HOUWING[2], M. AALDERINK[2], N. V. DRIEL[1], V. TREZZA[3], L. J. M. J. VANDERSCHUREN[2];
[2]Animals in Sci. and Society, [1]Fac. of Vet. Med., Utrecht, Netherlands; [3]Science, Section of Biomed. Sci. and Technologies, Univ. Roma, Rome, Italy

Abstract: Social play behaviour a characteristic form social behaviour displayed by the young of many mammalian species, including rats and humans, which is thought to be important for social and cognitive development. Being a rewarding activity, the performance of social play depends on its pleasurable and motivational properties. Opioids and endocannabinoids have an important role in social play behaviour, perhaps through modulation of reward processes. However, it is unknown whether opioids and endocannabinoids are also involved in the motivation for social play behavior. Therefore, we assessed the effects of opioid and cannabinoid (ant)agonists in an operant conditioning setup in which rats responded for social play under a progressive ratio schedule of reinforcement. This setup allowed for the parallel measurement of the performance of social play.
Treatment with the opioid receptor agonist morphine increased the performance of play behavior but did not affect operant responding. Blocking opioid receptors with naloxone reduced both responding for play and its performance. Enhancing endocannabinoid levels with the anandamide-hydrolysis inhibitor URB597 modestly reduced operant responding, without affecting social play performance. Treatment with the cannabinoid-1 receptor antagonist rimonabant non-specifically reduced operant responding, due to its pruritic effect, without affecting social play performance.

Consistent with previous work implicating opioid neurotransmission in social play behaviour, these data demonstrate also have an important role for opioids in the motivational properties of social play. However, although endocannabinoids are known to be involved in social play behaviour, they have only a minor role in the motivation for social play.

Disclosures: **M. Achterberg:** A. Employment/Salary (full or part-time): Utrecht Univerisity. **M.M.H. van Swieten:** None. **D.J. Houwing:** None. **M. Aalderink:** None. **N.V. Driel:** None. **V. Trezza:** A. Employment/Salary (full or part-time): University Roma "Tre". **L.J.M.J. Vanderschuren:** A. Employment/Salary (full or part-time): Utrecht University.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.14/HHH3

Topic: G.02. Motivation

Support: ERC starting grant

Simons Foundation SFARI Pilot grant

Israel Science Foundation

Title: Social representations in the mouse prefrontal cortex

Authors: ***D. R. LEVY**, A. WEISSBROD, O. YIZHAR;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The prefrontal cortex (PFC) is crucial for modulation of complex social functions in the mammalian brain. In autism spectrum disorders, functional and morphological abnormalities in this region are thought to be associated with diverse social deficits. Yet to date, it is unclear how the PFC encodes social information, and how such social representations might drive complex behavioral responses in health and disease. To address these questions, we utilized a novel apparatus to record unit activity in the medial prefrontal cortex (mPFC) of behaving male mice presented with rapid and precisely timed social and non-social olfactory cues. We found that a large fraction of all recorded mPFC units (117/278) responded exclusively to social over non-social odors. Many of these units were stimulus-specific, such that 33% responded solely to male cues, while 24% responded exclusively to female odors. We also observed distinct temporal response patterns to male and female cues. While presentation of male odors elicited a robust neuronal response within 150 ms of cue delivery, female-evoked responses developed more slowly, peaking within 1450 ms after stimulus onset. High-resolution analysis of behavior

revealed that activation of both female-specific and male-specific units, but not non-social units, was significantly correlated with the initiation of approach behavior. These patterns of social coding were significantly altered in capsr2 knockout mice, a well-established genetic model of autism. mPFC units in these mice showed decreased specificity for social odors, and blunted stimulus-evoked neuronal dynamics. Furthermore, while response magnitude was significantly larger for social over non-social odors in wild-type mice, units in caspr2 knockout mice responded with similar magnitude to all presented cues. Taken together, our results identify specific representation for salient social stimuli in the mPFC and reveal changes in mPFC information processing in a genetic model of autism.

Disclosures: D.R. Levy: None. A. Weissbrod: None. O. Yizhar: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.15/HHH4

Topic: G.02. Motivation

Support: NRF-2015048055

NRF-2014R1A1A3054603

NRF-GFP-CISS-2012M3A6A6054

KHIDI-HI14C2437

Title: Post-weaning social isolation of female mice delays maturation of parvalbumin-positive interneurons in the orbitofrontal cortex

Authors: *D. JEONG[1], E. KANG[1], D. JO[1,2], M. KIM[1,2], Y. KIM[1,2], S. KIM[1,3], S.-H. LEE[1]; [1]KAIST, Daejeon, Korea, Republic of; [2]Daejeon Sci. High Sch. for the Gifted, Daejeon, Korea, Republic of; [3]Univ. of California, Berkely, CA

Abstract: Social perception is important for animal survival and matures slowly after the birth. However, we still do not understand the brain mechanism of circuit maturation during the development of social behavior. Here, we adapt a mouse model of post-weaning social isolation (PWSI) to identify circuits matured during the social critical period. We found that the prolonged social isolation during the juvenile period significantly enhanced the sociability in female mice. Furthermore, the maturation of parvalbumin-positive (PV+) interneurons in the orbitofrontal cortex significantly delayed in the female PWSI mice. Interestingly, we did not observe any

impairment of PV+ neurons in male PWSI mice, even though they showed significant reduction in the social memory. Our results demonstrate that gender-specific PV+ circuit maturation in the prefrontal cortex is important for normal development of social behavior during the social critical period in mice.

Disclosures: D. Jeong: None. E. Kang: None. D. Jo: None. M. Kim: None. Y. Kim: None. S. Kim: None. S. Lee: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.16/HHH5

Topic: G.02. Motivation

Support: NIH 1R01HD059852

PECASE

Whitehall Foundation

Title: Single neuronal correlates of social bias

Authors: *R. BÁEZ-MENDOZA, F. BOUNNI, Z. M. WILLIAMS;
Neurosurg., Massachusetts Gen. Hospital-Harvard Med. Sch., Boston, MA

Abstract: Social groups can have a profound influence on their member's decisions, and can often bias an individual's behavior in both, positive and negative ways. Here, we explore the neuronal mechanisms through which social groups influence an individual's behavior. By using small groups of mice in a foraging task and covertly introducing biased participants, we discovered specific cells in the anterior cingulate cortex (ACC) that uniquely encoded social biases introduced by their group's decisions. This information was complemented by a second ensemble of cells that reflected conformity or opposition to the group. Neuronal responses to social bias were surprisingly insensitive to reward success and were most strongly modulated by group consensus, even when decisions were wrong. These effects were strongest when foraging in less reliable patches and were weakest when foraging democratically or when guided by non-social cues. Interestingly, electrical disruption of normal activity in ACC reduced the influence of the group on individual's behavior. These findings reveal a subgroup of cells in ACC that specifically encoded social biases introduced by group decisions while disrupting their normal activity reduced social biases. Overall, our results suggest an early phylogenetic process that may have allowed social animals to make decisions collectively as a group.

Disclosures: R. Báez-Mendoza: None. F. Bounni: None. Z.M. Williams: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.17/HHH6

Topic: G.02. Motivation

Support: NIH Grant DA033877

Title: Differential effects of oxytocin on maternal attachment in Sprague-Dawley and Fisher 344 preweanling rats.

Authors: D. E. HUMPHREY, A. TERAN, E. M. ALDERSON, Z. R. HARMONY, *C. A. CRAWFORD;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Fischer 344 (F344) rats exhibit reduced play behavior when compared to various other rat strains, including Sprague-Dawley (SD) rats. This finding suggests that F344 rats may be useful for studying social interaction deficits that are common in developmental psychiatric disorders. The purpose of the present study was to determine whether F344 and SD rats differ in their ability to form maternal attachment bonds during the preweanling period. In addition, we assessed whether the prosocial hormone oxytocin would alter attachment behavior in either rat strain. We measured attachment in F344 and SD rat pups on postnatal day (PD) 12 by using a maternal odor-induced conditioned place preference (CPP) procedure. The CPP procedure consisted of a habituation day, a conditioning day, and a test day, and was conducted in a Plexiglas three-compartment chamber. On the habituation day (PD 10), rats were given free access to the testing chamber for 1 min. On the conditioning day (PD 11), rats were given three 30-min conditioning sessions separated by 3 h of maternal deprivation. Sessions consisted of either placing the pups in a holding cage with a lemon-scented dam (maternal group) or in an identical cage with lemon-scented cotton balls (neutral group). On the test day (PD 12), all rat pups were given 5 min free access to the testing chamber, in which one side contained lemon scent and the other side was unscented. In Experiment 2, the identical procedures were used, with the exception that rat pups were injected with saline or oxytocin (250, 500, or 1000 ng, IC) prior to the first conditioning trial. In both experiments, time spent in the two compartments and the number of compartment entries were measured. As expected, rat pups in the maternal group spent more time in the lemon-scented compartment than rats in the neutral group. The maternal group also had more compartment entries. The latter effect was only evident in SD rats. In the second experiment, SD rats were unaffected by oxytocin treatment, while F344 rats were

impacted by oxytocin in a sex-dependent manner. Specifically, 250 ng oxytocin increased the odor preference of male F344 pups, while 1000 ng oxytocin increased odor preference in female F344 pups. The lower dose of oxytocin also caused a general increase in locomotor activity (i.e., increased entries into both compartments), but only in female F344 rats. In sum, these data indicate that SD and F344 rat pups exhibit a similar preference for dam-associated odors, but the preference shown by F344 rats could be enhanced by an exogenous application of oxytocin.

Disclosures: D.E. Humphrey: None. A. Teran: None. E.M. Alderson: None. Z.R. Harmony: None. C.A. Crawford: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.18/HHH7

Topic: G.02. Motivation

Title: Female mice social preference - interaction with the reproductive cycle

Authors: *L. F. MOREIRA, S. Q. LIMA;
Champalimaud Fndn., Lisboa, Portugal

Abstract: Cyclic changes in circulating sex hormones are known to guide females into sexual receptivity. On the other hand, in rodents, the midbrain dopaminergic circuit has been implicated in the appetitive aspects of sexual behavior with relevance to the pursuit of sexual cues.
We are interested in understanding how different hormonal states influence midbrain dopaminergic activity during social interactions, with either socially or sexually relevant stimuli (female vs male, respectively), as well as socially or sexually relevant stimulus predicting cues (pure tones that are associated with the different stimuli), sustaining a sexually receptive state.
To do so, we developed an operant task where female mice are rewarded with social interactions (each corresponding to 5 seconds of restricted nose-nose contact, odors and vocalizations) with either a male or a female conspecific, during 1 hour. In this task, trial initiation causes the delivery of a tone that indicates which of the two possible outcomes (male or female) is available for the female to cash. Vaginal smears are collected after every session to classify the physiological receptivity of each female. Therefore, we are able to investigate how hormone fluctuations influence female performance on the task.
Preliminary data indicates that sex hormones affect several behavioral parameters in female performance (such as reaction times and time spent with each stimulus). Using fiber photometry to record calcium transients in dopaminergic neurons of the ventral tegmental area, we are now

starting to investigate if the representation of reward predicting cues and social rewards are modulated by sex hormones.

Disclosures: L.F. Moreira: None. S.Q. Lima: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.19/DP08 (Dynamic Poster)

Topic: G.02. Motivation

Support: Collaborative and Multidisciplinary Pilot Research Grant from Columbia University

Title: Robust activation of nucleus accumbens neurons during selective affiliation in prairie voles

Authors: *J. L. SCRIBNER[1,2], E. M. KLEIN[4,7], J. C. JIMENEZ[1,2], E. CARAZO[1], A. M. CUNNINGHAM[3], M. A. KHEIRBEK[8], R. HEN[1], Z. R. DONALDSON[5,1,6];
[1]Div. of Integrative Neurosci., [2]Neurobio. and Behavior Grad. Program, [3]Columbia Univ., New York, NY; [5]Molecular, Cellular, and Developmental Biol., [6]Psychology and Neurosci., [4]Univ. of Colorado, Boulder, Boulder, CO; [7]Neurosci. Grad. Program, Brown Univ., Providence, RI; [8]Univ. of California, San Francisco, San Francisco, CA

Abstract: While strong evidence supports the effects social bonds have on numerous aspects of human health, efforts to understand the neural basis of bonding have left many open questions. This is in part due to the relative shortage of appropriate animal models; biomedical research relies heavily on rats and mice, but these animals do not form strong, selective bonds with other adult conspecifics. However, laboratory-amenable prairie voles offer further opportunities to understand how social bonds are formed and maintained because, as a monogamous species, they form highly selective affiliative bonds between mated partners. Multiple lines of converging evidence suggest that the nucleus accumbens (NAc) plays a critical role in pair bonding. Monogamous prairie voles exhibit high levels of oxytocin receptors in the NAc. Local antagonists or reduction of oxytocin receptors in the NAc inhibits bond formation, while increasing levels of these receptors can enhance bond formation. Moreover, neuroplastic changes within this region may underlie the patterns of bond maintenance. While the NAc has clear importance for pair bonding, very little is known about the neural circuit within the NAc that mediates this behavior, the degree of sexual dimorphism in patterns of circuit activity, and how this circuit changes upon bond formation and over time. In order to understand the neural circuits that underlie these social bonds, we have used miniaturized microscopes to perform freely-

moving calcium imaging in vivo to monitor activity of large populations of NAc neurons in male and female prairie voles during epochs of social interaction. This allowed us to observe population activity in response to interactions with their mate, a novel conspecific, and other non-social stimuli both before and at several time points following bond formation. We have found that NAc neurons are robustly activated in response to social interaction. Our ongoing efforts are aimed at further deciphering how the population code within the NAc is related to interactions with a bonded partner versus a novel conspecific, changes with time after bonding, and the extent to which population dynamics may differ between males and females.

Disclosures: J.L. Scribner: None. E.M. Klein: None. J.C. Jimenez: None. E. Carazo: None. A.M. Cunningham: None. M.A. Kheirbek: None. R. Hen: None. Z.R. Donaldson: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.20/HHH8

Topic: G.02. Motivation

Support: LABEX grant n° ANR-11—LABEX-0042

Agence Nationale de la Recherche grant n° BS4-0010-01

Title: Neural correlates of socio-emotional states in macaques

Authors: *M. JAZAYERI, S. WIRTH, J.-R. DUHAMEL;
Inst. of cognitive neuroscience, CNRS, Lyon, France

Abstract: Most non-human primates live in social environments, where appropriate decisions are believed to contribute to group fitness and seem to be crucial for survival and reproduction. Recent work in non-human primates shows that several brain regions, including prefrontal cortical, limbic and basal ganglia structures are implicated in the processing of motivational and emotional domains, extending to and integrating the social dimension of subjective experiences (1, 2, 3, 4). In our study we used a decision-making task akin to an iterated dictator game where, based on the monkeys' choices, positive or negative reinforcements could be delivered to self, another monkey or to nobody. Our previous behavioral results showed that, in terms of both pro-social decision rates and associated measures of social interaction like mutual gaze and eye blink rates, macaques take into account the welfare of their peers even when this has no impact on their own welfare (5). While monkeys were performing this task we investigated neuronal activity in orbitofrontal cortex (OFC), amygdala (AMY) and anterior insula (AI) by using

multiple contact electrodes. We recorded from one monkey at a time or from both simultaneously, as well as from one brain region or from two areas in parallel, as monkeys alternated in making social decisions. Preliminary neuronal findings show distinct populations of neurons responding differentially to outcomes for self and other, and to appetitive and aversive outcomes. Interestingly, information carried by neurons in the amygdala was highly specific and predicted not only outcome recipients and valences but also differentiated between self and other as the agent of social decisions. Together these results suggest that evaluating the social context of decisions about reward and punishment involves different, distributed subsets of neuronal specialization.

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[5] S. Ballesta, J.-R. Duhamel, Proc. Natl. Acad. Sci. 112 (2015) 15516-15521.

Disclosures: M. Jazayeri: None. S. Wirth: None. J. Duhamel: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.21/HHH9

Topic: G.02. Motivation

Support: NIH NICD K99HD077019

Leon Levy Center for Mind, Brain & Behavior

NSF Center for Brains, Minds & Machines, CCF-1231216/5710003506

NINDS 5 R01 MH105397-02

New York Stem Cell Foundation, Robertson Investigator Award to WAF NYSCF-R-NI23

Title: Neural mechanisms of communication via facial expression

Authors: *S. V. SHEPHERD, W. A. FREIWALD;
Rockefeller Univ., New York, NY

Abstract: Human communication abilities are unique, but build upon a common foundation we share with our primate kin. Primates live in complex social groups with which they must coordinate their behavior, exchanging signals comprising postures, movements, and vocalizations. Scientists have made progress in understanding how facial signals are decoded in primate brains, but know almost nothing about how these signals are produced. We recorded brain activity in monkeys seeing and responding to communicative signals in an MRI scanner. Stimuli were made from video recordings of six monkeys producing a variety of facial expressions. Stimulus videos were simultaneously recorded from multiple angles; a half-silvered mirror was used when recording direct gaze to facilitate naturalistic eye contact. Two 10-second clips were gathered from each monkey, synchronously from three viewpoints. Phase scrambled versions of each video were used as low-level visual controls, and were generated by randomly rotating each Fourier component a consistent amount across each frame. To reduce habituation, we limited exposure to each video. Long (14 second) gaps were placed between videos. Within each run, only one video per subject was shown. Within each session, each video (i.e. subject, event, perspective and scramble) was shown exactly once.
Monkeys produced an affiliative gesture, called a 'lipsmack', in response to brief video of subject-directed monkey expressions. Facial movements were recorded by MR-safe video camera and later analyzed both digitally and by manually scoring behavior while blind to stimulus condition. IRON-fMRI imaging was used to track changes in cerebral blood volume associated with neural activity. By comparing both perceived and produced facial behavior to these simultaneously-recorded brain images, we identified key regions involved in processing social interactions and translating perceived signals into appropriate expressive responses. We find that perception of social stimuli activated the extended face patch system, and that production of the macaque 'lipsmack' signal correlated with activation of facial motor regions. Finally, we examine functional correlations between perceptual and motor face patches to determine candidate pathways by which these socially-driven facial expression may arise.

Disclosures: S.V. Shepherd: None. W.A. Freiwald: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.22/HHH10

Topic: G.02. Motivation

Support: MRC Intramural Research Program MC-A060-5PQ14 (MRC Cognition and Brain Sciences)

Title: Neural encoding of dynamic social interactions

Authors: ***M. AINSWORTH**[1,3], D. KYRIAZIS[2], M. G. KELLY[2], O. JOLY[2], A. H. BELL[2,3]; [2]Dept. of Exptl. psychology, [1]Oxford Univ., Oxford, United Kingdom; [3]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom

Abstract: Over the past decade, numerous studies have identified regions within the primate brain that seem to be necessary for the representation of social information. These include, but are not limited to face-responsive regions, such as the fusiform face area, as well as regions responsive to body parts and species-specific vocalisations. These studies have predominantly, although not exclusively, utilised static images. However, social information in natural environments evolves continuously based on interactions within a group. Therefore, not only is using static images not representative of our daily experience. But more critically - it limits our ability to understand the neural mechanisms by which these regions facilitate our processing of social situations.

In the present study, we collected functional neuroimaging (fMRI) data from awake, behaving macaques presented with video clips. These clips contained a variety of social interactions between macaques. Interactions were classified as "positive" (e.g., grooming), "neutral" (e.g., indifference), or "negative" (e.g., fighting). Three macaques were trained to fixate on a cue and were rewarded while they maintained their gaze within the frame area of the video clip (approx 10º wide). We obtained fMRI data from all subjects (at 3T, 1.5 mm isotropic voxels, TR=2s, TE=19ms; 1320-1760 volumes per session, 12 sessions per macaque). Video clips were presented in 220s runs and consisted of four "ON" blocks (40s) in which clips 5-20s long, were randomly interleaved and three "OFF" blocks (20s) in which only a fixation cue was presented. To identify regions putatively involved in encoding social context and interactions, we contrasted the nature of the interaction (positive, negative) with neutral interactions as well as scenes featuring only one macaque. We included several additional regressors (e.g., number of macaques), as well as nuisance regressors (e.g., luminance changes, overall motion, etc.). Timeseries data were subjected to a general linear model. Consistent with previous studies changes in the number of macaques activated visual and temporal cortex including documented face and body part selective regions. Of note, we found regions that were significantly activated for proximal positive and negative interactions (over neutral). Specifically, ventral prefrontal cortex, inferior and superior banks of the superior temporal sulcus as well as subcortical activations (amygdala and basal ganglia). These data suggest the existence of a network of regions in the macaque brain extending beyond the face-selective patches of the temporal lobe that is responsible for encoding social interactions.

Disclosures: M. Ainsworth: None. D. Kyriazis: None. M.G. Kelly: None. O. Joly: None. A.H. Bell: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.23/HHH11

Topic: G.02. Motivation

Support: Funding from University at Texas at Austin

Title: Effects of varying social interaction parameters to optimize acquisition of a socially transmitted food preference

Authors: *L. AGEE, L. K. CORMACK, M.-H. MONFILS;
Psychology, Univ. of Texas At Austin, Austin, TX

Abstract: Learning through observation of conspecifics is an important form of information gathering in many species. The social transmission of food preference (STFP) paradigm is a common model for studying social learning in rodents, and involves pairing of an observer animal with a demonstrator that has recently eaten food containing a novel flavor (Galef, 2012 for review). Given the choice between two novel flavors, observers are found to show a preference for the flavor that was eaten by their demonstrator. As part of a larger study we will be running using the STFP paradigm, we compared the ability of observer rats to learn a food preference from a demonstrator when learned under two different conditions of either high or low social interaction with their demonstrator. The rats in the low social interaction condition were socially isolated during the demonstrators' food deprivation period and were then given only 15 minutes of social interaction after demonstrators were exposed to the novel food. In the high social interaction condition, rats were kept in the same cage separated by a cage barrier during the food deprivation period, given 30 minutes of social interaction, and the demonstrators' noses were powdered with the appropriate flavor after they ate. In both conditions, rats acquired preference for the demonstrated behavior. However, the effect size for the high social interaction condition, was higher than for the low social interaction condition, and this difference was statistically significant (10,000 bootstrap replications). We conclude that the higher social interaction condition yielded much better learning of the socially transmitted taste preference. This finding hints at the profound importance of the degree of social interaction in transmitting important behaviors amongst conspecifics.

Disclosures: L. Agee: None. L.K. Cormack: None. M. Monfils: None.

Poster

260. Social Behavior: Motivational Systems

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 260.24/HHH12

Topic: G.02. Motivation

Support: NIH Grant AA013983

Title: Aggressive arousal and escalated aggression are dissociated by CRF-amine VTA-DRN-LH microcircuits in mice: targets for alcohol

Authors: H. E. COVINGTON[1], D. STEIN[1], M. LEONARD[1], E. NEWMAN[1], L. M. DARNIEDER[2], M. DAWES[1], K. GOBROGGE[1], J. F. DEBOLD[1], *K. A. MICZEK[3]; [1]Psychology, Tufts Univ., Medford, MA; [2]Neurosci., Tufts Univ., Boston, MA; [3]Moses Hunt Professor of Psychology, Tufts Univ., Medford, MA

Abstract: More than half of violent acts are associated with the consumption of alcohol. However, types of aggression vary widely when considering impulsive vs. premeditated and calculated offenses towards another. Here, aggression and the motivation to commence aggressive acts were quantitatively examined in mice under the following conditions. First, access to the opportunity to engage in fighting behavior was permitted in separate groups of outbred (CFW) and inbred (C57 Bl/6J) mice, using a fixed-interval ten-minute (FI 10) schedule for aggression as reinforcement. Second, the role of corticotrophin releasing hormone (CRH) receptors was examined on aggressive arousal and performance of aggressive acts. Third, mice were trained to self-administer alcohol and then gain access to fighting by their successful completion of behavioral demands. Both inbred and outbred mice were highly motivated by the opportunity to fight and schedule-controlled aggression was significantly escalated (Fish et al. 2004, 2008). Increasing doses of the selective CRH receptor subtype 1 antagonist CP 376395 completely abolish motivational aspects of aggressive behavior without influencing the escalation of attack behaviors during confrontations with an intruder. As expected, self-administered alcohol dose-dependently modulates both the motivation to engage in aggression and fighting performance. Experiments using optogenetics further examine the particular role of CRH neuronal populations on alcohol-escalated aggressive behaviors. Both the dorsal raphe nucleus and hypothalamus modulate aggressive behavior, but CRH projections from these areas to the posterior ventral tegmental area appear to contribute uniquely towards aggressive arousal or the escalation of aggressive acts. In sum, these experiments are aimed at identifying the motivational components of premeditated violence and the neural elements that control reactive “hot” acts of aggression commonly associated with sympathetic activation.

Disclosures: H.E. Covington: None. D. Stein: None. M. Leonard: None. E. Newman: None. L.M. Darnieder: None. M. Dawes: None. K. Gobrogge: None. J.F. DeBold: None. K.A. Miczek: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.01/HHH13

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R15MH100689-01

Title: Post-retrieval midazolam inhibits ethanol memory reconsolidation in adolescent rats

Authors: *K. BYRKET, J. HICKEY, A. SIBERT, E. SMALLEY, J. J. QUINN;
Miami Univ., Oxford, OH

Abstract: Memory retrieval often induces a time-dependent period of destabilization followed by reconsolidation. Destabilization of the memory occurs upon retrieval. Together these processes allow long-term memories to be updated with new information. Both aversive and appetitive memories have been shown to undergo reconsolidation. The ability to update appetitive memories, in particular, has relevance to the treatment of substance abuse disorders. The present experiment addressed whether ethanol conditioned place preference (CPP) memories undergo reconsolidation following a non-reinforced retrieval session in male and female adolescent rats. On day 1, rats underwent baseline preference testing during which they were allowed unrestricted access to all three compartments of the conditioning chamber for 15 minutes. On days 2-9, rats were trained using alternating daily exposures to the ethanol-paired and control-paired compartments (four exposures to each). On ethanol days, rats were allowed access to 10g of ethanol gelatin while restricted to the ethanol-paired compartment for 30 minutes. On control days, rats received access to the control gelatin in the control-paired compartment for 30 minutes. On day 10, rats were placed into the ethanol-paired compartment for 10 minutes with no access to gelatin. Immediately following this reactivation session, rats received an injection of midazolam (MDZ; 3mg/kg) or vehicle. The next day, rats were tested for 15 minutes with access to all three compartments (identical to the baseline test). The number of entries into and time spent in each compartment were assessed. Vehicle-injected control rats did not appear to acquire a preference for either compartment. However, MDZ-injected rats spent significantly less time in the ethanol-paired chamber, compared to controls. These data suggest that the rats had acquired a memory for each compartment, and this memory was susceptible to reconsolidation upon retrieval. No sex differences were apparent. We are currently assessing

whether extended training will yield a strong preference for either chamber and whether this stronger memory will be susceptible to reconsolidation. Together, these investigations will provide valuable information regarding appetitive memory reconsolidation in adolescence, with relevance to the heightened susceptibility to substance abuse behaviors observed during this developmental period.

Disclosures: K. Byrket: None. J. Hickey: None. A. Sibert: None. E. Smalley: None. J.J. Quinn: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.02/HHH14

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R15MH100689-01

Title: The role of NMDA receptors in the retrieval and reconsolidation of context fear discrimination memory

Authors: *D. E. KOCHLI, A. F. POSTLE, R. S. LAB, E. W. HOLLINGSWORTH, T. L. CAMPBELL, V. C. MORDZINSKI, J. J. QUINN;
Psychology, Miami Univ., Oxford, OH

Abstract: Mounting evidence suggests that, under certain conditions, retrieved memories become destabilized and must be re-stabilized via a process termed reconsolidation. However, boundary conditions on the induction of the process are not well understood. In addition, the evidence for the role of NMDA receptors in reconsolidation is somewhat inconsistent; some studies suggest that NMDAr antagonism is sufficient to disrupt reconsolidation *per se*, while others suggest NMDArs play a role in memory destabilization but not reconsolidation. Further, there is mixed evidence as to whether or not NMDArs are necessary for the retrieval of a previously learned memory. The present work displays strong evidence that NMDArs are necessary for the retrieval of a context fear discrimination memory. While previous work from our lab demonstrates that well-learned context discrimination memories undergo protein-synthesis-dependent reconsolidation, the present work suggests that less well-learned discriminations do not. This points to a clear role for prediction error as a boundary condition of discrimination memory reconsolidation; if there is no clear prediction to violate, then there is no error present to initiate the reconsolidation process. Alternatively, it is possible that the memory for the shock-reinforced context may be overtrained. As animals reach asymptotic levels of

freezing to the reinforced context by approximately day four of training but do not reach appropriate levels of discrimination (i.e. low freezing in the non-reinforced context) until day 10, it is possible that the CTX+ association is being overtrained. It is well-documented that strong memories are resistant to reconsolidation. Current efforts are exploring this question with an alternative training approach aimed at minimizing overtraining by including a greater number of exposures to CTX- compared to CTX+ over the course of training. Demonstration of a reconsolidation deficit with this approach will provide further evidence supporting memory strength as a boundary condition, and will allow us to better assess the contributions of NMDArs to memory destabilization and reconsolidation.

Disclosures: D.E. Kochli: None. A.F. Postle: None. R.S. Lab: None. E.W. Hollingsworth: None. T.L. Campbell: None. V.C. Mordzinski: None. J.J. Quinn: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.03/HHH15

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R15MH100689-01

Title: The effect of extinction on the destabilization of an overtrained memory

Authors: *M. MCDANIEL, M. DUNN, M. MYERS, V. DIANA;
Psychology, Miami Univ., Oxford, OH

Abstract: Reconsolidation is a process by which memories are destabilized, updated, and then restabilized. Strong memories are resistant to undergoing reconsolidation. Therapeutically, this resistance has proven difficult since behavioral and pharmacological interventions that interfere with memory reconsolidation in the laboratory often are of little efficacy in treating strong emotional memories in the clinic. In the present experiment, we addressed whether an overtrained fear memory could be made susceptible to reconsolidation by first extinguishing, and then renewing, the memory. Rats were trained with ten tone-shock pairings, followed by eight days of tone extinction in the same context. The next day, rats were placed into a second context and memory for the tone was reactivated/renewed with a single tone presentation. Immediately following reactivation, rats received an injection of Midazolam or vehicle. Midazolam has previously been shown to disrupt the reconsolidation of fear memories. Rats were then tested for freezing to the tone in a third context. Results indicate that Midazolam had no effect in rats that did not undergo tone extinction. This is consistent with previous finding suggesting that strong

memories do not undergo reconsolidation upon retrieval. Rats that underwent tone extinction froze less than those that received no extinction. This is consistent with findings showing that renewal of an extinguished memory tends to be less that the original, non-extinguished, memory. Importantly, Midazolam significantly attenuated freezing to the tone in extinguished rats. This suggests that those rats that received tone extinction underwent tone memory reconsolidation following its renewal. These data suggest that strong emotional memories may, in fact, be capable of updating following weakening of memory expression through extinction. This could prove invaluable for therapeutic approaches that attempt to weaken the impact of debilitating memories.

Disclosures: M. McDaniel: None. M. Dunn: None. M. Myers: None. V. Diana: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.04/HHH16

Topic: H.01. Animal Cognition and Behavior

Support: NIH R15MH100689-01

Title: The role of the ventral tegmental area in the reconsolidation of context fear discrimination memory

Authors: M. M. PERRY, J. L. GENARO, H. E. LINDNER, C. N. DYE, D. E. KOCHLI, R. J. FLOYD, *J. J. QUINN;
Psychology, Miami Univ. Dept. of Psychology, Oxford, OH

Abstract: Under certain conditions, retrieved memories become destabilized and must be re-stabilized via the reconsolidation process. However, conditions necessary for the induction of the process are not well understood. A body of research suggests that a prediction error must occur to initiate memory destabilization. It has been suggested that dopamine signaling may critically support the signaling of a prediction error. Inactivation of the VTA, the principal source of dopaminergic input to forebrain and limbic structures, prevents destabilization of appetitive memories. However, much less is known regarding dopamine-mediated destabilization of aversive memories. The present work explored whether VTA inactivation with a muscimol/baclofen cocktail would prevent memory destabilization and subsequent disruption of reconsolidation via systemic administration of the $GABA_A$ agonist, midazolam. While earlier work from our lab shows that well-learned context discrimination memories are destabilized following a non-reinforced reactivation trial in CTX+, the present study suggests that less well-

learned discriminations are not. This pattern suggests that a prediction error is requisite for the initiation of the reconsolidation process. With no clear prediction to violate, omission of the US does not initiate reconsolidation. It is also possible that the memory for CTX+ was overtrained. Because animals reach asymptotic levels of freezing to CTX+ after five days of training but do not display robust discrimination (i.e. low freezing in CTX-) until days 10-12, it is possible that appreciable overtraining occurrs. It has been demonstrated that strong memories are resistant to reconsolidation. We are currently exploring this possibility with an alternative training approach designed to eliminate overtraining. This approach includes more exposures to CTX- relative to CTX+ during discrimination training. Demonstrating a reconsolidation deficit using this approach would provide further evidence that memory strength can serve as a boundary condition. Additionally, this would allow us to assess the role of dopaminergic signaling in memory destabilization.

Disclosures: M.M. Perry: None. J.L. Genaro: None. H.E. Lindner: None. C.N. Dye: None. D.E. Kochli: None. R.J. Floyd: None. J.J. Quinn: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.05/HHH17

Topic: H.01. Animal Cognition and Behavior

Support: Development Center of Research and Graduate Studies of the Faculty of Medicine of Itajubá

Foundation for Research Support of the State of Minas Gerais

Title: Effects of the swimming exercise on the consolidation and persistence of auditory and contextual fear memory

Authors: *G. T. REIS[1], A. B. BERETA[2], L. S. GUTIERRES[2], F. F. C. SOBRINHO[2], I. D. MIRANDA[2], J. DOS REIS[2], E. V. DIAS[2], C. R. SARTORI[3], R. S. DE FARIA[2], D. A. R. MOREIRA[2];
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Abstract: Exposure to negative environmental events triggers defensive behavior and leads to the formation of aversive associative memory. Cellular and molecular changes in the central nervous system underlie this memory formation, as well as the associated behavioral changes. In

general, memory process is established in distinct phases such as acquisition, consolidation, evocation, persistence, and extinction of the acquired information. After exposure to a particular event, early changes in involved neural circuits support the memory consolidation, which corresponds to the short-term memory. Re-exposure to previously memorized events evokes the original memory, a process that is considered essential for the reactivation and consequent persistence of memory, ensuring that long-term memory is established. Different environmental stimuli may modulate the memory formation process, as well as their distinct phases. Among the different environmental stimuli able of modulating memory formation is the physical exercise which is a potent modulator of neuronal activity. There are many studies showing that physical exercise modulates learning and memory processes, mainly in the consolidation phase of the explicit memory. However, there are few reports in the literature regarding the role of physical exercise in implicit aversive associative memory, especially at the persistence phase. Thus, the present study aimed to investigate the relationship between swimming exercise and the consolidation and persistence of contextual and auditory cued fear memory. Male Wistar rats were submitted to sessions of swimming exercise five times a week, over six weeks. After that, the rats were submitted to classical aversive conditioning training by a pairing tone/foot shock paradigm. Finally, rats were evaluated for consolidation and persistence of fear memory to both auditory and contextual cues. Our results demonstrate that classical aversive conditioning with tone/foot shock pairing induced consolidation as well as persistence of conditioned fear memory. In addition, rats submitted to swimming exercise over six weeks showed an improved performance in the test of auditory cued fear memory persistence, but not in the test of contextual fear memory persistence. Moreover, no significant effect from swimming exercise was observed on consolidation of both contextual and auditory fear memory. So, our study, revealing the effect of the swimming exercise on different stages of implicit memory of tone/foot shock conditioning, contributes to and complements the current knowledge about the environmental modulation of memory process.

Disclosures: G.T. Reis: None. A.B. Bereta: None. L.S. Gutierres: None. F.F.C. Sobrinho: None. I.D. Miranda: None. J. dos Reis: None. E.V. Dias: None. C.R. Sartori: None. R.S. de Faria: None. D.A.R. Moreira: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.06/HHH18

Topic: H.01. Animal Cognition and Behavior

Support: FAPEMIG

NDPPG

Title: Effects of the swimming exercise on the extinction of contextual fear memory

Authors: *A. L. BERETA[1], G. H. T. REIS[2], M. S. GOMES[2], L. B. BRAGA[2], E. V. DIAS[3], C. R. SARTORI[3], R. S. FARIA[2], D. A. R. MOREIRA[2];
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Abstract: OBJECTIVE:The present study aimed to investigate the relationship between swimming exercise and the extinction memory. METHODS: Male Wistar rats were submitted to sessions of swimming exercise five times a week, over six weeks. After that, the rats were submitted to aversive conditioning training. After 28 days rats were evaluated for exctintion of fear memory to contextual cues. The following groups were formed: Sedentary NAIVE: Not exercised rats, unconditioned;Trained NAIVE: Exercised swimming rats , unconditioned; Sedentary COND: Not exercised rats, submitted to fear conditioning and evaluated 28 days after the conditioning training, Trained COND: Exercised swimming rats, submitted to fear conditioning and evaluated 28 days after the conditioning training. RESULTS: It was observed that on day one of extinction test the rats of the groups conditioned to aversive training: Sedentary Conditioned, Trained and Conditioned showed no statistical differences between them ($p > 0.05$; $F3.31 = 1.71$; $P = 0.4901$). The analysis post hoc Tukey's multiple comparison tests indicated that the mice Sedentary Naive groups and trained Naive differ significantly from Sedentary Conditioned groups. Trained and Conditioned ($p < 0.0001$). It was observed that the fifth day of extinction test, unlike the first day, the rats of Group Trained Conditioned demonstrated lower freezing value when compared to the group of rats Sedentary Conditioned ($F3.31 = 71.38$; $p < 0.001$; one-way ANOVA followed by Tukey-Kramer test for multiple comparisons). Additionally, it was observed that member groups that did not go through aversive conditioning training (Sedentary Naive, Trained Naive) had a lower percentage of freezing when compared to mice conditioned Sedentary groups Conditioned, Trained Conditioned ($F3.31 = 71.38$; $p < 0.001$; one-way ANOVA followed by Tukey-Kramer test for multiple comparisons). CONCLUSION: Our results showed that exercised rats had the extinction of the context memory first than the sedentary ones. Demonstrate that swimming exercise induced a positive effect in the reconsolidation memory. So, our study, revealing the effect of the swimming exercise on different stages of implicit aversive memory, contributes to and complements the current knowledge about the environmental modulation of memory process.

Disclosures: A.L. Bereta: None. G.H.T. Reis: None. M.S. Gomes: None. L.B. Braga: None. E.V. Dias: None. C.R. Sartori: None. R.S. Faria: None. D.A.R. Moreira: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.07/HHH19

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Saliency and distributed conditioning contribute to making context fear memory independent of the hippocampus

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Abstract: Damage to the hippocampus (HPC) following contextual fear conditioning in rats typically causes retrograde amnesia, but distributing/repeating the conditioning over several sessions can mitigate the retrograde amnesic effects. Hence, context fear memory can rapidly become independent of the HPC over repeated learning episodes. It is unclear, however, whether repetition of the learning episodes alone is sufficient or whether a combination of repetition and high conditioning saliency is necessary to make the context fear memory become HPC independent. To examine this possibility, rats were trained under a distributed contextual fear conditioning protocol (10 single shock sessions; two per day for five days) using either a 0.4, 0.7, or 1.0 mA shock intensity, which respectively corresponded to low, intermediate, and high saliency. Three to five days after the last conditioning session, the rats received sham surgery or neurotoxic lesions of the HPC and 12-14 days later were tested for memory of the context fear. During the retention test, the rats with HPC damage from the low saliency condition (0.4 mA) froze significantly less than their respective sham control group, suggesting that the damage caused retrograde amnesia despite the distributed conditioning. The same pattern was observed with the rats in the intermediate saliency condition (0.7 mA). In contrast, the rats with complete HPC damage in the high saliency condition (1.0 mA) did not freeze significantly less than their respective sham control group, suggesting that the lesions failed to cause retrograde amnesia in this instance. Thus, repeated contextual fear conditioning episodes can cause context fear memory to become independent of the HPC, but only when the saliency of the event is high and causes strong conditioning.

Disclosures: S. Kishun: None. K. Jungkunz: None. C. Carter: None. H. Lehmann: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.08/HHH20

Topic: H.01. Animal Cognition and Behavior

Title: Persistence of long-term contextual fear memory in the absence of the hippocampus.

Authors: *D. C. GIDYK, R. J. MCDONALD, R. J. SUTHERLAND;
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Abstract: Contextual fear memory is among the most robust long-term memories (LTM). During one conditioning session, intact rats rapidly form a memory that is maintained for very long intervals (for example, 180 days, Lehmann et al. 2007). The pattern of results with complete HPC damage includes: 1) if HPC damage follows the learning episode, there is profound retrograde amnesia, regardless of how long after the conditioning session the damage takes place (Broadbent & Clark, 2013; Sutherland et al., 2010); 2) if HPC damage precedes the learning episode, there is little if any evidence of anterograde amnesia (Maren et al. 1997, Wiltgen et al. 2006). Thus, LTM for contextual fear can be supported by the HPC and non-HPC networks. However, it is likely that that some properties of HPC and non-HPC long-term memories differ. One recent study measured whether non-HPC contextual fear memory in rats decays more rapidly over time (Zelikowsky et al., 2012). Briefly, prior to contextual fear conditioning, rats received either neurotoxic lesions to the dorsal hippocampus (dHPC), or sham lesions. Rats were then tested for retention of learned fear 1, 3, 10, or 30 days later. Intact rats displayed high levels of freezing at all time points. Rats with dHPC damage displayed memory of the experience one day, but not 30 days later, indicating accelerated decay of the contextual fear memory. These data were interpreted as evidence for the dHPC being necessary for consolidation of contextual fear memory in non-HPC regions. Given that the vHPC was intact, it is unclear if the reported pattern of memory decay was due to a non-HPC memory being less resilient to decay, or whether a dysfunctional memory was acquired by the partially compromised HPC and was more prone to decay. The present study is an attempt to replicate Zelikowsky et al (2012), with the addition of a 16 site complete HPC lesion group. Neither lesion group exhibited rapid decay of a context fear memory. Our results suggest that non-HPC systems create a long-lasting context fear memory in the absence of HPC.

Disclosures: D.C. Gidyk: None. R.J. McDonald: None. R.J. Sutherland: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.09/HHH21

Topic: H.01. Animal Cognition and Behavior

Support: CONACYT (Grant 130524, Scholarship to RP-L 342154)

PAPIIT-UNAM IN214111, IN202414 and IN208812.

Title: Glucocorticoid receptor phosphorylation in the amygdala and hippocampus after acquisition of contextual fear conditioning

Authors: R. PONCE-LINA, M. CARRANZA, N. SERAFIN, R. A. PRADO-ALCALÁ, *G. L. QUIRARTE;
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Abstract: Glucocorticoid release during the acquisition process facilitates memory consolidation, especially of highly emotional or stressful experiences. This effect depends on several factors, among them is glucocorticoid receptor (GR) activation. The GR is a transcriptional factor whose transcriptional activity and cellular localization depends on its phosphorylated state. The present work aimed to investigate the effect of contextual fear conditioning training on the phosphorylation of GR at serine 211(Ser 211) and serine 226 (Ser 226) in the amygdala and the hippocampus. Male Wistar rats (250-350 g) were trained in contextual fear conditioning in a single session (11 min) under different foot-shock intensities (0.0, 0.5, or 1.5 mA). There were two groups for each intensity, in the first one the subjects were tested for memory retention at 48 h and the other group was sacrificed 1 h after training, and the amygdala and the hippocampus were extracted. Total GR and GR phosphorylated at Ser 211 and Ser 226 levels were measured by SDS-PAGE/WB. Rats trained with 0.5 and 1.5 mA learned the task. The optical density of total GR was the same for all the intensities in the amygdala and the hippocampus, with higher levels in the hippocampus. Density of GR phosphorylated at Ser 211 was higher than Ser 226 in the two brain areas and there were no differences among intensities. Phosphorylation at Ser 211 was higher in dorsal hippocampus and amygdala, whereas phosphorylation at Ser 226 was higher in ventral hippocampus. Phosphorylation ratio Ser 211/Ser226 showed that dorsal hippocampus and amygdala had the highest levels. These results indicate that in the acquisition phase of the contextual fear conditioning task, GR phosphorylation is not a process that depends on the stressor intensity during training, and that those areas involved in context-footshock association (dorsal hippocampus and amygdala) have the highest levels of GR phosphorylated at Ser 211, which probably could be related to an increase of transcription of molecules involved in plasticity. We acknowledge the technical assistance of, Cristina Medina, Bertha Islas Rivas Martín García, Leonor Casanova, Omar

González, Sandra Hernandez and Lourdes Lara. This work was funded by CONACYT (Grant 130524, Scholarship to RP-L 342154) and PAPIIT-UNAM IN214111, IN202414 and IN208812.

Disclosures: R. Ponce-Lina: None. M. Carranza: None. N. Serafin: None. R.A. Prado-Alcalá: None. G.L. Quirarte: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.10/HHH22

Topic: H.01. Animal Cognition and Behavior

Support: 973 2013CB835100

Title: Maternal seperation exaggerates spontaneous recovery of extinguished contextual fear in adult female rats

Authors: *W. LIPING[1], G. XIONG[2], L. XU[2];
[1]Kunming Inst. of Zoology, Yunnan Province, China; [2]Kunming Inst. of Zoology, CAS, Kunming, China

Abstract: Early life stress increases the risk ofposttraumatic stress disorders (PTSD). Patients with PTSD show impaired extinction of traumatic memory, and in women, this occurs more often when PTSD is preceded by child trauma. However, it is still unclear how early life stress accounts for extinction impairment.Here, we studied the effects of maternal separation (MS, postnatal day 2 to 14)on contextual fear extinction in adult female rats. Additionally,to examinechangesin synaptic functionaffected by MS, we measured long-term potentiation (LTP) in prefrontal cortex and hippocampus in vitro, both of which have been implicated in fear extinction. We found that adult female rats had been subjected to MS exhibited significantspontaneous recovery of fearto the extinguished context.Furthermore, MS exposure resulted in LTP impairment in both infralimbic prefrontal cortex layer 2/3-layer 5 and hippocampal SC-CA1 pathways. Interestingly, no obvious effectsof MS on contextual fearconditioning, fear recall as well as extinction training and recall were observed. Innate fear in the elevated plus maze or open fieldtest remained nearly unaffected. These findings provided the first evidence that MSmayexaggerate spontaneous recovery after contextual fear extinction, forwhich LTP impairmentin the medial prefrontal cortex and hippocampus may be responsible, thereby possibly leading to impaired extinction associated with PTSD.

Disclosures: W. Liping: None. G. Xiong: None. L. Xu: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.11/HHH23

Topic: H.01. Animal Cognition and Behavior

Support: Call for Research Proposals Orlando Fals Borda 2015-2016. Research Division Bogotá Campus, National University of Colombia. Project No. 32928.

Title: Effects of stress and corticosterone on spatial memory consolidation and Arc protein expression in hippocampal dentate gyrus in rats

Authors: *J. M. CERÓN GONZÁLEZ, M. LAMPREA;
Psychology, Natl. Univ. of Colombia, Bogota, Colombia

Abstract: Memory consolidation is the process of gradual stabilization of long-term memory after learning has occurred. This process requires activation of intraneuronal molecular cascades that regulate patterns of gene expression and protein synthesis in specific brain regions. Previous work has shown that memory consolidation can be modulated by different post-training interventions, either impairing or facilitating long-term retention of recently acquired information. The current work shows that exposure to a mild stressor or the exogenous administration of the glucocorticoid corticosterone immediately after training in the Barnes maze, enhances long-term retention in this task. Thus, during the memory retention test, stress and corticosterone-treated animals exhibited decreases in exploration errors and escape latency as well as an enhancement of spatial preference compared to their respective control groups. Additionally, it was found that exposure to a stressor or corticosterone administration alone did not modify the basal levels of the activity-regulated cytoskeletal-associated protein (Arc) in the hippocampal dentate gyrus. Nevertheless, the eight-trial training session in the Barnes maze led to a significant increase in Arc protein expression in this brain region predominantly observed in the dorsal blade and measured two hours after training had finished. Importantly, this induction was not significantly modified by post-training stress or corticosterone. The present findings suggest that post-training exposure to stress and glucocorticoids enhance spatial memory consolidation and this effect does not seem to involve a modulation in the expression pattern of Arc protein in the dentate gyrus of the hippocampus in the time point evaluated.

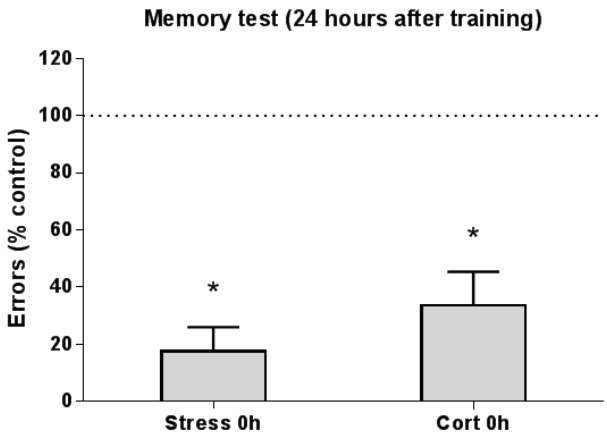

Figure 1. Non-goal hole explorations during the memory test (number of times the animal pokes its nose into a hole that did not contain the escape box during training). Data are expressed as normalized to the respective control group performance during the test (*): p<0.05 (n=8 each group). Abbreviations: Cort: corticosterone injection; 0h: treatment immediately after training.

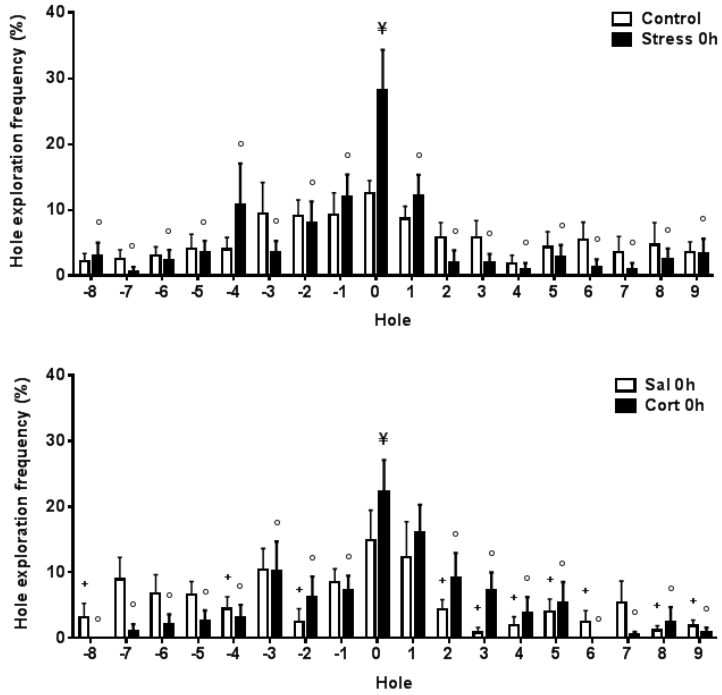

Figure 2. Hole exploration frequency (escape-hole is numbered as hole 0 and the remaining holes are numbered 1 to 9 clockwise, and −1 to −8 counterclockwise). (+): p <0.05 compared to escape-hole exploration frequency within the control group. (°): p <0.05 compared to escape-hole exploration frequency within the experimental group. (¥): p <0.05 compared to escape-hole exploration frequency in the respective control group.

Disclosures: J.M. Cerón González: None. M. Lamprea: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.12/HHH24

Topic: H.01. Animal Cognition and Behavior

Title: The dynamic nature of systems consolidation stress during learning as a switch guiding the rate of the hippocampal dependency and memory quality

Authors: *L. K. PEDRAZA CORREA[1], R. SIERRA[2], F. BOOS[2], J. HAUBRICH[2], J. QUILLFELDT[2], L. ALVARES[2];
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Abstract: Memories fade after a time point, becoming more schematic or abstract. The loss of contextual detail in memory may reflect a time-dependent change in the brain structures supporting memory. It is well established that contextual fear memory relies on the hippocampus for expression shortly after learning, but becomes hippocampus-independent at later time point, a process called systems consolidation. This time-dependent process correlates with the loss of memory precision. Here, we investigated whether the training intensity predicts the gradual decay of the hippocampal dependency to retrieve memory and the quality of the contextual memory representation overtime. We found that learning intensity modulates the progressive decay of the hippocampal dependency and memory precision. Strong training intensity accelerates systems consolidation and memory generalization in a remarkable timeframe match. The mechanisms underpinning such process are triggered by the glucocorticoid and noradrenaline released during training. These results suggest that the stress/arousal levels during emotional learning act as a switch, guiding the fate of memory quality. A moderate stress will create a detailed memory, whereas a highly stressful training will develop a generic gist-like memory.

Disclosures: L.K. Pedraza Correa: None. R. Sierra: None. F. Boos: None. J. Haubrich: None. J. quillfeldt: None. L. Alvares: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.13/HHH25

Topic: H.01. Animal Cognition and Behavior

Support: ANPCYT Grants PICT2010 1528 and 1482

ANPCYT Grant PICT2012 2369

ANPCYT Grant PICT2013 0375

CONICET Grant PIP 2010-2012- 00005

UBACYT Grant B018; 2011-2013 – 20020100100683

UBACYT Grant 2014-2017 - 20020130100881BA and 20020130200283BA

Title: Reconsolidation-induced memory persistence: participation of late phase hippocampal ERK activation

Authors: *M. KRAWCZYK[1], N. NAVARRO[1], M. BLAKE[2], A. ROMANO[3], M. FELD[3], M. BOCCIA[1];
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Abstract: The reconsolidation hypothesis posits that after reactivation,memory goes from an inactive to an active,labile state, and it needs a re-stabilization-storage process as during consolidation. Persistence is an attribute of long-term memories (LTM) that has recently caught researcher´s attention in search for mechanisms triggered by experience that assure memory perdurability. However, up-to-date, scarce evidence of relationship between reconsolidation and persistence has been described. We have recently shown that reconsolidation-induced cytosolic ERK activation can be modulated in mice hippocampi to regulate memory strength using a pharmacological approach on the inhibitory avoidance task (Krawczyk et al. 2015). Here, we broadened our studies by further characterizing ERK participation in long-term memory stabilization for longer periods. Although intra-dorsal-hippocampal (dHIP) administration of an ERK inhibitor (PD098059, PD) 3 h after retrieval did not affect reconsolidation, when tested at 24 h, it impaired performance when animals were tested 7 days after memory reactivation, regardless of the training´s strength; and being specific to memory reactivation. To the best of

our knowledge, this is the first report showing that persistence might be triggered after memory reactivation involving an ERK/MAPK-dependent process.

Disclosures: M. Krawczyk: None. N. Navarro: None. M. Blake: None. A. Romano: None. M. Feld: None. M. Boccia: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.14/HHH26

Topic: H.01. Animal Cognition and Behavior

Support: NHMRC Grant

ARC Grant

Title: Role of NMDA receptors in the storage of conditioned fear memory

Authors: *S. YAN, F. WINDELS, P. SAH;
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Abstract: The N-methyl-D-aspartate (NMDA) receptor is a ligand-gated ion channel that plays important roles in synaptic plasticity and learning. Studies have shown that activation of NMDA receptors during fear conditioning is essential for the acquisition of conditioned fear memory. However, whether NMDA receptors are activated after fear conditioning is unclear. To address this question, we injected NMDA receptor competitive antagonist 3-[(+)-2-carboxypiperazin-4-yl]-propyl-l-phosphonic acid (CPP) intraperitoneally (10mg/kg) into adult male Sprague-Dawley rats immediately after discriminative auditory fear conditioning. Conditioned memory was tested 24 h after conditioning, and freezing measured during the presentations of danger (CS+) and safety (CS-) cues. The results showed that post-conditioning blockage of NMDA receptors partially disrupt storage of discriminative memory: animals showed less freezing to CS+, while freezing was not affected during CS-. We then investigated the effect of local NMDA receptor inactivation in a series of brain regions that have been proposed to be involved in the storage of fear memory. Guide cannulas were bilaterally implanted into the basolateral amygdala (BLA), the medial division of medial geniculate nucleus (mMGN), or the perirhinal cortex (PRh) in different groups of rats. Either 0.5 μL or 1 μL CPP solution (60ng/μL) was injected into the target areas (0.5 μL/min) immediately after discriminative fear conditioning. Animals receiving post-conditioning CPP infusion into the PRh showed reduced fear response to CS+ and unaffected freezing during CS-, similar to the result observed after post-conditioning CPP

intraperitoneal injection, suggesting that the amnesiac effect of systemic CPP injection was partly due to inactivation of NMDA receptor in the PRh. In contrast, post-conditioning CPP infusions into the BLA or MGN did not significantly affect the retention of discriminative memory. Our data suggest a post-learning consolidation phase that is NMDA receptor dependent, and the activation of NMDA receptors in the PRh is involved in the processing of fear memory after learning.

Disclosures: S. Yan: None. F. Windels: None. P. Sah: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.15/HHH27

Topic: H.01. Animal Cognition and Behavior

Title: Assessment of memory mechanisms in rat brain by administration of the NMDA receptor antagonist AP5 using *In vivo*, *In vitro* and behavioral methods.

Authors: *C. W. SPANIS[1], R. R. BEYER[2];
[1]Neurosci., Univ. San Diego, San Diego, CA; [2]Neurosci., Univ. of San Diego, San Diego, CA

Abstract: The first set of experiments were designed to evaluate memory by observing an emotionally learned task and how and when the task was impaired, using AP5, considered causative of impairing NMDA receptors in the basolateral amygdala. Our work adds to past studies on impairment to the hippocampus. Pre-training infusion of AP5 did not affect the training acquisition but blocked the retention performance, given adequate training trials. Posttraining administration of AP5 also impaired retention performance in a staggered time frame, which lessened from significant impairment at five minutes post administration to no impairment at administration of drug given at 30 minutes post administration time. The second study involved in vivo injections of AP5 into the hippocampus in regions between CA1 and CA3. We noted a comparable result to study one, with impairment of LTP formations following injections of the drug. In the third series of experiments involving use of in vitro methods, we noted total loss of LTPs formed at loci between CA1 and CA3 following injections of AP5. The employment of AP5 in three different experimental procedures has allowed us to offer additional information on how memory works; in this case, at the level of activation or inhibition of NMDA receptors either in the BLA or the hippocampus using behavioral and/or in vivo and in vitro methods.

Disclosures: C.W. Spanis: None. R.R. Beyer: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.16/HHH28

Topic: H.01. Animal Cognition and Behavior

Support: Fondation pour la recherche médicale

ARN

CNRS

Title: Observational learning of a spatial discrimination task in the rat

Authors: *P. MEYRAND[1], B. JURA, Jr[2], B. BONTEMPI[1], T. BEM, s[2];
[1]Inst. des Maladies Neurodégénératives, CNRS UMR5293 Univ. de Bordeaux, Pessac Cedex, France; [2]Lab. of Physiol. and Modelling of Neural Networks, Nalecz Inst. of Biocybernetics and Biomed. Engin. Polish Acad. of Sci., Warsaw, Poland

Abstract: Learning by observing others has long been acknowledged as a powerful learning strategy. In several species, it has been reported that prior observation of fear conditioning or other operational procedures can improve subsequent acquisition. However, what an animal may gain by simply observing a conspecific performing a spatial discrimination task remains to be further explored. To this end, we have developed an original procedure in which Long Evans adult rats are submitted to spatial memory testing in an 8-arm radial maze. Inexperienced food-deprived observer rats (rats observing a demonstrator, RODs) were placed in a mesh cage positioned above the maze while a demonstrator rat was trained to locate the spatial position of three constantly baited arms of the maze. Each trial was terminated after the demonstrator retrieved the third food pellet and had returned to the central platform of the maze. During the observational period, each ROD was assigned a set of two naive demonstrators performing 6 trials per day during 9 days. The control group consisted of rats placed in the observation cage and looking at the empty maze (rats observing the maze, ROMs) for the same amount of time as RODs. After 9 days of observation, RODs and ROMs were placed in the maze and tested according to the same protocol as the demonstrators (9 daily sessions of 6 trials). Our findings reveal that RODs were capable of integrating relevant spatial information during the observational period and of using it efficiently when later submitted to spatial discrimination testing in the maze. Whereas both ROMs and demonstrator rats needed 9 days of learning to achieve mastery of the task with a low level of reference memory errors (less than 1.5 per trial), RODs needed only 4 days of testing to reach a similar level of performance. This beneficial effect of observation relied primarily on observing errors made by demonstrator rats. Indeed, RODs did not express any gain of observation when able to observe previously trained

demonstrators performing the task perfectly since the very first day of demonstration. These data challenge previously reported results using operant conditioning and suggesting that the improvement in performance of observers is related to the absence of any mistake during demonstration.

Disclosures: P. Meyrand: None. B. Jura: None. B. Bontempi: None. T. Bem: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.17/HHH29

Topic: H.01. Animal Cognition and Behavior

Support: NSF DGE-0808392

NSF Grant #1146708

NIH Grant NS045260

ONR Grant N00014-10-1-007

Title: Past experience with complexity enables strategy formation and rapid learning by rats in novel environments, without affecting synapse counts.

Authors: *C. D. COX, L. C. PALMER, D. T. PHAM, B. H. TRIEU, J. HUI, K. NGUYEN, I. MANFREDO, C. M. GALL, G. LYNCH;
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Abstract: Most learning in nature occurs in complex environments with minimal obvious reward or punishment. Whether animals transfer information and strategies gained from past experience with complexity to novel, challenging environments, as is routine with humans, is a fundamental question that has received little attention. In the present study, we used a free exploration task that was too complex to be learned in a 30 minute session by rats given extensive handling or several days of free exercise. In contrast, rats that had spent an equivalent period in a social, multilevel complex environment prior to exploration rapidly encoded both short- and long-term memory in the apparatus. The three groups were not detectably different on various measures of arousal and anxiety. Markov Sequence Analysis showed that rats with prior exposure to the enriched environment (EE) were less predictable with regard to future locations after long movement sequences than the handled or exercise groups. Consistent with this, the EE animals also repeated long sequences to a greater degree. Both the predictability and sequence effects

were markedly greater in a second 30-minute long session conducted 24 hours later. We propose that the results reflect a shift from local to global processing of information due to the transfer of search strategies formed during prior experience with a different complex environment. We tested if the extensive, experience based learning that occurred during EE exposure was accompanied by an increase in synapse numbers in hippocampus, as assayed by quantifying PSD95 immunoreactive elements. The results were negative across 14 hippocampal sampling fields; there was, however, a detectable shift towards larger synaptic volumes in the dentate gyrus molecular layer of EE rats. In sum, these findings demonstrate that rats transfer past experience-based learning to new circumstances resulting in the rapid formation of information processing strategies and rapid learning. The material acquired during past interactions was not encoded by increased numbers of synapses.

Disclosures: C.D. Cox: None. L.C. Palmer: None. D.T. Pham: None. B.H. Trieu: None. J. Hui: None. K. Nguyen: None. I. Manfredo: None. C.M. Gall: None. G. Lynch: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.18/HHH30

Topic: H.01. Animal Cognition and Behavior

Title: Novel object recognition: importance of counterbalancing and retention intervals

Authors: *M. CANEN, A. M. BABCOCK;
Psychology, Montana State Univ., Bozeman, MT

Abstract: Murine novel object recognition paradigms have presented challenges to researchers for many years. Previous studies have used a variety of techniques that have yielded inconsistent results in the novel object recognition paradigm. The present study evaluated the importance of counterbalancing in murine models of object recognition. Using a one-trial test in Balb-C mice, counterbalancing was evaluated using tasks that manipulated object location, object color and size, and unique side preference of the individual mice. Five studies were conducted to test the significance of each individual technique, all three variations combined, and a model with no counterbalancing. Mice spent significantly more time with the novel object compared to the familiar object when counterbalancing was present. No preference was observed when no counterbalancing was present. The current study demonstrates the importance of counterbalancing in studies of object recognition in mice.

Disclosures: M. Canen: None. A.M. Babcock: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.19/HHH31

Topic: H.01. Animal Cognition and Behavior

Support: MOST #100-2410-H-006-085-MY2

MOST #101- 2320-B-006-007-

MOST #102-2410-H-006-016-MY2

MOST #104-2410-H-006-025-MY3

Title: The mechanism of rimonabant's facilitatory effect on cocaine-associated memory consolidation in mice: A sex difference study

Authors: *S.-J. HU, H.-A. CHANG, W. DAI;
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Abstract: Cannabinoid CB_1 receptors are implicated in various forms of learning and memory, including cocaine-associated memory. We previously found that systemic or intra-medial prefrontal administration of the CB_1 receptor antagonist/inverse agonist rimonabant facilitates the consolidation of conditioned place preference (CPP) induced by a low dose (2.5, 5, or 10 mg/kg) of cocaine in male wild-type mice. The current study aimed to investigate the receptor and hormone mechanisms underlying the facilitatory effect of rimonabant on cocaine-induced CPP. We first examined whether CB_1 receptors mediates rimonabant's effect by using CB_1 knockout mice. We found that rimonabant did not facilitate memory consolidation of a low-dose (10 mg/kg) cocaine-induced CPP in both genders of the CB_1 knockout mice. Moreover, rimonabant did not enhance the low-dose cocaine-induced CPP in female wild-type mice. Intriguingly, the facilitatory effect of rimonabant on cocaine-induced CPP was found to be mediated by the increased level of plasma corticosterone in male wild-type mice. Next, to test whether the sex hormone estrogen is involved in the sex difference effect, we examined rimonabant's effects on the low-dose cocaine-induced CPP in vehicle-treated *vs.* estrogen-treated ovariectomized (OVX) female wild-type mice and found two interesting results. First, estrogen replacement in the OVX mice *per se* enhanced the low-dose cocaine-induced CPP memory. Second, rimonabant facilitated cocaine-induced CPP memory in the OVX mice supplied with sesame oil, while impaired the same memory in the OVX mice supplied with estrogen. Taken together, our results indicate: 1) The facilitatory effect of rimonabant on cocaine-induced CPP is mediated by CB_1 receptors and corticosterone in male wild-type mice; 2) Estrogen is involved in a sex difference in the facilitatory effect of rimonabant on cocaine-associated memory.

Disclosures: S. Hu: None. H. Chang: None. W. Dai: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.20/HHH32

Topic: H.01. Animal Cognition and Behavior

Support: National Research Foundation 2009-0090188 2011-0030737 2013R1A2A1A01015228

Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT and future Planning(grant number)

Title: Novel function of histone demethylase of JHDM in spatial learning and memory

Authors: *H.-J. KIM[1], S.-Y. KIM[1], M.-H. KIM[1], J. PARK[2], S. KIM[1], Y.-S. CHUN[1];
[1]Dept. of Biomed. Sci., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Ctr. for Cognition and Sociality, Inst. for Basic Sci. (IBS), Daejeon, Korea, Republic of

Abstract: Dynamic changes in histone modification play a role in regulating the gene expression program linked to the memory formation. Among them, however, very little is known about the role of Jumonji-containing histone demethylases which erase the methyl group of H3K4, H3K9, H3K27, H3K36 and H4K20 that are associated with memory formation. Here, we documented the physiological role of JHDM (H3K9 demethylase) in both learning and memory at the adult stage of mice. It is of interest that the expression of JHDM is shown to be significantly increased in the hippocampus. In agreement, we found out that the JHDM overexpressed transgenic mice displayed enhancement in both spatial-memory formation and contextual fear conditioning compared to the wild-type mice. On the other hand, JHDM-deficient mice derived by Lenti-viral vector in the hippocampus showed impairment in the hippocampus-dependent memory function. Therefore, our results suggest that JHDM has an important role in memory formation in the hippocampus.

Disclosures: H. Kim: None. S. Kim: None. M. Kim: None. J. Park: None. S. Kim: None. Y. Chun: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.21/HHH33

Topic: H.01. Animal Cognition and Behavior

Support: Israel Science Foundation Grant 1379/12

Title: Synthesis of a memory blocker during *Aplysia* sleep prevents maladaptive long-term memory formation

Authors: *R. LEVY[1], A. SUSSWEIN[2];
[1]Goodman Fac. Of Life Sci., Bar Ilan Univ., Ramat Gan, Israel; [2]Bar |ilan Univ., Ramat Gan, Israel

Abstract: Training *Aplysia* with inedible food during the active phase of their daily cycle, but not during the sleep phase, produces long-term memory. During the active phase, inhibiting protein synthesis before training with a short-acting dose of anisomycin prevents memory formation. However, during the sleep phase this treatment allows memory to be formed, indicating that memory formation after training during the sleep is blocked by a protein synthesis-dependent process. Even a brief training that is ineffective at other times in forming long-term memory is effective during the sleep phase, after treatment with the anisomycin. Proteins already present before the anisomycin treatment are required for memory formation, and elimination of these proteins prevents long-term memory formation. After an effective training during the sleep phase, subsequent long-term memory formation requires transcription and a later stage of translation, as shown by blocking memory formation with the transcription blocker DRB, or with a longer-lasting dose of anisomycin. C/EBP transcription in the buccal ganglia was increased both after treatments leading to long term memory, and after treatments not leading to long term memory, indicating that increased C/EBP transcription could not account for memory formation. By contrast, increased CREB1 transcription was seen only after training that led to long-term memory. Increased CREB2 transcription was not seen after either effective or ineffective training. These findings suggest that molecular processes during sleep that underlie consolidation of experiences from the previous active phase enhance the ability of new experiences to form new memories. However, new memories formed at this time may not be adaptive. To prevent the formation of new, maladaptive memories, and to prevent new experiences from interfering with the ongoing consolidation, the formation of new long term memories during sleep is actively blocked by a protein synthesis-dependent process.

Disclosures: R. Levy: None. A. Susswein: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.22/HHH34

Topic: H.01. Animal Cognition and Behavior

Support: Israel Science Foundation Grant 1379/12

Title: New learning while consolidating memory during sleep is actively blocked by a protein synthesis dependent process

Authors: *A. J. SUSSWEIN[1], I. HURWITZ[2], R. LEVY[2];
[1]Bar-Ilan Univ., Ramat-Gan, Israel; [2]Bar Ilan Univ., Ramat Gan, Israel

Abstract: The effects of a new experience while a memory of a previous experience is being consolidated have provided insight into the nature of the consolidation process: 1) an transitory experience that would generally not be consolidated may capture the ongoing consolidation, producing long-term memory (a process known as synaptic or behavioral tagging and capture); 2) a new experience may interfere with the consolidation (the original observation showing that a memory requires consolidation). However, interactions between consolidation and a new experience may cause problems: 1) it may not be adaptive for a weak experience that captures a consolidating process to create a long-term memory; 2) it may not be adaptive to interrupt the consolidation by a new experience. It is well known that after a period of consolidation immediately following an experience, an additional stage of consolidation occurs while animals sleep. Why divide consolidation to different stages? Why defer aspects of consolation for many hours? We propose that partially deferring consolidation to sleep, when animals are relatively inactive, is a mechanism to minimize new experiences that might interact with the molecular processes underlying consolidation. However, when animals are awakened from sleep, these molecular processes are unlikely to be terminated immediately. At this time, even fleeting experiences could produce unwanted long-term memory, because of the ongoing consolidation. New data from our lab indicate that potential maladaptive memory formation from experiences when animals are awakened from sleep is prevented because new experiences at this time initiate an active protein synthesis dependent block of long-term memory. Removing the block allows even fleeting experiences to capture molecular processes underlying sleep phase consolidation, thereby leading to maladaptive long-term memory.

Disclosures: A.J. Susswein: None. I. Hurwitz: None. R. Levy: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.23/HHH35

Topic: H.01. Animal Cognition and Behavior

Support: CNPq

CAPES

Title: Retuning memory valence: novel reconsolidation-mediated updating procedure to disrupt fear memories

Authors: *J. HAUBRICH[1], A. CRESTANI[2], L. CASSINI[2], F. SANTANA[2], R. ORDONEZ SIERRA[2], L. DE OLIVEIRA ALVARES[2], J. QUILLFELDT[2];
[1]Federal Univ. of Rio Grande Do Sul, Porto Alegre, Brazil; [2]Federal Univ. of Rio Grande do Sul, Porto Alegre, Brazil

Abstract: When a consolidated memory is retrieved, it can return to a labile state and undergo reconsolidation, becoming sensitive to pharmacological and psychological interferences. This process can lead to memory updating through the integration of new information into a previously consolidated memory background. Thus reconsolidation provides the opportunity to modify an undesired fear memory by updating its emotional valence to a less aversive level. Here we evaluated whether a fear memory can be reinterpreted by the presentation of appetitive stimuli during its reactivation. We found that this procedure resulted in the suppression of a fear response that was not amenable to reinstatement, spontaneous recovery and rapid reacquisition. This effect was prevented by systemic injections of the L-type voltage-gated calcium channels blocker nimodipine or intra-hippocampal infusion of the GluN2B antagonist ifenprodil, what suggests that memory updating was mediated by the reconsolidation process and hippocampal neuronal plasticity. Taken together, this study shows that reconsolidation allows for a re-signification of unwanted aversive memories through the incorporation of information of opposing valence. It brings a new promising cognitive approach to treat fear-related disorders.

Disclosures: J. Haubrich: None. A. Crestani: None. L. Cassini: None. F. Santana: None. R. Ordonez Sierra: None. L. de Oliveira Alvares: None. J. Quillfeldt: None.

Poster

261. Animal Cognition: Memory Consolidation and Reconsolidation in Behavior

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 261.24/HHH36

Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Title: Potential interplay between the lysine acetyltransferase PCAF and estrogen receptors in the rat hippocampus for short-term object-in-place memory

Authors: *K. A. MITCHNICK[1], S. D. CREIGHTON[1], R. JAMIESON-WILLIAMS[2], A. LACOUSIERE[1], J. M. CLOKE[1], S. CASTELLANO[4], C. MILITE[4], G. SBARDELLA[4], B. E. KALISCH[3], E. CHOLERIS[1], B. D. WINTERS[1];
[1]Psychology, [2]Cell. and Mol. Biol., [3]Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada; [4]Med. Chem., Univ. of Salerno, Salerno, Italy

Abstract: The actions of lysine acetyltransferases (KATs), which include chromatin remodeling via histone acetylation as well as modifications to non-histone proteins, are increasingly implicated in mnemonic processes. We have previously found that antagonism of the KAT p300/CBP-associated factor (PCAF) by embelin impairs long-term (24h) object-in-place (OiP) memory in rats when administered to either the perirhinal cortex or hippocampus (HPC). However, intra-HPC embelin also impairs short-term (20min) OiP memory, and PCAF knockout mice display short-term spatial and object memory deficits. As PCAF has been shown to activate nuclear hormone receptors, and nuclear estrogen receptors (ERs) are known to be involved in short-term memory processes, the present study explored the potential interaction of PCAF and ERs in the rapid regulation of OiP memory in the HPC of rats. Behavioural results were consistent with this interpretation, as intra-HPC administration of the non-selective ER antagonist, fulvestrant, impaired short-term OiP memory, the PCAF activator, SPV-106, enhanced short-term memory in a sub-optimal learning version of the task, and co-administration of an otherwise ineffective concentration of fulvestrant blocked the enhancing effects of SPV-106. Although intra-HPC SPV-106 also facilitated long-term OiP memory, no evidence was found for ER involvement in this effect. Finally, intra-HPC administration of ERα agonist PPT enhanced short-term OiP memory, whereas the ERβ agonist DPN did not. Ongoing molecular analyses are examining the potential interaction between PCAF and ERα following learning. Thus, PCAF appears to regulate short-term and long-term memory via dissociable mechanisms. The present data are consistent with the notion that PCAF can influence short-term memory through a non-epigenetic process related to the stimulation of ERα, which has been shown to induce relatively rapid effects on hippocampus-dependent learning and dendritic spine density.

Disclosures: K.A. Mitchnick: None. S.D. Creighton: None. R. Jamieson-Williams: None. A. LaCousiere: None. J.M. Cloke: None. S. Castellano: None. C. Milite: None. G. Sbardella: None. B.E. Kalisch: None. E. Choleris: None. B.D. Winters: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.01/HHH37

Topic: G.01. Appetitive and Aversive Learning

Title: Examining sex-based differences in unconditioned and conditioned fear

Authors: *M. VOULO, R. G. PARSONS;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Fear conditioning is one of the dominant models of learning and memory, and one that may help shed light on fear-based psychopathology, yet it's been estimated that less than 2% of research related to fear conditioning has been conducted in females (Lebron-Milad & Milad, 2012). Furthermore, the limited studies that have investigated sex-based differences in fear have produced mixed results. The current study investigated sex-based differences in both unconditioned and conditioned fear in male and female Sprague-Dawley rats. In the first experiment, animals were tested in a light-enhanced startle paradigm (LES) in which acoustic startle responses were measured in the dark and in the presence of a bright light, which has been shown to be anxiogenic in rodents (Walker & Davis, 1997). During Phase 1 rats received 30 startle trials in a darkened chamber. Five minutes later this procedure was repeated in the presence of a bright light (experimental) or in the dark (control), and percent potentiation was calculated for each animal and compared across sessions (control vs. experimental) to investigate the degree of LES. The results showed that while both sexes showed significant light-enhanced startle, there was no difference in the magnitude between males and females. In the second set of experiments, animals were fear conditioned with 2 pairings of a tone and foot shock. The next day they were exposed to 20 presentations of the tone alone to undergo fear extinction. They were tested for extinction retention 24 hours later with 8 presentations of the tone alone, and on the final day they were tested for contextual fear. Freezing behavior was recorded and fear acquisition, extinction training, extinction testing, and context testing data were compared between males and females. Our findings show that males and females acquire fear to a similar degree, but females showed significantly greater extinction during the extinction training session. In addition, females showed a trend for greater retention of extinction learning during the following testing session, although the difference between males and females did not reach

statistical significance. Consistent with prior studies, males exhibited higher levels of freezing during the contextual fear test. Current studies are investigating fear conditioning and extinction in both sexes using a fear-potentiated startle paradigm to determine if a similar pattern of data emerges using another measure of fear.

Disclosures: M. Voulo: None. R.G. Parsons: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.02/HHH38

Topic: G.01. Appetitive and Aversive Learning

Title: Acoustic startle response as a predictive index of a PTSD-like phenotype in rats

Authors: *A. S. RUSSO, R. G. PARSONS;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Although a large portion of the population is exposed to a traumatic event at some point, only a small percentage of the population develops post-traumatic stress disorder (PTSD). Identification of factors that make some individuals more likely to develop PTSD than others is crucial to the further understanding of the mechanisms underlying the development of PTSD, but prospective models that aim to identify susceptible populations prior to trauma exposure are limited. Abnormal acoustic startle response (ASR) has been shown to be associated with PTSD, so it is of interest to investigate this reflex as a potential predictor of the development of PTSD-like symptoms. Since poor extinction and retention of extinction learning are characteristic of PTSD patients, it is of interest to determine if abnormal ASR is predictive of development of such deficits. To determine whether baseline ASR has utility in predicting the development of PTSD-like symptoms, the relationship between baseline ASR and extinction of fear response was analyzed in 45 adult, male Sprague-Dawley rats. Baseline ASR was measured for each rat as the average startle amplitude across 30 trials of a 50 msec, 95 dB white noise burst. The rats were then exposed to a Pavlovian fear conditioning paradigm consisting of training with 2 pairings of a 30 sec, 4 kHz tone and a 1 sec, 1 mA footshock. Following conditioning, the rats received extinction training and testing sessions, consisting of 20 and 8 additional presentations of the tone, respectively. Freezing behavior was measured throughout the experiment as an indicator of fear, and was used to calculate values for magnitude of extinction learning and retention of extinction learning for each rat. Analysis of the relationships between baseline ASR and measures of extinction and retention revealed that low baseline ASR was associated with poor within-session extinction learning and poor retention of extinction learning. Furthermore,

the rats were ranked based on their baseline ASR and assigned to “low,” “medium,” or “high” startle groups. The “low” startle rats had poorer retention of extinction learning than the “high” startle rats, but the groups did not differ in retention of the conditioning memory or rate and magnitude of extinction learning. The results show that low baseline ASR is associated with poor retention of extinction learning following fear conditioning. Since impaired ability to recall extinction learning is characteristic of patients with PTSD, these results suggest that the ASR may have value as a predictive index of the development of a PTSD-like phenotype.

Disclosures: A.S. Russo: None. R.G. Parsons: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.03/HHH39

Topic: G.01. Appetitive and Aversive Learning

Support: Focus Program Translational Neuroscience (FTN, State of Rhineland-Palatinate)

Title: Good made better: L-DOPA reduces return of fear after successful within-session extinction

Authors: *R. KALISCH, A. M. V. GERLICHER, O. TÜSCHER;
Deutsches Resilienz-Zentrum (DRZ), Johannes Gutenberg Univ. Med. Ctr., Mainz, Germany

Abstract: Background
Relapse, or return of fear (ROF), is a frequent problem after exposure-based fear therapy. One promising counter-strategy is adjunctive treatment with a pharmacological enhancer of the extinction memory formation presumed to occur during and after exposure. Accumulating evidence suggests that the effects of enhancers may depend on successful fear reduction during exposure. However, there is currently no controlled laboratory study systematically investigating a potential dependency of pharmacological ROF prevention on extinction success using objective physiological measures. We employed the dopamine precursor L-DOPA, which is stable, safe and well-tolerable and powerful in preventing ROF when given after extinction training in rodents. First data indicate it may also work in humans.
Methods
In a three-day paradigm including fear conditioning (day 1), extinction (day 2) and spontaneous recovery of fear (day 3, ROF test), N=79 (experiment 1) and N=32 (experiment 2) healthy male participants were pseudo-randomly assigned to a post-extinction administration of 150 mg L-DOPA or placebo. We tested whether end-fear after short (10 trials, experiment 1) and long (25

trials, experiment 2) extinction moderated the effect of L-DOPA on ROF. Skin conductance responses were used as primary outcome.
Results
In both experiments, L-DOPA, but not placebo, participants showed low ROF when extinction end-fear was low, but high ROF when end-fear was high.
Conclusions
These results point towards a common boundary condition for pharmacological interventions targeting extinction memory consolidation and emphasize the importance of limiting their clinical use to successful exposure sessions. They also confirm the potential of L-DOPA as an adjunct to exposure treatment.

Disclosures: R. Kalisch: None. A.M.V. Gerlicher: None. O. Tüscher: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.04/HHH40

Topic: G.01. Appetitive and Aversive Learning

Support: HHMI grant to Swarthmore College

Title: Propranolol as a potential adjunct to exposure therapy in the treatment of pathological fear memory: an animal model

Authors: *A. M. SCHNEIDER[1], P. E. SIMSON[3], Z. FRANK[1], N. PALADUGU[1], V. GOMES[1], R. ABISHEK[1], J. KANG[1], C. H. WONG[1], C. EVERBACH[2], L. G. KIRBY[4]; [1]Dept Psychol, [2]Dept Engin., Swarthmore Col., Swarthmore, PA; [3]Dept Psychology and Ctr. for Neurosci., Miami Univ., Oxford, OH; [4]Ctr. for Substance Abuse Res. and Dept. Anat. and Cell Biol., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Clinical studies have shown that exposure therapy is effective in reducing abnormal fear memory such as in post-traumatic stress disorder (PTSD). The treatment, however, is limited and abnormal fear memory often recovers. Using an analogue of exposure therapy in rats—hereafter referred to as the brief exposure procedure—we investigated the extent to which the effectiveness of the brief exposure procedure in reducing retention of fear could be enhanced or impaired by the ß-adrenergic blocker propranolol, a drug used to treat PTSD. Male Long Evans rats underwent contextual fear conditioning followed 24 hr later by the brief exposure procedure; the next day the animals received a retention test. Contextual fear conditioning consisted of placing rats in a dark compartment for 120 sec followed by a single footshock; the brief exposure

procedure, in contrast to the standard extinction procedure, consisted of confining the animals to the dark compartment for 30 sec in the absence of shock and removing them before an opportunity to recall fear developed to a significant degree; the retention test consisted of returning the animals to the dark compartment for 180 sec. Freezing behavior provided a measure of fear during the brief exposure and the subsequent retention test. Propranolol (4 mg/kg or 10 mg/kg) or saline was injected 20 min prior to the brief exposure procedure or was injected in the absence of the brief exposure procedure yielding a total of 6 groups. The doses of propranolol chosen were based on electrophysiological evidence from a previous study indicating that propranolol has opposing dose-dependent effects on amygdala (central nucleus, CeA) activity: a relatively high dose (10 mg/kg) increases CeA activity; a relatively low dose (4 mg/kg) suppresses spontaneous CeA activity. The results were as follows: 1) Neither the brief exposure procedure administered in the absence of propranolol nor propranolol (4 mg/kg or 10 mg/kg) administered in the absence of the brief exposure procedure affected retention of the fear memory. 2) Propranolol administered prior to the brief exposure affected freezing behavior in a dose-dependent manner: the CeA activity-suppressing dose (4 mg/kg) did not affect freezing behavior either during the brief exposure or during the retention test 24 hrs later; the CeA activity-increasing dose (10 mg/kg) increased freezing behavior and did so during both the brief exposure and the retention test 24 hrs later. In conclusion, the present results indicate that the efficacy of propranolol as an adjunct to exposure therapy may be dose-dependent and suggest the importance of minimizing amygdala activation and resulting fear during exposure therapy.

Disclosures: A.M. Schneider: None. P.E. Simson: None. Z. Frank: None. N. Paladugu: None. V. Gomes: None. R. Abishek: None. J. Kang: None. C.H. Wong: None. C. Everbach: None. L.G. Kirby: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.05/III1

Topic: G.01. Appetitive and Aversive Learning

Support: CONACyT (176639)

DGAPA-UNAM (IA200313 and IA200715)

Fundación Miguel Alemán A.C

IBRO Return-Home award to FS-B

postdoctoral fellowship CONACyT to AM-C

postdoctoral fellowsihip DGAPA-UNAM to AM-C

Title: Neurogenesis regulates fear recovery after extinction by recruiting hippocampal and prefrontal activity

Authors: *A. MARTINEZ-CANABAL[1], G. LOPEZ-OROPEZA[1], A. GAONA-GAMBOA[1], P. BALLESTEROS-ZEBADUA[2], O. GALVAN DE LA CRUZ[2], S. MORENO-JIMENEZ[2], F. SOTRES-BAYON[1];
[1]Cell Physiol. Inst. - Neurosci., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico;
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Abstract: Learning to extinguish a defensive response to a threat (fear) associated to a context, leads to the formation of a new memory that inhibits a previously acquired fear memory to the context. This fear inhibition, however, is temporary as fear responses often return spontaneously with the passage of time. It is not clear what brain mechanisms underlie this recovery of fear. Given that contextual fear and extinction memories are hippocampus-dependent and adult-born hippocampal neurons (neurogenesis) have been reported to modify preexisting fear memories, we hypothesized that neurogenesis-mediated modification of a preexisting extinction memory modifies fear recovery levels. To test this, rats previously trained to press a bar to obtain food underwent contextual fear conditioning (day 1), extinction training (day 2) and an extinction memory retrieval test in the same context (day 3). Next, we manipulated hippocampal neurogenesis levels by exposing rats to an enriched environment (EE) to enhance neurogenesis or by X-ray focal irradiation (IRR) to ablate neurogenesis. One month after these manipulations, rats were tested to evaluate spontaneous fear recovery with respect to the previous retrieval test, and their brains collected for immunostaining. We found that EE after extinction prevented fear recovery, as indicated by low freezing and high bar pressing levels as compared to controls during memory retrieval test. In contrast, we found that IRR after extinction promoted fear recovery, as indicated by high freezing and low bar pressing levels as compared to controls during memory retrieval test. These effects on fear levels were not present when EE or IRR occurred before or without extinction learning, suggesting that the observed effects are extinction-dependent. By using the expression of doublecortin as a neurogenesis marker, we show that neurogenesis levels increased with EE, decreased with IRR and were correlated with freezing and bar-pressing at test. Importantly, by using the expression of c-Fos as neuronal activity marker, we found that low fear recovery mediated by increased neurogenesis recruited CA3 hippocampal region and decreased prelimbic prefrontal cortex activity, whereas high fear recovery mediated by ablated neurogenesis decreased CA3 and infralimbic prefrontal cortex activity. Together, our findings suggest that manipulation of hippocampal neurogenesis levels after extinction learning modifies the strength of the extinction memory by modifying hippocampal-prefrontal activity, and thereby altering fear recovery levels.

Disclosures: A. Martinez-Canabal: None. G. Lopez-Oropeza: None. A. Gaona-gamboa: None. P. Ballesteros-zebadua: None. O. Galvan de la cruz: None. S. Moreno-jimenez: None. F. Sotres-Bayon: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.06/III2

Topic: G.01. Appetitive and Aversive Learning

Support: 5R01MH105398-03

Title: A novel DNA modification associated with extinction learning and memory formation

Authors: *X. LI[1], C. MAGNAN[2], W. WEI[5], M. EMAMI[1], L. E. WEARICK-SILVA[6], T. VIOLA[6], R. GRASSI-OLIVEIRA[6], S. NAINAR[3], C. B. VÅGBØ[7], M. BJØRÅS[3], P. BALDI[2], R. SPITALE[3], T. BREDY[4];
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Abstract: DNA methylation, once considered static and restricted to directing cellular lineage specificity during early development, is now recognized to be highly dynamic and reversible across the lifespan. Although it is known that there are more than 20 DNA modifications, nearly all research aimed at elucidating the role of these chemical modifications in the brain has focused on either 5-methylcytosine (5mC) or the recently rediscovered 5-hydroxymethycytosine (5hmC), which is a functionally distinct oxidative derivative of 5mC. 5mC and 5hmC are highly prevalent in neurons relative to other cell types and both modifications are regulated in response to learning. Despite these exciting threads, a complete understanding of how DNA methylation controls neuronal gene expression to facilitate memory formation is severely lacking. Here we report that the novel eukaryotic DNA modification N6-methyl-2'-deoxyadenosine (m6dA), which is beyond cytosine methylation, drives activity-induced gene expression and is associated with fear extinction memory in the infralimbic prefrontal cortex (ILPFC) of adult C57/Bl6 mice. In primary cortical neurons, m6dA accumulates within the P4 promoter of the gene encoding brain-derived neurotrophic factor (bdnf) via a putative m6dA-specific methyltransferase, N6amt1. An N6amt1-dependent increase in the deposition of m6dA is associated with an active chromatin state, as well as the recruitment of the activating transcription factor Yin-Yang 1 and RNA polymerase II, which promote bdnf exon IV mRNA expression. The same process regulates learning-induced bdnf exon IV mRNA expression in the adult brain. Viral-mediated knockdown of N6amt1 in the ILPFC blocks the effect of extinction learning on m6dA deposition and related chromatin and transcriptional activity, resulting in a significant impairment in extinction memory.

Disclosures: X. Li: None. C. Magnan: None. W. Wei: None. M. Emami: None. L.E. Wearick-Silva: None. T. Viola: None. R. Grassi-Oliveira: None. S. Nainar: None. C.B. Vågbø: None. M. Bjørås: None. P. Baldi: None. R. Spitale: None. T. Bredy: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.07/III3

Topic: G.01. Appetitive and Aversive Learning

Support: 5R01MH105398-03

Title: ADAR1 dual function as RNA editing and potential DNA binding enzyme in the activity-dependent regulation of adaptive behaviour in the mouse

Authors: *P. MARSHALL[1,2], X. LI[2], L. WEARICK[3], T. VIOLA[3], T. BREDY[2];
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Abstract: RNA editing enzymes have been known for some time to affect the qualitative and quantitative nature of RNA products. RNA editing has also been correlatively linked to the mediation of behaviour of organisms ranging from flies to humans, with the highest levels of these enzymes in humans. Mechanistically, this has been suggested to occur by their ability to bind to RNA and participate in deamination of: cytosine in the case of APOBECs', or adenosine in the case of ADARs'. However, two caveats arise in the proposed relationship. One is that not all variants of these enzymes appear to operate solely by this mechanism; they can also bind to DNA. And two, that much of the mechanistic work for this latter point has been divorced from a behaviourlly relevant context. Therefore, in order to assess the function of these domains in the context of behavioural adaptation, genome-wide DNA sequencing for ADAR1 was performed both with cultured primary cortical neurons (PCN's) and 6-8 week old C57 mice subjected to fear conditioning and extinction. An shRNA was also designed against ADAR1 and transfected with PCN's and into the infralimbic cortex of the trained mice. It has been observed that ADAR1 appears to bind a number of targets on DNA. Additionally and surprisingly, it was observed that although mRNA and protein levels appeared elevated specifically to extinction behaviour, following knockdown of ADAR1 in the infralimbic cortex the expression of fear was enhanced, while fear extinction remained unaffected. It remains to be seen whether this effect is mediated primarily by ADAR1's RNA editing activity or the binding capacity to DNA.

Disclosures: P. Marshall: None. X. Li: None. L. Wearick: None. T. Viola: None. T. Bredy: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.08/III4

Topic: G.01. Appetitive and Aversive Learning

Support: CDMRP PT090344

NIH T32AA007573

NIH T32NS007431

Title: Infralimbic projections to the basolateral amygdala, but not the nucleus accumbens, are required for the extinction of fear memories

Authors: *D. W. BLOODGOOD[1], J. A. SUGAM[1], H.-J. JO[1], M. TORRUELLA SUAREZ[1], T. L. KASH[2];
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Abstract: The prefrontal cortex (PFC) has been shown to be involved in the inhibition of learned emotional responses. Deficits in emotional learning are a key feature of Post-Traumatic Stress Disorder and are thought to be characterized by a loss of top-down control over subcortical regions. Importantly, distinct outputs control different domains of behavior whereby outputs to the nucleus accumbens (NAc) act in the appetitive domain to reduced cued drug seeking while outputs to the basolateral amygdala (BLA) act in the aversive domain to reduce cued fear responses. There is also a dorsal-ventral of distribution of function in the PFC whereby the prelimbic cortex (PLC) is required for the expression of fear behavior while infralimbic cortex (ILC) is required for the extinction of fear behavior. Previously it has been shown that pharmacologic and optogenetic inhibition of ILC impairs fear extinction. However these studies have not examined the role of PFC neurons with respect to output region. To functionally assess this, we preformed retrograde anatomic tract tracing experiments and found that BLA and NAc outputs are discrete non-overlapping populations in both the PLC and ILC. We next used patch clamp electrophysiology with fluorescent retrobeads to assess changes in excitability following fear acquisition and extinction. We found increased excitability following extinction specifically in BLA projecting neurons. This change was subregion dependent and increased excitability was found only in ILC but not PLC. Finally we used a multiplexed chemogenetic technique to inhibit

PFC neurons based on output and found that projections to BLA are required for extinction of conditioned fear responses while NAc projections are not. Our findings are consistent with previous results and highlight that behavioral responses are controlled by the specificity of output.

Disclosures: D.W. Bloodgood: None. J.A. Sugam: None. H. Jo: None. M. Torruella Suarez: None. T.L. Kash: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.09/III5

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH 086591(RWS)

NSF Grant IBN 0630522 (RWS)

National NSF (China) Grant 81271217 (GZ)

National NSF (China) Grant 81471161 (GZ)

Ph.Anhui Medical University Award XJ201405 (GZ)

Title: Fear memory extinction is associated with an increased expression of synaptic small conductance calcium-activated potassium channels, type 2 (SK2) in male C57BL/6J mice

Authors: *R. W. STACKMAN, JR[1,2], G. ZHANG[2], D. A. CINALLI, Jr.[3], C. RICE-KUCHERA[4], X. HUANG[5], T.-F. YUAN[5], R. HUA[6], Y.-M. ZHANG[6];
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Abstract: Small conductance Ca2+-activated K+ channels (SK channels) are widely expressed in the central nervous system and regulate neuronal functions at all levels, including synaptic plasticity, neuronal excitability, sensory, behavior, emotion and cognition. We previously demonstrated that pharmacological blockade of SK channels with apamin enhanced learning and memory, while activation of SK channels with EBIO produced the opposite effect. Further, SK2 channels expressed on dendritic spines of pyramidal neurons in the hippocampus and amygdala shape glutamatergic EPSPs and modulate the induction of long-term potentiation. Excessive fear

is a hallmark of several emotional and mental disorders such as phobias and panic disorders. Considerable effort has been given to defining the neurobiological mechanisms of the extinction of conditioned fear memory as such mechanisms may hold clinical significance for remediating aberrant fear memory. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. We theorized that fear memory extinction is associated with a dynamic alternation in SK channel expression and/or functioning. Male C57BL/6J mice received trace fear conditioning (8 CS-US pairings with a stimulus-free gap between the onset of each stimulus of the pair) followed by extinction (8 CS). 24 h after extinction, mice that experienced fear conditioning and extinction exhibited a significantly greater sensitivity to the locomotor suppressing effect of systemic EBIO treatment during an open field test. Western blot results revealed that mice that experienced fear conditioning and extinction presented a higher expression of SK2 channel protein in synaptosome fractions, but not the cytosolic fractions prepared from amygdala samples. Voltage clamp recordings from amygdala neurons in slices prepared from mice after conditioning, revealed that fear conditioning was associated with an increase in the amplitude of slow and medium components of the AHP current (sIAHP, mIAHP). Together, these data suggest that fear extinction is associated an upregulation of SK2 channels in amygdala synapses, which may hold a significant influence on the behaviorally-triggered synaptic plasticity required for fear memory extinction.

Disclosures: R.W. Stackman: None. G. Zhang: None. D.A. Cinalli: None. C. Rice-Kuchera: None. X. Huang: None. T. Yuan: None. R. Hua: None. Y. Zhang: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.10/III6

Topic: G.01. Appetitive and Aversive Learning

Support: National Institutes of Mental Health (1R01MH096764)

Ruth L. Kirstein National Research Service Award (NRSA) Predoctoral Fellowship F31 MH105237-01

Office of Research Infrastructure Programs/OD P51OD011132 [formerly National Center for Research Resources (NCRR) P51RR000165]

Title: Mechanisms underlying the structural, functional and epigenetic responses in the primary sensory system of adult mice accompanying olfactory fear learning

Authors: *F. G. MORRISON[1,2], B. G. DIAS[2], K. J. RESSLER[1,2];
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Abstract: Olfactory sensory neurons (OSNs) within the main olfactory epithelium (MOE) provide a rich model to study the perception of external cues and the underlying mechanisms regulating structural plasticity within the olfactory system. Using the M71-LacZ mouse line, we have previously demonstrated an increased number of M71+ OSNs in the olfactory epithelium and increased M71+ glomerular area in the olfactory bulb (OB) following cue-specific olfactory fear conditioning to acetophenone, an odorant shown to specifically activate the M71 receptor. Functionally, mice exhibit enhanced freezing to the conditioned odor stimulus following olfactory fear conditioning. We sought to determine whether the behavioral and structural changes observed after olfactory fear conditioning may be reversed with extinction training. Using native chromatin immunoprecipitation (N-ChIP) protocols on the MOE, we investigate the dynamic alterations in histone marks around the M71 gene locus following both olfactory fear acquisition and extinction. Additionally, to understand cell dynamics as a function of fear learning, we used 5-Ethynyl-2'-deoxyuridine, 5-Du Alkyne (EdU) labeling to investigate cell birth and turnover. To understand rates of cell death and survival, we used cell death assays such as terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). With these parallel assays, we sought to determine the role of cell turnover dynamics in the structural plasticity accompanying olfactory learning. Male mice were trained to associate mild footshocks with acetophenone. Three weeks after the last conditioning session, animals were handled only or exposed to an extinction session and 3 weeks after the last extinction session, animals were sacrificed. Extinction training specific to the conditioned odorant cue reversed the conditioning-associated increases in freezing and M71-specific OSN number and glomerular area. We also demonstrated a dynamic regulation of histone marks around the M71 locus associated with both cue-specific fear learning acquisition and extinction. Our observations shed light on how the olfactory sensory system responds dynamically to extinction learning after fear conditioning.

Disclosures: F.G. Morrison: None. B.G. Dias: None. K.J. Ressler: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.11/III7

Topic: G.01. Appetitive and Aversive Learning

Support: Start-Up funds

Title: The role of cholinergic input from the medial septum in cued and contextual fear extinction memory

Authors: *J. M. STAIB[1], D. KNOX[2];
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Abstract: In classical fear conditioning, a neutral stimulus (CS) is paired with an aversive stimulus (US), causing the animal to associate the US with CS, and display a fear response to the CS. Fear extinction occurs when the CS is presented without the US and the animals learn that the CS no longer predicts the US, thus learning to no longer show fear with CS presentation. Ventral medial prefrontal cortex inhibition of neural activity in basolateral and central amygdala nuclei is critical for extinction memory formation. Recently, we observed that cholinergic lesions in the Medial Septum and Diagonal Bands of Broca (MS/DBB), induced with 192-IgG saporin results in fear extinction memory deficits and contextual fear memory generalization between the conditioning and extinction contexts. While this suggests that MS/DBB cholinergic neurons may be a component of the fear extinction circuit, these neurons project to many brain regions. As a result, the MS/DBB cholinergic efferents that are critical for mediating extinction memory and contextual fear memory discrimination are unknown. The goal of the present study is to isolate the exact MS/DBB efferents that mediate extinction memory and contextual fear memory discrimination. While the study is in progress, some results have been collected. Cholinergic lesions in the dorsal hippocampus, ventral hippocampus, and medial prefrontal cortex have no effects on fear extinction memory or contextual fear memory discrimination. This is surprising because all of these regions are components of the fear extinction circuit and the dorsal hippocampus is critical for contextual learning during acquisition of fear and extinction memory. The MS/DBB also projects to habenula nuclei, and there are cholinergic interneurons in the MS/DBB as well. For the remainder of the study, we explore the potential role of MS/DBB cholinergic input to the habenula and MS/DBB cholinergic interneurons in mediating extinction memory and contextual fear memory discrimination. Isolating a region that has a direct role in mediating extinction memory could help focus future research in fear memory disorders like post traumatic stress disorder.

Disclosures: J.M. Staib: None. D. Knox: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.12/III8

Topic: G.01. Appetitive and Aversive Learning

Support: F31 MH106288

MBRS-RISE R25 GM082406

R15 MH101700

G12 MD007579

Title: Fear conditioning and extinction alter ventral hippocampus activation of nmdar currents in infralimbic neurons

Authors: O. SOLER-CEDENO[1], O. TORRES-RODRIGUEZ[3], L. MALDONADO-LABOY[4], A. HERNANDEZ-LOPEZ[2], *J. T. PORTER[5];
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Abstract: After exposure therapy, some patients with posttraumatic stress disorder (PTSD) relapse when trauma-associated cues are experienced outside of the therapeutic context. Similar to humans, fear extinction in rodents, which mimics human exposure therapy, is context-dependent such that extinguished fear of a cue renews when the cue is presented in a context different from where the extinction occurred. Recent evidence suggests that synaptic plasticity between the ventral hippocampus (vHPC) and the medial prefrontal cortex (mPFC) could be mediating the context-specificity of fear extinction memory. Therefore, we investigated whether extinction in the conditioning context causes different synaptic changes in vHPC synapses in the mPFC than extinction in a different context. To address this, we assigned male Sprague Dawley rats to one of the following experimental groups: pseudo-conditioning, fear conditioning, same context extinction, and different context extinction. We did whole-cell patch-clamp recordings of prelimbic (PL) and infralimbic (IL) pyramidal neurons in the mPFC to assess AMPA and NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) evoked by optical stimulation of channelrhodopsin expressing vHPC axons. Surprisingly, we found in IL that NMDA to AMPA ratios were significantly reduced after fear conditioning due to a reduction in NMDA EPSCs. Moreover, extinction in the conditioning context caused a larger reversal of changes induced by fear conditioning than extinction in the different context. Interestingly, animals that failed to recall same context extinction had smaller NMDA to AMPA ratios and NMDA EPSCs than animals with successful extinction recall. These synaptic alterations were not observed in vHPC connections to PL. Taken together, our data suggest that fear conditioning weakens NMDA receptor-mediated excitation of IL neurons by vHPC synapses, and that extinction in the conditioning context strengthens the weakened vHPC synapses. The inability of different context extinction to inducing similar strengthening of vHPC synapses in IL might account for fear renewal, which could explain relapse in certain patients with PTSD.

Disclosures: O. Soler-Cedeno: None. O. Torres-Rodriguez: None. L. Maldonado-Laboy: None. A. Hernandez-Lopez: None. J.T. Porter: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.13/III9

Topic: G.01. Appetitive and Aversive Learning

Support: ARC Grant DP150104835

NHMRC Grant APP1031688

Title: Early life stress does not result in impaired generalized extinction in adult rats

Authors: *N. ELLIOTT, R. RICHARDSON;
Univ. of New South Wales, UNSW Sydney, Australia

Abstract: Anxiety disorders have a severe and long-lasting impact on an individual's quality of life and overall functioning. A growing body of research suggests that alterations in fear generalization processes are involved in the emergence of psychopathology. Indeed, human studies have found that individuals diagnosed with anxiety show increased generalization of fear after conditioning. Although fear generalization is a well-researched phenomenon in the animal literature, few studies have examined how generalization contributes to the development of anxiety using animal models. Further, no research to date has examined how the generalization of extinction learning influences the emergence of psychopathology. Given that anxiety disorders are thought to stem from both increased fear learning, as well as impaired fear inhibition, it is expected that rats prone to anxiety will show decreased generalized extinction. In this study, we examined generalized extinction after early life stress, which has been shown to be associated with anxiety. Rats were exposed to maternal separation from postnatal day 2-14 (MS; a rodent model of early life stress) or reared as normal. In adulthood, rats received fear conditioning to two distinct tone conditioned stimuli (i.e., CS1 and CS2). The following day, rats received extinction training to one CS (i.e., CS1). Standard reared rats tested the next day showed generalized extinction, such that they demonstrated low levels of fear to both the extinguished CS (i.e., CS1) and the non-extinguished CS (i.e., CS2). It was predicted that MS rats would exhibit less generalized extinction, but they performed similarly to the standard-reared animals. That is, the MS rats also exhibited generalized extinction, showing low levels of fear to both the extinguished and non-extinguished CS. Both MS and SR rats that received extinction training showed lower levels of fear to both CS1 and CS2 compared with non-extinguished controls. That is, contrary to expectations, animals prone to the development of anxiety were not impaired at generalized extinction. These findings contribute to the current body of research on the role of generalization in anxiety disorders, but suggest that perhaps altered generalization of fear inhibition does not influence to the development of psychopathology. **Support:** ARC grant DP150104835 and NHMRC grant and APP1031688.

Disclosures: N. Elliott: None. R. Richardson: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.14/III10

Topic: G.01. Appetitive and Aversive Learning

Support: FAPESP 2013/04741-5

FAPESP BEPE 2015/20487-7

Title: Systemic administration of 5-HT receptor agonist m-chlorophenylpiperazine (mCPP) modulates fear extinction

Authors: *A. E. REIMER[1,2,3], A. R. DE OLIVEIRA[4,2], M. R. MILAD[3], M. L. BRANDÃO[1,2]; [1]Dept. de Psicologia, Univ. de Sao Paulo, Ribeirao Preto, Brazil; [2]Inst. de Neurociências e Comportamento, Ribeirão Preto, Brazil; [3]3Department of Psychiatry, MGH – Harvard Med. Sch., Boston, MA; [4]Dept. de Psicologia, Univ. Federal de São Carlos, São Carlos, Brazil

Abstract: Although much is now known about the serotonergic (5-HT) mechanisms involved in fear conditioning, less is known about 5-HT mechanisms underlying fear inhibition. The neurocircuitry involved in fear inhibition is impaired in anxiety and mood disorders. Considering that the leading treatments to diverse mental disorders include the use of pharmacological compounds aimed at the 5-HT neurotransmission, understanding how the modulatory 5-HT mechanisms influence fear inhibition can contribute to the developing of better approaches to treatment. The aim of this study is to evaluate fear extinction of rats acutely treated with the 5-HT agonist m-chlorophenylpiperazine (mCPP). Male Wistar rats underwent a 3-day fear conditioning and extinction protocol. On day 1, rats received tone-footshock pairings (conditioning). On day 2, rats received either mCPP (0.5 or 1.0 mg/kg) or vehicle prior to extinction learning. On day 3, extinction memory was assessed. Locomotor activity and exploratory behavior were also assessed using the open-field test. mCPP did not affect extinction learning, compared to vehicle. However, mCPP decreased freezing response during extinction recall, suggesting enhanced extinction memory consolidation. Both mCPP doses decreased ambulation in the open-field test, while only the higher dose increased immobility. Given the widespread of 5-HT1A, 5-HT2A and 5-HT2C receptors expression through brain areas involved in both fear expression and inhibition, it is challenging to specify the exact target responsible for the facilitation of fear extinction in the present study. However, given the presence of these receptors in both medial pre-frontal cortex (mPFC) and amygdala, we hypothesize that mCPP

enhanced fear extinction memory via influence of the 5-HT receptors within the mPFC-BLA circuit.

Disclosures: A.E. Reimer: None. A.R. de Oliveira: None. M.R. Milad: None. M.L. Brandão: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.15/III11

Topic: G.01. Appetitive and Aversive Learning

Support: ARC grant DP150104835

NHMRC grants APP1086855 and APP1031688

Title: Impaired fear extinction retention in adolescent rats: Pharmacological evidence for a failure to recruit NMDA receptors during extinction

Authors: *K. D. BAKER, R. RICHARDSON;
UNSW Australia, Sydney, Australia

Abstract: Adolescents, both humans and rodents, exhibit a marked impairment in extinction of learned fear relative to both younger (e.g., juvenile) and older (e.g., adult) groups. This impaired fear inhibition could be caused by NMDA receptors not being recruited efficiently during fear extinction in adolescents as these receptors are critical for the acquisition and consolidation of fear extinction. It is well-established that systemic administration of NMDA receptor antagonists (e.g., MK801) before fear extinction training impairs the retention of extinction in adult and juvenile rodents but it is unknown whether this is also the case for adolescents. In the present experiments we investigated the effect of pharmacologically manipulating the NMDA receptor on fear extinction retention in adolescent rats. In Experiment 1, rats were only given one day of extinction training (which typically results in impaired extinction retention) while in Experiment 2 animals were given two days of extinction training (which usually results in good extinction retention). In Experiment 1, animals were given either saline, D-cycloserine (DCS; a partial NMDA receptor agonist), or MK-801 (an uncompetitive antagonist) while in Experiment 2 animals were given either saline or MK-801 (only on the second day of extinction training). In Experiment 1 saline-treated rats showed the typical impairment of retention of extinction after one day of extinction training whereas DCS-treated rats showed enhanced extinction retention. In contrast, MK801-treated rats showed a similar level of freezing at test as saline-treated rats,

indicating there was no further impairment of extinction retention induced by NMDA receptor blockade. However, when adolescent rats were given twice the amount of extinction training in Experiment 2 so that saline-treated rats showed good retention of extinction at test, MK801 given before the second day of extinction training caused impaired extinction retention to a level comparable to that of rats given only one day of extinction training. The findings of these experiments suggest that extinction in adolescence does not initially involve NMDA receptors and this is a likely mechanism that contributes to the impaired fear inhibition observed at this age. However, NMDA receptors appear to be recruited with extended extinction training which leads to effective extinction retention.

Disclosures: K.D. Baker: None. R. Richardson: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.16/III12

Topic: G.01. Appetitive and Aversive Learning

Support: Baker Foundation Women In Science Fellowship awarded to DEG

NHMRC Career Development Fellowship awarded to JHK

Title: The neural correlates of fear extinction in adults and adolescents: an fMRI study

Authors: *D. E. GANELLA[1], E. P. GANELLA[2], J. H. KIM[1], S. L. WHITTLE[2]; [1]Florey Inst. of Neurosci. and Mental Hlth., Melbourne University, Australia; [2]Dept. of Psychiatry, Melbourne Neuropsychiatry Centre, The Univ. of Melbourne, Melbourne, Australia

Abstract: Anxiety disorders are the most common of all mental disorders worldwide, with 75% of those suffering from an anxiety disorder being diagnosed during childhood or adolescence. Fear extinction deficits have been suggested to be a fundamental mechanism underlying anxiety disorders. While rodent research suggests that deficits in adolescent fear extinction may be caused by abnormal ventromedial prefrontal cortical (vmPFC) function, no similar work has been done in human adolescents. Using a novel paradigm, we investigated the neural correlates of fear extinction learning and recall using functional magnetic resonance imaging (fMRI) in adolescent and adult humans. Given that females are twice more likely to suffer from an anxiety disorder than males, we also investigated gender differences within the adolescent group. Healthy adults (15, aged 25-35) and healthy adolescents (9, aged 14-16), underwent fMRI using a novel fear learning paradigm involving the pairing of a neutral face (CS) with a fear face plus

scream (US). The paradigm involved four phases: (A) Trace conditioning, where one of two neutral faces (CS+) was paired with an aversive sound (female scream, US) on 100% of trials. The other neutral face was a control stimulus (CS-) that was never paired with the US, (B) Extinction, CSs were presented without the US, (C) Reinstatement, two presentations of the US, and (D) Extinction recall, where both CSs were again presented without the US.
Analyses using SPM12 revealed that adults demonstrated the expected vmPFC activation during extinction (cs+ > cs-); however adolescents showed decreased vmPFC and increased rostral/dorsal anterior cingulate cortex activity during both extinction learning and recall, as compared to adults. We found that male adolescents showed reduced vmPFC activity during extinction learning compared with females. When tested for fear recall, however, the pattern reversed, such that female adolescents showed reduced vmPFC activity compared to males. These findings suggest that vmPFC deficits may not appear until after the initial extinction learning phase in female adolescents. These findings have implications for understanding risk factors and treatments for adolescent anxiety. They highlight the importance of investigating extinction learning and recall both across development and gender in order to elucidate the mechanisms of anxiety disorders.

Disclosures: D.E. Ganella: None. E.P. Ganella: None. J.H. Kim: None. S.L. Whittle: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.17/III13

Topic: G.01. Appetitive and Aversive Learning

Support: NHMRC Australia Career Development Fellowship APP1083309

NHMRC Australia Principal Research Fellowship 1020737

Baker Foundation Fellowship

Australian Postgraduate Award

Victorian Government's Operational Infrastructure Support Program

Title: Characterizing adolescent fear extinction learning in a rodent model of youth anxiety

Authors: *I. ZBUKVIC[1,2], J. PARK[1,2], D. GANELLA[1], A. J. LAWRENCE[1,2], J. H. KIM[1]; [1]Florey Inst. of Neurosci. & Mental Hlth., Melbourne, Australia; [2]Univ. of Melbourne, Melbourne, Australia

Abstract: Treatment for anxiety disorders frequently involves cue exposure therapy (CET), based on the principle of extinction. Extinction learning involves dopamine signaling in the prefrontal cortex (PFC), a system undergoing dramatic alterations during adolescence. Developmental changes in the infralimbic cortex (IL) of the PFC are implicated in adolescent extinction deficits, which model youth resistance to CET in the clinical setting. Importantly, resistance to CET due to extinction impairments may help explain adolescent vulnerability to anxiety disorders. The present study aimed to elucidate the role of prefrontal dopamine in extinction learning across adolescent development. Using a fear conditioning paradigm, adolescent (postnatal[P]35 and P53) and adult (P70 and P88) rats were conditioned using 3 tone-footshock pairings. The next day, rats underwent a single extinction session consisting of 30 tone presentations in absence of the footshock, designed to model CET. On testing the next day, long-term extinction was most effective in P88 rats and least effective in P35 rats. In separate subjects, changes in PFC dopamine 1 receptor (D1R) and dopamine 2 receptor (D2R) gene expression were measured following cue extinction. At age P35, PFC D1R/D2R ratio was lower for rats that received cue extinction ($p<0.05$), while at age P88 PFC D1R/D2R ratio was higher for rats that received cue extinction compared to handled controls ($p<0.05$). In a second study, age P35 and P88 rats underwent conditioning then received a bilateral intra-IL infusion of vehicle, the D1R agonist SKF-81297 (0.1ug/hemisphere), or the D2R agonist quinpirole (1.0ug/hemisphere) immediately before extinction. Acutely enhancing D1R signaling in the IL at the time of extinction had no effect on extinction learning in either age group. In contrast, acutely enhancing IL D2R signaling impaired extinction learning in adults, whereas it significantly enhanced long term extinction in adolescents ($p<0.05$). Results highlight a differential role for PFC D2R signaling in extinction learning across development, and offer important insights into the molecular mechanisms of fear extinction deficits during adolescence.

Disclosures: I. Zbukvic: None. J. Park: None. D. Ganella: None. A.J. Lawrence: None. J.H. Kim: None.

Poster

262. Learning and Memory: Extinction

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 262.18/III14

Topic: G.01. Appetitive and Aversive Learning

Support: Office of Naval Research grant N00014-09-1-0598

Office of Naval Research grant N00014-12-1-0366

National Institute of Mental Health R01MH104261

Pritzker Neuropsychiatric Disorders Research Consortium

Hope for Depression Research Foundation (HDRF)

Title: The role of heritable phenotype and social environment on fear extinction learning in rats

Authors: *K. E. PRATER[1,2], E. L. AURBACH[2], H. K. LARCINESE[2], P. BLANDINO, Jr.[2], S. J. WATSON[2], S. MAREN[3], H. AKIL[2];
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Abstract: Heritable propensities, such as temperament, that promote vulnerability or resilience in certain individuals are likely to interact with environmental factors (like trauma) to predict the development of PTSD. However, we know little about these interactions in humans or in animal models. The social buffering hypothesis posits that social support can mediate the effects of stress on an individual. We manipulated the social environment of rats to examine whether this variable would affect extinction behavior in male outbred and selectively bred animals. We compared outbred Sprague Dawley rats to rats selectively bred for high (bHR) or low (bLR) locomotor response to a novel environment. We demonstrated that the heritable phenotype of selectively bred animals leads to stable differences in fear extinction behavior across generations, indicating that these differences are similar to "genetic" propensities. We also observed that social environment significantly influenced the extinction learning of both outbred and selectively bred rats. We then manipulated the locomotor phenotype of the selectively bred animals through selective breeding and observed the interaction of this change in their heritable phenotype with the social environment. We found evidence that manipulating both the heritable locomotor phenotype and the social environment of selectively bred rats leads to additive effects, demonstrating a possible gene-by-environment interaction between novelty-seeking phenotype and social environment during extinction learning. Our work provides strong evidence that social environment significantly affects the extinction of learned fear in outbred and selectively bred rats, and has implications for both experimental design considerations and developing further understanding of gene-by-environment interactions in animal models.

Disclosures: K.E. Prater: None. E.L. Aurbach: None. H.K. Larcinese: None. P. Blandino: None. S.J. Watson: None. S. Maren: None. H. Akil: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.01/III15

Topic: H.01. Animal Cognition and Behavior

Support: NIH 5R01MH092925-02

W.M. Keck Foundation

Title: The independent contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats

Authors: *R. SANDLER[1,2,3], S. DHINGRA[1,3,2], C. VUONG[1,2,3], L. ACHARYA[7], M. MEHTA[4,2,3,5,6];
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Abstract: While the spiking activity of hippocampal place cells is well known to be modulated by spatial position, it is also simultaneously modulated by other factors including speed (McNaughton et al., 1983) and internal cellular dynamic processes such as refractory period, bursting, and theta oscillations. Furthermore, it has been traditionally believed that head direction does not influence hippocampal single units during 2D random foraging, but our group has recently demonstrated significant head direction modulation in this task (Acharya et al., 2016).These studies did not estimate the independent contribution of running speed on place cells. Such estimation is crucial since many theories of hippocampal function require the existence of robust speed modulation of place cells firing rates.
Importantly, the *independent and relative* contributions of each of these factors has not yet been disentangled. Such independent estimation is crucial yet difficult due to correlations amongst these factors which bias traditional method of calculating rate maps, such as binning. For example, a cell with a place field at the edge of a table may show a spurious negative speed correlation due to lower running speeds there. Furthermore, due to different methods of estimating rate maps from different factors, assessment of their relative importance is difficult. Here we develop a unified framework for identifying and quantifying the independent influence of these features on hippocampal place cells using generalized linear models (GLMs) along with basis expansions. The method yielded not only the independent contribution of position, head direction and running speed to neural spiking, but also provided an estimate of their relative magnitudes.
This framework was used on hippocampal neurons recorded from freely behaving rats during a 2D random foraging task with various types of visual cues. Surprisingly, we found that only a small fraction of neurons showed significant speed modulation. Further, speed modulation of place cells was weaker than both positional and head directional modulation. These results thus provide novel insights about the mechanisms governing place cells, and have important implications for theories of hippocampal function.

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McNaughton, B. L., Carol A. Barnes, and J. O'keefe. "The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats." *Experimental*

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Acharya, Lavanya, et al. "Causal Influence of Visual Cues on Hippocampal Directional Selectivity." *Cell* 164.1 (2016): 197-207.

Disclosures: R. Sandler: None. S. Dhingra: None. C. Vuong: None. L. Acharya: None. M. Mehta: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.02/III16

Topic: H.01. Animal Cognition and Behavior

Support: W. M. Keck foundation

NIH 5R01MH092925-02

Title: Emergence of hippocampal half-theta in virtual reality.

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Abstract: The rodent hippocampus shows robust theta rhythm (Green & Arduini 1954, Buzsaki & Vanderwolf 1983), which plays a crucial role in synaptic plasticity, learning and memory, and hippocampal information processing. Prominent theories of hippocampal function critically depend on the precise frequency of theta rhythm. Hence, it is important to understand the mechanisms governing theta rhythm. While theta rhythm is generated internally, it is also influenced by other variables including behavior. For example, during immobility, hippocampus shows Type 2 theta that has low frequency (~6Hz), that is quite irregular and has low amplitude. During spatial exploration, Type 2 theta is replaced by Type 1 theta with higher frequency (~8Hz) that has much larger amplitude and regularity. Further, the amplitude and frequency of Type 1 theta rhythm increases with running speed. In addition to locomotion, several other variables, such as vestibular and sensory cues, change between immobility and spatial exploration. To disentangle these different contributions, we compared the nature of hippocampal theta rhythm when rats were running either in the real world or in visually similar virtual reality (Cushman et al. 2013). Consistent with our recent findings (Ravassard et al. 2013) we found that the frequency of Type 1 theta was slightly smaller in virtual reality than in the real

world. Surprisingly, in the local field potential (LFP) on many electrodes we also discovered a novel theta rhythm, whose frequency (~4Hz) is nearly half of the Type 1 theta (~8Hz). Hence, we called this Half-Theta. Unlike Type 2 theta which only appears during immobility and does not coexist with Type 1 theta, Half-Theta was present during locomotion and coexisted with Type 1 theta. Half-Theta was only found in virtual reality and not in the real world. Unlike both Type 1 and Type 2 theta that are found on all electrodes in the hippocampus, significant Half-Theta was found on only about a third of electrodes. Both Type 1 theta and Half-Theta amplitudes increased with running speed in virtual reality. These results thus reveal a novel, behavior- and environment-dependent rhythm in hippocampal LFP, which would help elucidate the mechanisms of hippocampal theta rhythm. The Half-Theta in rodents in virtual reality has similar frequency and amplitude characteristics as that seen in primates and humans in virtual reality, thus bridging the gap in theta properties across species.

Disclosures: K. Safaryan: None. Y. Shen: None. M.R. Mehta: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.03/III17

Topic: H.01. Animal Cognition and Behavior

Support: W. M. Keck foundation

NIH 5R01MH092925-02

T32 Neural Microcircuits Training Grant

Title: Place cells encode path dependent episodic distance during virtual navigation

Authors: *J. J. MOORE[1,2,3,4], L. ACHARYA[8], J. D. CUSHMAN[3,5], C. VUONG[1,2,3,6], M. R. MEHTA[1,2,3,6,7];
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Abstract: The Morris Water Maze is a classic, widely-used behavioral test of spatial memory and navigation, as it tries to minimize non-specific local cues such that only distal visual cues can guide navigation. But this elimination of local cues is often not complete, and the neural basis of this behavior is not well-characterized. Hence, we have previously described a virtual

reality (VR) apparatus where only distal visual cues define spatial location[1,2]. Rats readily learn to successfully navigate in the virtual (water) maze task[2], displaying different trajectories based on the start position in the virtual environment. Here we characterized the responses of dorsal hippocampal CA1 single units while rats performed the virtual navigation task.

Surprisingly, pyramidal cells, the traditional "place cells" thought to underlie the cognitive map, do not exhibit allocentric spatial selectivity during virtual navigation. This is similar to the lack of an allocentric map during virtual random foraging tasks[3]. Instead, many cells are selective to the distance travelled from the start point of a trial, termed the episodic distance. However, episodic distance alone is not enough to uniquely define the position of the goal location in the virtual water maze when the rat starts from different positions. Hence we compared the episodic distance coding from different start locations. Episodic distance coding neurons exhibited three different patterns: I. Tuning for the same distance with the same peak firing rate regardless of start position; II. Tuning for the same episodic distance with differential firing rates based on start position (i.e. rate remapping); and III. Tuning for different episodic distances based on start position (i.e. global remapping). This modulation of rate by start position could be influenced by the distal visual cues defining head angle within the virtual space[4]. Additionally, this may be influenced by prospective coding based on the planned future path; it is unlikely to represent retrospective coding based on the previous path, because start positions were randomized across trials, and did not follow a reliable pattern. These results demonstrate that hippocampal single unit responses during virtual (water) maze navigation contain episodic information both about the distance along a trajectory as well as the position from which a trajectory started. This information could allow rats to successfully navigate in this task, even in the absence of an allocentric cognitive map.

1. Ravassard, P. *et al. Science* (2013). 2. Cushman, J. D. *et al. PLoS One* (2013). 3. Aghajan, Z. M. *et al. Nat. Neurosci.* (2015). 4. Acharya, L. et al. *Cell* (2016).

Disclosures: J.J. Moore: None. L. Acharya: None. J.D. Cushman: None. C. Vuong: None. M.R. Mehta: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.04/III18

Topic: H.01. Animal Cognition and Behavior

Support: W. M. Keck foundation

NIH 5R01MH092925-02

Title: Comparative influence of visual versus other multisensory cues on hippocampal spatial and directional tuning

Authors: *S. DHINGRA[1,2,3,4,5], R. SANDLER[1,2,3,4,5], C. VUONG[1,2,3,4], L. ACHARYA[6], M. MEHTA[1,2,3,4,5];
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Abstract: Hippocampal neurons show robust spatial selectivity during free behavior, i.e. during two dimensional (2D) random foraging. It has been commonly believed that place cells in rodents do not show any directional tuning (*McNaughton et al. 1983*), despite the inclusion of polarizing visual cues (*Muller et al. 1994*). However, methodological issues in experiments (e.g. presence of uncontrolled, nonspecific, multisensory cues) and analysis (e.g. interaction between spatial and directional variables) can strongly impact spatial and directional selectivity estimates. We recently developed a virtual reality system that eliminates spatially informative nonspecific multisensory cues, and generalized linear model (GLM) techniques which provide independent estimate of spatial and directional selectivity (*Aghajan et al., 2014; Acharya et al., 2016*). Using these we discovered that nearly a third of hippocampal neurons show significant directional selectivity in both real world and virtual reality, and visual cues alone are sufficient to generate directional tuning, in a causal manner, in the virtual world.
However, during natural behavior in the real world, both visual and other cues (e.g. vestibular, olfactory etc.) are present. In this case, it is important to understand if directional selectivity could exist even without visual cues, e.g. supported by vestibular cues, as is the case in the head direction system.
We conducted rodent experiments with different distal visual cues conditions. These conditions ranged from having no visual stimuli, to abundant visual stimuli but without any directional information and, to having varying amounts of directional visual information. We then estimated the independent contribution of position, head-direction and speed using a GLM framework (*Sandler et al., SfN Poster, 2016*).
We find that distal visual cues are not necessary for spatial selectivity in the hippocampus, though the spatial modulation seems to be significantly reduced under conditions with minimal distal visual stimuli, consistent with previous works (*Quirk et al., 1990*). We also find that even though spatial modulation might exist with non-distinct distal visual cues, they are significantly less coherent than with distinct cues. Importantly, we found a profound decrease in directional tuning in the absence of directionally informative visual cues. This shows that vestibular cues alone are insufficient for hippocampal directional selectivity. These results thus elucidate the relative contribution of visual versus other sensory cues in governing hippocampal spatial and directional selectivity, and constrain theories of hippocampal function.

Disclosures: S. Dhingra: None. R. Sandler: None. C. Vuong: None. L. Acharya: None. M. Mehta: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.05/III19

Topic: H.01. Animal Cognition and Behavior

Support: BMBF 01GQ1004A

Title: Hippocampal place cells differentially integrate visual and locomotor inputs

Authors: *O. V. HAAS[1,2,3], J. HENKE[1,2,3], C. LEIBOLD[1,2], K. THURLEY[1,2];
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Abstract: Hippocampal place cells provide a neural representation of an animal's location within its environment. To accomplish this, the hippocampus integrates signals from multiple modalities. However, it is unclear how these different inputs are combined into place fields. We investigated how visual spatial cues and an animal's locomotion are integrated by place cells. We recorded from the hippocampus of Mongolian gerbils (Meriones unguiculatus), while animals moved on a virtual linear track by rotating an air-suspended sphere. In virtual reality, we could decouple locomotion from visually perceived movement by changing the gear ratio between movement of the sphere and the visual projection. Place cells both in CA1 and CA3 responded differentially to this manipulation. We find that a subset of cells mostly encodes visual information, as it keeps their place fields in accord with the virtual environment. Whereas in another subset of cells, place fields remain at the same running distance. An in-depth analysis revealed that visual fields predominantly occur at locations at which visual textures change and that place field width decreases with prominent visual landmarks. For locomotion-induced place fields, we find that their width increases with running distance. Some place cells even exhibited two place fields, each driven by one of the two different modalities. A small fraction of cells changed their field's position in a way that could not be attributed to changes in gear ratio (remapping). All place cells adjusted their firing field according to the gear ratio on a single run basis; no adaptation over multiple runs was necessary, arguing for a mechanism that acts on a (milli-)second time scale. Altogether our results show that hippocampal place cells retain strong sensory influence from multiple modalities suggesting a role of the hippocampus as a multisensory associator.

Disclosures: O.V. Haas: None. J. Henke: None. C. Leibold: None. K. Thurley: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.06/III20

Topic: H.01. Animal Cognition and Behavior

Support: Gatsby Charitable Foundation

Wellcome Trust

Title: The Honeycomb Maze: a new behavioral apparatus for testing spatial navigation

Authors: *J. M. O'KEEFE, M. BAUZA, M. BERTELLI, S. BURTON, A. DELEKATE, A. HASTINGS, D. HOWETT, J. KRUPIC, R. WOOD;
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Abstract: The Morris Water Maze has been the workhorse for testing hippocampal-dependent allocentric spatial learning in rodents for over 30 years. It has the advantage that animals are started from different locations on each trial and therefore must approach the goal from different directions, ruling out directional and cue-approach strategies. Animals with damage to the hippocampus are deficient in learning the task. Its drawbacks are that it does not offer a series of discrete choices on the path to the goal making it difficult to score performance, it does not easily lend itself to varying the level of task difficulty, successive choices along the path to the goal are not independent, and it is not compatible with chronic single unit recording. We have devised a new behavioral test which retains the advantages of the water maze and overcomes these disadvantages. The Honeycomb Maze consists of 37 platforms organized in an overall hexagonal shape. Each platform is situated on top of a pneumatic tube which can be raised or lowered independently of the others. In the standard allocentric spatial navigation task one platform is designated as the goal and the animal is started from different platforms on each trial. Each trial consists of the food-deprived rat being placed on a raised start platform and after a short period 2 adjacent platforms raised to offer it a binary choice. Selection of one of these results in the start platform and the non-chosen platform being lowered and two new choice-platforms being raised. This continues until the animal has reached the goal where it is fed or after five minutes has elapsed. The animal's knowledge of goal direction can be assessed at each choice, and results from trials in which neither choice-platform points in the goal direction support the idea that animals can perform vector computations. Test difficulty can be varied by varying the angle between the two choice-platforms. Control animals and animals with ibotenic acid lesions of the hippocampus were given four trials per day for 17 days. Controls learned rapidly, on average reaching a criterion of 90% correct choices in 18.5 trials while lesioned animals were significantly impaired, with 4/8 of animals not reaching this criterion within 68 trials. While

primarily designed to test allocentric spatial navigation to a goal location, the Honeycomb Maze is easily adapted to test other forms of spatial and non-spatial learning.

Disclosures: J.M. O'Keefe: None. M. Bauza: None. M. Bertelli: None. S. Burton: None. A. Delekate: None. A. Hastings: None. D. Howett: None. J. Krupic: None. R. Wood: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.07/III21

Topic: H.01. Animal Cognition and Behavior

Support: DFG EXC 307

Title: Structural determinants of granule cell activity in the dentate gyrus of freely-moving rats

Authors: *M. DIAMANTAKI[1,2], M. FREY[1], P. PRESTON-FERRER[1], A. BURGALOSSI[1]; [1]Werner-Reichardt Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Grad. Training Ctr. of Neurosci. - IMPRS, 72074 Tübingen, Germany

Abstract: To understand how neural circuits contribute to behavior, it is crucial to relate in-vivo activity patterns to the underlying neuronal elements. In the dentate gyrus – a key component of the spatial memory circuits – granule cells (GCs) are known to be morphologically diverse and to display heterogeneous activity profiles during behavior. To resolve structure-function relationships, we juxtacellularly recorded and labeled single GCs in freely-moving rats. We found that, in line with previous observations, the large majority of neurons were silent during exploration (196 out of 228). Active GCs displayed a unique spike waveform, which could be used for assigning unidentified recordings to the GC population with high accuracy. Most identified and classified GCs fired at low rates and showed spatial activity. Compared to silent neurons, identified active GCs displayed a higher degree of dendritic complexity, and contributed ~2 times more high-order dendrites within the molecular layer. Spine densities were not different between active and silent GCs, but increased as a function of dendritic branch order; as a consequence, active neurons displayed significantly more spines than silent ones. Average firing rates strongly correlated with dendritic complexity ($r=0.68$; $p= 0.01$) and the total number of spines ($r=0.62$; $p= 0.02$). Our data thus provide the structural basis for functional heterogeneity among the GC population. We propose that the in-vivo activity of GCs is determined by their dendritic organization, which quantitatively constrains the total amount of postsynaptic sites available to excitatory entorhinal inputs.

Disclosures: M. Diamantaki: None. M. Frey: None. P. Preston-Ferrer: None. A. Burgalossi: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.08/III22

Topic: H.01. Animal Cognition and Behavior

Support: Human Frontier Science Program

Department of Biotechnology, India

Department of Science and Technology, India

Title: Degenerate mechanisms mediate decorrelation and pattern separation in the dentate gyrus

Authors: *P. MISHRA, R. NARAYANAN;
MOLECULAR BIOPHYSICS UNIT, INDIAN INSTITUTE OF SCIENCE, BANGALORE, India

Abstract: A prominent hypothesis regarding the physiological significance of neurogenesis is its role in pattern separation, a process by which similar afferent inputs impinging on a network result in distinct (decorrelated) population responses. In this computational study involving heterogeneous conductance-based models of dentate granule (GC) and basket cells (BC), we quantitatively delineated the specific contributions of different forms of neuronal diversity to response decorrelation. We first employed a random-sampling procedure to build physiologically and biophysically constrained populations of BCs and GCs that exhibited significant parametric variability and weak pair-wise correlations. We constructed a dentate gyrus (DG) network of these models (500 GCs and 75 BCs) with connectivity defined by anatomical data. Based on a virtual animal's traversal in a square arena (1 m × 1 m) at rodent speeds, this DG network received external inputs from the medial (grid-like) and lateral (contextual) entorhinal cortices. In one set of experiments, we fed identical external inputs to the network, and assessed response decorrelation in networks built with different combinations of intrinsic, synaptic and neurogenesis-induced variability. We demonstrate that the mere presence of experimentally constrained intrinsic biophysical variability was sufficient to introduce significant decorrelation of GC responses to identical inputs. Additionally, correlations between population responses decreased when the strengths of excitatory or inhibitory synapses were increased. Next, we incorporated synaptic and neurogenesis-induced diversity (in addition to intrinsic diversity) into

the network, and found that population correlations achieved with either form of diversity were comparable to those attained solely with intrinsic variability. In a second set of experiments, we designed two distinct arenas and gradually morphed one to the other, fed spatial information from these morphed arenas to the network and computed response correlations of the same cells across different arenas. Here, we show that efficient pattern separation could be achieved by the presence of intrinsic and synaptic variability, with non-significant contributions from an additional layer of variability introduced by neurogenesis. Our results suggest that degenerate mechanisms (1), involving disparate combinations of intrinsic, synaptic, connectivity and neurogenesis-induced forms of variability, contribute to pattern separation/decorrelation in the DG.

Reference

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Disclosures: P. Mishra: None. R. Narayanan: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.09/III23

Topic: H.01. Animal Cognition and Behavior

Title: Measuring the topological dimensions of hippocampal place cell firing rate space generalizes to all classes of neurons

Authors: *S. E. FOX, J. B. RANCK, Jr;
Dept Physiol & Pharmacol, State Univ. of New York Downstate Med. Ctr., Brooklyn, NY

Abstract: The firing of a class of neurons can be represented as a manifold embedded in a larger dimensional space. For N neurons, the space has N dimensions, each point a vector whose components represent the persisting effect of each action potential (a generalized rate space). We know that there is typically a small dimensional embedded space, because the activity of most neural classes can be described in a few words.
The inductive method to measure the topological dimension, which is always an integer, exploits the property that a boundary of a subspace with unknown topological dimension n, in an N-dimensional space has n-1 dimensionality. A randomly selected point in the rate space becomes the center of an n-sphere with a diameter small enough to fit inside that N-space. A point on its boundary is then selected as the center of a new (n-1)-sphere. The number of times this process must be applied in order to be left with only 2 points is the inductive dimension of the embedded

manifold.
This can be appreciated intuitively in a 3D space that is uniformly populated with points. The 1st step produces a 2D surface of a sphere, the 2nd, a 1D curve (a circle) and the 3rd, 2 points: 3 steps = inductive dimensionality of 3. The firing rate space of N neurons during behavior is a sparsely populated N-space having an embedded manifold with an inductive dimensionality ($n \leq N$, usually $n<<N$) that represents the number of terms necessary to describe the firing in the subspace. To deal with the sparsity, the inductive method was modified to use a thick shell for the boundary, rather than a surface.
We measured the inductive dimension of an 89D space describing the firing rate vectors for 89 place cells (courtesy of Dr. Eva Pastalkova) recorded simultaneously for 1 hr while the rat foraged for food pellets scattered on a 1 m square open field. Initially, we expected the dimensionality of the embedded manifold to be 2, representing the coordinates of the open field, but the modal result was 3, which is, upon further consideration, the right answer.
Whereas the conclusion was predictable for place cells during foraging because the sensory-behavioral correlates are well known, there are many neuronal populations and conditions for which there are an unknown number of parameters necessary to account for the firing rate vectors over time, e.g. firing with no change in behavior, as in sleep. The topological dimension of the manifold is useful because: 1) it allows more extensive mathematical analyses to be used, e.g. maps between manifolds, combining manifolds, and 2) it allows us to define circumstances in which the experiential curve intersects itself, i.e. in which something is the "same", as is required in learning and recognition.

Disclosures: S.E. Fox: None. J.B. Ranck: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.10/III24

Topic: H.01. Animal Cognition and Behavior

Support: CNPq

CAPES

Title: Asymmetry of temporal and rate codes for space by hippocampal place cells

Authors: *A. B. TORT, B. C. SOUZA;
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Abstract: The rodent hippocampus plays a role in spatial memory and navigation. Some hippocampal neurons, called place cells, increase their firing rate when the animal is at a specific location of the environment, known as the 'place field' of the cell. As the animal crosses place fields, place cells form spike sequences coordinated by the hippocampal theta rhythm - a prominent field potential oscillation at 5-12 Hz - by firing action potentials coupled to earlier phases of the theta cycle, a phenomenon known as 'phase precession'. Place fields and phase precession are considered canonical examples of rate and temporal codes, respectively, in which the firing rate of the neuron and the exact spike timing relative to the theta cycle provide information about space. Whether temporal and rate coding are governed by independent or related mechanisms is currently unknown and the subject of wide debate. Here we show that the spike timing of place cells couples to theta phase before major increases in firing rate (i.e., before the animal enters the classical, rate-based place field). In contrast, theta-phase coupling rapidly ceases as the animal leaves the place field and firing rate decreases, revealing that temporal coding has strong asymmetry around the place field centre. Interestingly, place cells are not coupled to theta phase at positions distant from the place field centre; therefore, the dynamics of place cell activity can be separated into three stages: phase coupling, phase precession and phase decoupling. These results suggest independent mechanisms of temporal and rate coding by hippocampal place cells.

Disclosures: A.B. Tort: None. B.C. Souza: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.11/III25

Topic: H.01. Animal Cognition and Behavior

Support: JSPS KAKENHI 16K07009

MEXT KAKENHI 16H01557

Title: A nonlinear unsupervised-learning method extracts spatial information and more from hippocampal population activity of freely-moving rats

Authors: *M. ITO, K. DOYA;
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Abstract: How to extract information coded in large population of neuronal activities has become an important issue, with the recent progress in neural recording technology, especially in

calcium imaging. Currently, a decoding method is one of the most common techniques to analyze a large population data. This supervised-learning method uses a data set of population activity X and a target variable Y, for example, a parameter of stimulus animal is receiving, to construct a decoder (mapping from X to Y). Information of Y coded in X can be quantified from the accuracy of estimation Y for new X. However, in this analysis, Y should be selected as a candidate of coded information in advance. Thus, it is impossible to find out unknown information that researchers don't think of.
In this study, we propose that unsupervised-learning methods can extract information purely from X without Y. We focused on the population activity of hippocampal pyramidal cells, because they are well known to convey spatial information. We recorded activities of 338 CA1 pyramidal neurons expressing calcium indicator (GCaMP6f) simultaneously with a miniature fluorescence microscope (inscopix) while a rat was exploring in an open space (45 cm x 45 cm). We applied a nonlinear unsupervised-learning method, called t-SNE (t-distributed stochastic neighbor embedding) to the population activity X. t-SNE extracted three-dimensional structure from X in 338-dimensional space spanned by each neuron's activity. Then, we added a new coordinate system for the three-dimensional structure by a principal component analysis.
We found that 1st and 2nd components represented the position of the rat in the environment. The mean-squared error from the actual x-y trajectories was significantly lower than the chance level (7.2 cm < 22.5 cm) after the adequate transformation (mirror, rotate, and scale). Surprisingly, the error was significantly smaller than that of a supervised-learning method (a grid-based Bayesian method) when the data size was smaller than 300. Furthermore, we found that the remaining 3rd component significantly correlated with the moving speed of the rat. When the 3rd component was a lower value, the tendency of staying at the same position was stronger.
These results demonstrated that an unsupervised-learning method can be a strong tool to extract unknown information from the population activity. By this method, we newly found that the moving speed was also represented in the hippocampus as well as spatial information.

Disclosures: M. Ito: None. K. Doya: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.12/III26

Topic: H.01. Animal Cognition and Behavior

Title: Dynamical properties of hippocampal reactivation of two-dimensional spatial representations

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Abstract: Sharp wave/ripples (SWR) are high-frequency oscillatory events that dominate periods of slow wave sleep in the CA1 region of the hippocampus. Importantly, the bursts of highly synchronized neuronal activity that accompany these events are thought to promote the consolidation of memory patterns across multiple areas. Indeed activity observed during SWRs is highly structured and often corresponds to the firing patterns of place cells expressed during previous environment exploration. More specifically, it has been shown that the reactivation of spatially-modulated hippocampal cells is organized into sequences that replay previous experience by reproducing the order of place fields in the environment.
A wide breath of studies have characterized reactivation and replay of hippocampus place cells, yet the link between this information-rich activity and the neural mechanisms regulating its expression over the course of a SWR is still missing. As a consequence it is still unclear how the findings of sequential activation, mostly studied in one-dimensional environments (e.g. linear track), may be extended to two-dimensional environments with fewer geometrical constraints (e.g. open field).
To address this question, we analysed activity of CA1 neurons during sleep following the exposure to a large open field environment. By using Bayesian decoding techniques we are able to track the evolution of space-related activity during each reactivation event and to reconstruct the set of environment locations expressed during each SWR. We find that reactivation events properties distribute on a spectrum ranging from the expression of a single-location to the sequential activation of distant, unrelated sets of positions. The statistical properties of these events appear to reflect those characterizing stochastic processes like random walks, suggesting that such a heterogeneous phenomenology might actually emerge from a common, shared process.

Disclosures: F. Stella: None. P. Baracskay: None. J. Csicsvari: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.13/III27

Topic: H.01. Animal Cognition and Behavior

Support: Kaken-hi (15H05569)

Keken-hi (15H01417)

Title: Hippocampal neuron firing during a cue preparation period for trajectory planning

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Abstract: Animals adaptively navigate to remembered goals according to instantaneous demands of external environments. To attain such online navigation, the brain circuit needs to plan future possible routes based on current environmental/idiothetic cues. Recent studies have demonstrated that hippocampal circuits not only encode present locations (so-called place fields) but also generate organized firing patterns corresponding with future behavior. However, the detailed spatio-temporal characteristics of such prospective activity are not fully understood. In this study, we designed a spatial task in which rats learned to traverse specific locations in a two-dimensional open field. The rats began a trial with their active nose poke in a small box where guidance cues with or without a light cue were presented for five seconds. A start door was then opened to allow the rats to enter into the field. If the rats could travel along a correct trajectory toward a goal door corresponding with the cue pattern, the goal door was opened so that they obtained a reward. During the task, we performed multiunit recordings from hippocampal CA1 neurons. A certain population of cells exhibited trajectory-dependent place-selective firing in the open arena. In addition, during the 5-s cue presentation period, we observed cue-specific firing sequences of neuronal ensembles, including cells that would be both active and silent during the subsequent spatial running. We will further analyze the detailed characteristics of the cue-driven firing sequences, such as which types of cells are involved in the sequence, whether the sequences are associated with task performance, and how the sequences are modulated by local field oscillations of the hippocampus. The results will help understand the contribution of internal activity patterns stored in the hippocampal network to online planning of upcoming behavior in response to continuously changing situations.

Disclosures: H. Igata: None. T. Sasaki: None. Y. Ikegaya: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.14/III28

Topic: H.01. Animal Cognition and Behavior

Support: ONR MURI N000141310672

NSF IIS 1464349

UBRP

Title: The role of hippocampal replay in a computational model of path learning

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Abstract: Hippocampal place cells play an important role in spatial navigation in rodents, but the exact mechanism by which networks of place cells store and process spatial information is poorly understood. “Replay” events, in which behaviorally relevant place cells are reactivated offline during sharp-wave ripples (SPWs), are likely critical to spatial information storage. However, the mechanisms by which cells are selected to fire within SPWs are unknown.
In this study, we use a mobile robot and a biophysically realistic network model of place cells and interneurons with spike timing-dependent plasticity (STDP) to simulate hippocampal activity during spatial navigation. The robot is comparable in size to a rat and generates paths that are approximations of typical rat trajectories in spatial noise and velocity. These paths are used as inputs to the model and cause specific patterns of activation in the network, which, coupled with the STDP rule, generate a synaptic matrix that contains path-specific information. When SPWs are simulated in a trained network, its synaptic matrix lays constraints on which cells are reactivated, and may explain why some cells are selected for replay firing and others are not. The firing patterns of these cells during path traversal are then decoded in an ideal observer framework to estimate the path taken by the robot. We hypothesize that place cells that participate in trajectory replay events will convey more information about the path than non-replaying cells. We use the mean squared error (MSE) of the estimated location to test this hypothesis by comparing the MSE of decoding between different ensembles of place cells—all cells, replaying cells, and non-replaying cells.
Recent work suggests that grid cells and place cells are spatially coherent during replay events. In a separate set of simulations, we investigate the effects of including modular or non-modular grid cell information to path decoding with the aforementioned place cell ensembles. We test the robustness of different code ensembles with nominal paths and paths that contain significant perturbations. Our study shows the extent to which replay is governed by the synaptic weights of the network as learning occurs across trials, and how replay may affect the encoding of a learned path in the face of perturbations.

Disclosures: M.J. Ragone: None. S. Gianelli: None. D. Schwartz: None. L. Su: None. O.O. Koyluoglu: None. J. Fellous: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.15/III29

Topic: H.01. Animal Cognition and Behavior

Title: Real-time identification and closed-loop control of hippocampal replay content in freely behaving rats

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Abstract: The formation and retrieval of new memories is thought to be supported by a complex dialogue among different brain structures coordinated by the hippocampus. In the rodent hippocampus, signatures of memory processing at the cellular and network level can be found in the experience-modulated firing of principal cells and in the accelerated replay of firing patterns that occur during characteristic sharp-wave ripple (SWR) events. Previous work has shown that non-specific interruption of SWR events in both awake and rest state impairs the acquisition of spatial tasks. However, no direct evidence exists yet that causally links specific spike patterns (replay content) to the ability to learn and execute a memory-guided behavioral task. In order to bridge this gap, we developed a brain-computer interface (BCI) that performs real-time identification of replay content and enables content-specific manipulation of replay events in freely behaving rats.

Our BCI deploys an efficient spike-sorting-free neural decoding algorithm and a simple but effective strategy to estimate replay content using the initial portion of a replayed spike sequence. Both algorithms are implemented in a custom-made, general purpose multi-threaded software. This software executes a user-defined signal processing graph in soft real-time and provides the critical advantage of computing parallelization. The software framework only adds a small millisecond-scale latency to the computation time required by the decoding and replay content identification algorithms, which is negligible for our purposes.

For online replay content identification, we continuously searched in a short sliding window of decoded positions for fragments of target trajectories in a multi-segment track (y-maze or radial-arm maze). In order to reduce spurious detections of non-SWR associated sequences, the content identification is complemented with concurrent detection of a multi-unit burst and a SWR event. In tests on pre-recorded datasets, the replay content algorithm positively identifies target replay content within less than 50 ms after SWR start with >80% sensitivity and specificity.

Finally, we demonstrate real-time identification of replay content in live streaming neural data recorded in a freely behaving rat.

Disclosures: D. Ciliberti: None. F. Michon: None. F. Kloosterman: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.16/III30

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI16H01290

KAKENHI16K14561

Title: Hippocampal representations of spatial information of self and other

Authors: *T. DANJO, S. FUJISAWA;
Lab. for Systems Neurophysiol., Riken Brain Sci. Inst., Wako, Japan

Abstract: When animals explore an open field, how do they recognize their own place and other positional information in the field? Amounts of studies have shown that such spatial memory is founded on the hippocampal place cells, activated specifically when the one is located in their place fields. The assemblies of the place cells are hypothesized to establish the cognitive map, by which animals are able to find a place and navigate to go there, same as we find a place by referring a map. In this map-based navigation, spatial representations are allocentric, and thus place cells should represent not only the self's position, but also the positions of external cues which the animals pay attention to. However, neurons with such a feature have never been identified yet. Moreover, the mechanisms for the representation of a place where the animal is not located remain to be elucidated.
The main purpose of this study is to identify the mechanisms of how spatial information of other animals is represented in the brain. To this end, we record electrophysiological activities from the hippocampus while the rat is required to observe the position of the other rat, and then isolate neuronal activities that contribute to represent the position of the other. We show that the spatial information of self and other is jointly represented in the hippocampus.

Disclosures: T. Danjo: None. S. Fujisawa: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.17/III31

Topic: H.01. Animal Cognition and Behavior

Support: NIH 2R01MH080007

NIH R01MH093807

Simons Foundation 325467

WaNPRC Base Grant P51 OD010425

Title: Spatial non-grid cells in the primate entorhinal cortex

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Abstract: In primates, vision is the primary modality used to gather information from the environment, and cells representing visual space and eye position are found in many brain areas. Previous work identified neurons in the entorhinal cortex (EC) which exhibit grid-like visuospatial representations reflecting fixation locations across image space (Killian et al., 2012). To further examine the extent of spatial representation and to identify the spatial reference frame for the primate EC, we determined whether spatial activity was locked to the bounds of a visual image or instead locked to body position.

The spiking activity of 349 EC neurons from two rhesus macaque monkeys was recorded as they viewed complex images (30° x 25° or 30° x 15°) on a monitor. In alternating trial blocks, the images appeared in one of two locations that were laterally offset from each other by 4°. Firing rate maps for each neuron were computed for each of the two image positions, and the spatial correlation between the maps was computed for a range of incremental spatial offsets between 0 and 100% lateral shift. A high correlation at 0% implies no shift (egocentric reference frame), whereas one at 100% implies a complete shift of spatial activity with the image bounds (allocentric reference frame). Spatial stability was assessed within each image location. Spatial correlations were considered significant if they surpassed the 95th percentile of bootstrapped values (shuffling the spike train relative to eye position).

A large proportion of the neurons (138/349, 40%) demonstrated stable spatial activity, and this proportion was similar across animals. Both monkeys showed an even distribution of cells with an allocentric (49%) and egocentric (51%) reference frame, and reference frames were not always identical for simultaneously recorded cells. An analysis of reference frame that included the full population of cells (i.e., cells that were not pre-selected for spatial activity) revealed that

most had maximum correlation values at either 0% or 100% lateral shifts rather than at intermediate spatial offsets.
These results indicate that a large proportion of entorhinal neurons represent eye position. While some cells represent eye position relative to the bounds of an examined image, other cells simultaneously represent eye position relative to body position (or other elements that were invariable in our experiment like the room itself). These findings show that visuospatial representation is a fundamental property of entorhinal neurons and suggest these neurons may support relational memory and motor planning by coding attentional locus in distinct, behaviorally relevant frames of reference.

Disclosures: M.L. Meister: None. E.A. Buffalo: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.18/III32

Topic: H.01. Animal Cognition and Behavior

Support: NIH 5T90DA032436

NIH 2R01MH080007

NIH R01MH093807

Simons Foundation 325467

WaNPRC Base Grant P51 OD010425

Title: View cells in the primate hippocampus during visual exploration and directed saccades.

Authors: *S. D. KOENIG[1,2], E. A. BUFFALO[3,4];
[2]Grad. Program in Neurosci., [3]Physiol. and Biol., [1]Univ. of Washington, Seattle, WA;
[4]Washington Natl. Primate Res. Ctr., Seattle, WA

Abstract: Decades of studies in humans, non-human primates, and rodents have demonstrated that the hippocampus and surrounding cortex are important for memory formation. Single unit recordings in rodents have revealed place cells in the hippocampus and grid cells in the entorhinal cortex that are hypothesized to support the spatial aspects of episodic memories. However, spatial representations in the primate hippocampus are not fully understood. Notably, Rolls and colleagues (1997) identified neurons in the primate hippocampus that fired selectively

when a monkey viewed portions of the environment, independent of the monkey's physical position. Recent work in our lab identified visual grid cells in the primate entorhinal cortex which fired in a grid-like pattern reflecting fixation location during the free-viewing of complex images presented on a computer monitor (Killian, Jutras, and Buffalo, 2012). To further explore visuospatial representations in primates, we recorded the spiking activity of 282 hippocampal neurons from two monkeys as they freely viewed complex images. We found that 82 (29%) of these neurons were spatially stable and had significant (all p's < 0.05) spatial information scores (Skaggs et al., 1993). These spatial view cells were more prevalent in the posterior 2/3^{rds} of the hippocampus (36% of cells compared with 19% in the anterior 1/3rd). In between free-viewing trials, monkeys performed a directed saccade task in which they received reward for fixating a sequence of 4 items presented in different spatial locations against a black background. Unique sequences were used for each recording session. Of the 70 view cells that contained at least 1 of these sequence items in their view field, 58 (83%) showed significantly higher firing rates for items placed inside versus outside their view field (t-test, $p < 0.05$). These data suggest that spatial view responses are not driven selectively by free exploration compared to directed saccades. However, many view cells additionally demonstrated contextual modulation, with significant differences in firing rates during free-viewing as compared to the directed saccade task. Taken together, these data suggest that neurons in the primate hippocampus are modulated by viewing location. These neurons are found most prominently in the posterior 2/3^{rds} of the hippocampus, and many of these neurons are additionally modulated by behavioral context. Further, these results suggest that visual exploration in primates and physical exploration in rodents may engage similar neural mechanisms in the hippocampus.

Disclosures: S.D. Koenig: None. E.A. Buffalo: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.19/III33

Topic: H.01. Animal Cognition and Behavior

Support: NIH Training Grant in Computational Neuroscience, 5T90DA032436-04

Washington Research Foundation Innovation Graduate Fellowship in Neuroengineering

NIH 2R01MH080007

NIH R01MH093807

Simons Foundation 325467

WaNPRC Base Grant P51 OD010425

Title: Spatial representations in the monkey hippocampus during free-foraging in virtual reality

Authors: *Y. BROWNING[1,2,3], M. J. JUTRAS[4,5], K. L. MORRISROE[5], C. LEWIS[6], E. FIEDLER[7], T. STIEGLITZ[7], P. FRIES[6], A. L. FAIRHALL[4,2], E. A. BUFFALO[4,2,5]; [2]Washington Res. Fndn. Inst. for Neuroengineering, [3]Grad. Program in Neurosci., [4]Physiol. and Biophysics, [5]Washington Natl. Primate Res. Ctr., [1]Univ. of Washington, Seattle, WA; [6]Ernst Strüngmann Inst. for Neurosci., Frankfurt am Main, Germany; [7]Dept. of Microsystems Engin., Univ. of Freiburg, Freiburg im Breisgau, Germany

Abstract: A long history of research has identified exquisite spatial representations in the rodent hippocampus. However, the extent to which similar representations can be demonstrated in the primate hippocampus is not fully understood. In order to address this, we trained rhesus monkeys to perform a virtual reality task analogous to the rodent free-foraging task. Here, head-fixed monkeys were trained to use a joystick to navigate a first-person avatar towards targets (bananas) that were randomly scattered around a courtyard in virtual space. Bananas appeared in sets of ten, with new bananas appearing only after the previous set had been collected. The monkeys received food reward for each collected banana. For neural recordings, one monkey was prepared with chronically implanted, flexible, polyimide arrays which enabled recordings from 36 electrode contacts positioned throughout the anterior-posterior axis of the hippocampus. These arrays enabled stable recordings from single cells across multiple virtual environments and across days. Within a given recording session, in alternating blocks of trials, the monkey foraged in either a square or circular virtual arena. In initial analyses of 21 isolated neurons, we were unable to identify clear spatially selective responses related to the location of the monkey in virtual space. Instead, we identified 9 cells (42%) that were selective for the maneuver made by the monkey in the virtual world. Two types of maneuver cells were identified: “turning cells” and “speed cells”. Each of the turning cells fired preferentially for either left or right turns, irrespective of the monkey’s position within the virtual arena. Speed cells had firing rates that correlated significantly with the speed of the monkey’s avatar. For individual cells, these response preferences were consistent across environments (square or circle) and across multiple recording days, suggesting stable representations of maneuvers independent of context. Importantly, attention to spatial features and distal landmarks in the virtual environment was not required for successful performance in this virtual foraging task. Indeed, behavioral analyses suggest that monkeys complete this task using a set of stereotyped maneuvers, rather than explicit route planning. Taken together, these data suggest that in this task, hippocampal responses reflect parameters necessary for successful task performance, even though these parameters are not explicitly spatial. Future work will focus on the use of spatial memory-dependent tasks to further elucidate the role of the monkey hippocampus in encoding representations of space and memory.

Disclosures: Y. Browning: None. M.J. Jutras: None. K.L. Morrisroe: None. C. Lewis: None. E. Fiedler: None. T. Stieglitz: None. P. Fries: None. A.L. Fairhall: None. E.A. Buffalo: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.20/III34

Topic: H.01. Animal Cognition and Behavior

Support: Fondation pour la Recherche Médicale

Marie Curie reintegration grant

CNRS PEPII

Agence Nationale pour la Recherche (Brain GPS)

Title: Logical representation of space in monkey hippocampus

Authors: P. BARADUC[1], J.-R. DUHAMEL[1], A. PLANTÉ[1], S. PINÈDE[1], *S. C. WIRTH[2];
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Abstract: Two macaque monkeys were trained to navigate with a joystick a virtual reality maze, in search of a hidden liquid reward. The reward was obtained at the end of one of the 5 identical arms of the star maze, and could only be located with respect to the 5 distinct landmarks that were placed at a distance between the arms. Animals started their search at an arm end, and after reaching another arm end, they were passively translocated to a new start in a straight path outside the maze arms, preventing them from retracing their steps to the reward. Each training session comprised two periods; in the first, the animals learned a novel maze layout, in the second period they navigated a previously learned, familiar maze. These periods were run in counterbalanced order. The activity of 270 neurons in the hippocampus was recorded concurrently with the animals' gaze and joystick motions.
The monkeys' oculomotor behavior was consistent with a perception of the 3-dimensional environment and an anticipation of their subjective motion. Animals quickly found the reward and performed above chance in 12.3 ± 2 trials (monkey S) or 14.8 ± 2.4 trials (monkey K). They also successfully generalized from an incomplete exploration of the maze, suggesting a correct internal representation of the environment.
A great majority of hippocampal cells were sensitive to place, but these also responded to one or more variables like head direction, point of gaze, or task context. Many cells fired at the sight of a single landmark in a viewpoint- or task-dependent manner, simultaneously encoding the animal's logical situation within a set of actions leading to the goal. Overall, hippocampal activity was best fit by a logical state-space comprising current position, view and action contexts: 44% of the task-active recorded cells contained significant information in that space, and this information content was statistically higher than in positional, viewpoint or point-of-

gaze space. Some cells even distinguished the same position, orientation, and next action depending on the action context. Furthermore, correlation between the activity maps in the familiar and novel environments was highest in the reward-referenced logical state space. In this space, 24% of the cells were significantly correlated between the familiar and the environment, some of them coding novel and familiar mazes in a strikingly similar way, although the two mazes had no common landmark. This shows that while animals forage for food, hippocampal cells can represent space in a task-situated, goal-referenced, logical manner.

Disclosures: P. Baraduc: None. J. Duhamel: None. A. Planté: None. S. Pinède: None. S.C. Wirth: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.21/III35

Topic: H.01. Animal Cognition and Behavior

Support: John Templeton Foundation

Title: A novel shortcut task for analyzing hippocampal sequences

Authors: *E. M. IRVINE, M. A. VAN DER MEER;
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Abstract: The ability to take novel shortcuts towards a goal location is a signature behavior of the hippocampal “cognitive map,” and model-based behavior more generally. Despite a long history of behavioral work, there is currently little known of the neural processes that underlie successful shortcut-taking. Previous work examining neural activity in the hippocampus has focused on changes in place fields following experience with the shortcut, leaving open how the shortcut was chosen in the first place. It has been widely noted that internally generated sequences in the hippocampus, such as those associated with sharp wave-ripple complexes (SWRs) have properties suitable for model-based behavior, and therefore may be associated with successful shortcut behavior. Testing this idea requires a task in which (1) neural activity can be measured before rats take a session-unique shortcut, and (2) neural representations of the shortcut trajectory can be compared with those of trajectories that are novel but not shortcuts. To this end, we developed a three-phase shortcut task using a modular, flexibly reconfigurable track system. In Phase 1, subjects traversed a variant of a roughly U-shaped track, receiving food reward at both ends. In Phase 2, two novel segments were added to the maze. These segments were blocked with clear barriers, making them visible but not physically accessible. One of the

segments was a shortcut between the two reward sites; the other segment led to a "dead-end" away from the rewards. In Phase 3, the barriers were lifted and subjects could traverse the novel segments. The modular nature of the maze allowed for session-unique configurations of both shortcut and “dead-end” novel segments. Crucially, the rats need to choose both novel segments a certain number of times so that place fields on both track segments can be estimated; we then retrospectively use these place fields to determine to what extent these novel segments were represented in hippocampal sequences prior to taking them. In Phase 3, rats (n = 4), implanted with 16-tetrode arrays recording from CA1, explored both novel segments. They showed a preference for the shortcut over both the U-shaped and “dead-end” tracks, demonstrating the shortcut is behaviorally relevant. Cells with place fields on both novel trajectories participated in SWR events prior to Phase 3, suggesting that animals represented both trajectories prior to direct experience, enabling further statistical comparison of the relative frequencies and temporal dynamics of shortcut vs. non-shortcut novel paths.

Disclosures: E.M. Irvine: None. M.A. van der Meer: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.22/III36

Topic: H.01. Animal Cognition and Behavior

Support: Supported by NSF-ANR CRCNS #1429937 Grant - A replay-driven model of spatial sequence learning in the Hippocampus-PFC network using reservoir computing

Title: Intra-hippocampal cell synapse learning may support pre-exposure based latent learning by means of improved replay events

Authors: *M. LLOFRIU ALONSO[1], P. SCLEIDOROVICH[2], T. PELC[3], N. CAZIN[4], P. F. DOMINEY[4], J.-M. FELLOUS[3], A. WEITZENFELD[2];
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Abstract: Early on, Small (1901) recognized the importance of unrewarded pre-exposure of animals to the environment to facilitate learning. Tolman (1948) analyzed Blodgett’s (1929) experiments on a multiple-T maze, which showed how unrewarded pre-exposure of a rat to the environment can increase the learning speed once rewarded trials begin, a latent learning phenomenon. In Blodgett’s experiment, 2 groups of rats were trained to find food on a multiple-T labyrinth, one with pre-exposure and one without. He observed that after the exploration

period, the rate at which the pre-exposed group of rats learnt the path to the food was much higher than control. We hypothesize that this higher learning rate could be explained by an improved quality of replay events due to a better “cognitive map” built during the unrewarded pre-exposure trials. Building a cognitive or experience map with greater coverage would allow the rat to generate longer replay events, which could improve offline learning. To test this hypothesis we implement a model of navigation based on the three main characteristics stated in Johnson & Redish (2005): the learning of intra-hippocampal synaptic weights, the generation of replay events based on this connectivity and the application of reinforcement learning to the replay trajectories. We apply this model to Blodgett’s experiments and assess whether the model is able to explain the observed difference in learning rates. We predict that the modulation of intra-hippocampal synapses and replay could constitute a concrete plausible biological mechanism for the latent learning phenomenon observed by Tolman in Blodgett’s experiments. We also predict that pre- exposure animals would present replay events that cover longer distances. Supported by NSF-ANR CRCNS #1429937 Grant - A replay-driven model of spatial sequence learning in the Hippocampus-PFC network using reservoir computing

Disclosures: M. Llofriu Alonso: None. P. Scleidorovich: None. T. Pelc: None. N. Cazin: None. P.F. Dominey: None. J. Fellous: None. A. Weitzenfeld: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.23/III37

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 16H01289

15H04265

Title: A hippocampal-entorhinal microcircuit model for dynamic communication in a memory-based navigation task.

Authors: *T. KURIKAWA, T. FUKAI;
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Abstract: Neural rhythm plays an important role in communication between different cortical areas. Such a communication is not constant in time, but dynamically changed depending on demands of memory-consolidation, memory recall, and so forth. Gamma and theta rhythms in hippocampus and entorhinal cortex are critical for memory-based navigation task. A recent study

(Yamamoto, et al., 2014, Neuron) has shown that coherence of these rhythms is dynamically changed during a T-maze task in which a rat is required to choose one of arms depending on the previous choice. They have found that neural activities locked with theta rhythm is selectively higher in a test trial than in sample trial and also found that gamma synchrony between hippocampus and entorhinal cortex which only occurs around a decision point is necessary for the correct choice. Blocking this connection reduces success rate to chance level. This study implies that information of previous choice is transported through the gamma synchrony. What mechanism, however, underlies such a dynamic and flexible communications remains unclear. For understanding this mechanism in microcircuit level, in this study, we model a local neural circuit including pyramidal neurons and some types of inhibitory neurons. This model network has three sub networks corresponding to CA1 and entorhinal cortices (EC3 and EC5) with input from CA3 to CA1 and that from a thalamus nucleus (reuniens). We assume that working memory of the previous choice is stored in EC3 and/or EC5 neurons as persistent activities and the decision is triggered by medial PFC via reuniens. By this model, we analyze the functional role of theta and gamma rhythms and conditions of each type of neurons under which the synchronization occurs in appropriate timing.We found that persistent activity with locked to theta rhythm is triggered by non-linear interactions of inputs from CA3 and EC3 to CA1 through CA1-EC5-EC3 loop. Because CA3 input encodes spatial information, this theta rhythm is generated in specific location on the maze. We also found that gamma synchronization that transfers the information stored in entorhinal cortices to CA1 is generated by interaction between feed-back and feed-forward inhibitions with triggered by reuniens. These results implies different roles of theta and gamma rhythm: theta-locked activity stores information in longer time scale (during a trial) and gamma synchronization triggered by the thalamus input controls more detailed timing to transfer the stored information.

Disclosures: T. Kurikawa: None. T. Fukai: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.24/III38

Topic: H.01. Animal Cognition and Behavior

Support: MEXT Strategic Research Program for Brain Sciences (11041047)

JSPS Grants-in-Aid for Scientific Research (25710003, 25116531, 15H04264, 16H01291) grants

Title: Hippocampal place cell remapping encodes alterations in task contingencies rather than valence in an aversive decision making task

Authors: *J. ORMOND, J. P. JOHANSEN;
Lab. for Neural Circuitry of Memory, RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Aversive experiences produce fear of contexts in which they occur and avoidance of those contexts when other options are available. In humans, contextual fear creates an expectation of potential future danger that characterizes anxiety, suggesting its study in rodents may provide important information about the neural mechanisms which underlie anxiety disorders. Contextual fear requires an intact hippocampus, likely due to the hippocampus's central role in spatial memory. Both physical and contextual changes in an environment can lead to changes in the properties of hippocampal place fields, a phenomenon termed remapping. A number of studies have demonstrated that aversive experiences cause remapping in hippocampal subregion CA1, but it not known whether remapping also occurs in hippocampal area CA3, which is distinguishable from CA1 based on its partially distinct anatomical connectivity. Furthermore, it is not clear if this remapping encodes a general change in the valence of the context from neutral to aversive, or whether it reflects changes in task contingencies and/or behavioral decisions. To address these questions, we developed a behavioral paradigm in which food-deprived rats chose one of three routes leading to food reward; two routes were paired with aversive eye-lid shock, and rats learned to avoid these routes in favor of a "safe" route. Notably, rats voluntarily ran along "shock" routes during forced-choice trials (i.e. when the "safe" route was blocked), allowing for an assessment of remapping on all routes after changes in the locations of the aversive stimuli. We show that introducing aversive stimuli to a subset of routes after first allowing the animals to run all routes in the complete absence of aversive stimuli induces remapping. We also observed remapping in subsequent sessions when the locations of "safe" and "shock" arms were switched but the overall aversiveness of the task environment was kept constant; this did not occur when the shock contingencies were left unchanged, demonstrating that place fields do not simply become unstable in an aversive context. These data suggest that place field remapping reflects changes in environmental/behavioral contingencies during decision making and not simply changes in the overall valence of the environment. This remapping was apparent in both areas CA1 and CA3, constraining hypotheses on the underlying afferent circuits which produce this reorganization of place cell coding in response to aversive experiences.

Disclosures: J. Ormond: None. J.P. Johansen: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.25/III39

Topic: H.01. Animal Cognition and Behavior

Support: China Scholarship Council

Bioscience PhD Student

Title: Role of dopamine in spatial acquisition and reversal learning

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Abstract: Background: Dopamine is a key neurotransmitter in the brain. Distinct dopamine systems play different roles, either in reward-motivated behavior or in motor control and other hormones release. Dopamine's effects on higher cognitive function have also been studied in monkeys and rodents. But the mechanisms are still unclear. Methods: Three months old C57BL6/J mice were trained to perform a flexibility-involved water maze task. At some key time points of learning, mice brains were dissected immediately and the certain dopamine content levels in ROIs were measured by punch tissue and HPLC method. Results: Significantly decreased path lengths and escape latency show that all mice can learn the spatial learning task. For the HPLC analysis results, dopamine and its metabolites levels in nucleus accumbens increased when the mice were at the beginning of reversal learning. Conclusion: The results suggested that when the goal position was switched, a mismatch between expectation and outcome occurred. The new goal position worked as a novel stimulus and lead to elevated dopamine content.

Disclosures: L. Zhang: None. A. Garthe: None. C. Winter: None. G. Kempermann: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.26/III40

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R21NS082956

Postdoctoral NRSA 5 F32 NS077840-03

Title: A subset of CA1 and subiculum neurons selectively encode rewarded locations

Authors: *J. L. GAUTHIER[1,2,3], D. W. TANK[1,2,3];
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Abstract: The hippocampus is known to be critical for the retention of spatial memories, but it remains unclear which features of hippocampal physiology encode memories of known locations, or how that information is transmitted to other brain areas. Previous studies have shown that when rewards are presented at fixed locations, firing fields in CA1 form at an increased density near the rewards. We set out to distinguish whether the "excess" firing fields encode place per se, or whether they are instead associated with the reward. Mice were trained to run on a virtual linear track with a reward at a fixed location, and simultaneous optical recordings were made from two major hippocampal output structures, CA1 and the subiculum. Consistent with previous studies, an increased density of fields was found near the rewarded location. When the reward was moved to a new location, some of the reward-associated fields also shifted to the new location, suggesting they encoded the reward itself rather than a specific region of space. These fields continued to be reward-associated even when the mouse was trained to run on a different linear track and most CA1 fields underwent global remapping. These observations reveal a previously undescribed feature of hippocampal remapping: a distinct neural population that shifts its firing fields to consistently predict a rewarded location, despite unrelated restructuring of simultaneously recorded place fields. Population activity was also tracked across several weeks as animals became familiar with the new reward location. A preliminary analysis suggested that development of reward-associated activity tracked behavioral learning, and reward-associated fields developed in CA1 earlier than subiculum. A further trial-by-trial analysis revealed that activity of reward-associated cells correlated with behavioral anticipation of reward, raising the possibility that these cells transmit memory of the rewarded location to other brain areas.

Disclosures: J.L. Gauthier: None. D.W. Tank: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.27/III41

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R37NS081242

Title: Micro-organization of grid cells in layer II of medial entorhinal cortex

Authors: *Y. GU[1], A. A. KINKHABWALA[2], D. W. TANK[1];
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Abstract: The medial entorhinal cortex (MEC) is important for spatial representation and episodic memory. Grid cells are abundant in the superficial layers (II and III) of MEC and exhibit firing fields on the vertices of a triangular lattice as rodents navigate in open arenas (Moser et al., 2014). Previous studies using tetrode recordings showed that grid cells form discrete functional modules. Grid cells within a module share similar scale, orientation, asymmetry and theta-frequency modulation. Anatomically, although modules with larger scale generally distribute more ventrally in MEC than modules with smaller scales, most modules show significant overlap (Stensola et al., 2012). Since the resolution of tetrode recordings prevents identification of the anatomical location of individual cells, it is unknown how cells in different modules are organized at microstructural level. In addition, layer II of MEC contains two excitatory cell types: pyramidal cells and stellate cells (Kitamura et al., 2014; Ray et al., 2014). The contribution of the two cell types to the grid cell population is still unclear. Here, we addressed these two questions by cellular resolution two-photon imaging of calcium dynamics in neurons in layer II of MEC while mice navigated on virtual linear tracks. The same population of grid cells was imaged in two behavior sessions (the two sessions could be on the same track or different tracks). Grid cells were then assigned to different modules based on their field spacings and widths, which were calculated from their activity patterns in the two sessions. Stellate and pyramidal neurons were simultaneously imaged and identified *in vivo* based on the diameters of their cell bodies. We found that co-modular grid cells generally clustered and separated from cells in other modules, although some degree of intermingling existed at the boundaries between modules. Most grid cells were stellate cells (~80%). More specifically, 43% of stellate cells were identified as grid cells, whereas only 17% of pyramidal cells were grid cells. Stellate cells showed significantly more speed-modulated activity than pyramidal cells. In summary, the anatomical clustering of cells within grid modules raises the possibility that co-modular cells form strong functional connectivity and receive common inputs during navigation. Our results

also support the idea that the stellate cell population encodes more position information than the pyramidal cell population.

Disclosures: Y. Gu: None. A.A. Kinkhabwala: None. D.W. Tank: None.

Poster

263. Cortical and Hippocampal Circuits: Place Cells

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 263.28/III42

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R37NS081242

NIH Grant 5K99NS093071

Simons Foundation SCGB

Title: Representation of an abstract non-spatial coordinate by firing fields in the hippocampal/entorhinal circuit

Authors: *D. ARONOV, R. NEVERS, D. W. TANK;
Princeton Univ., Princeton, NJ

Abstract: Neurons in the hippocampus and the medial entorhinal cortex (MEC) fire in discrete fields within a spatial environment. However, these areas are critical not only for spatial navigation, but for a variety of memory-guided behaviors. How does hippocampal/entorhinal activity implement the more general function of these brain regions? One possibility is that the activity of neurons like place and grid cells is not fundamentally a framework for representing location; rather, such activity patterns may generally serve to represent the values of behaviorally-relevant variables.
To test this idea, we performed tetrode recordings in CA1 and MEC of rats trained in an acoustic virtual reality apparatus to “navigate” along a non-spatial “linear track” defined by sound frequency. Animals deflected a joystick to increase the frequency of a pure tone and were required to release it in a target zone between two frequency values. The scaling factor between joystick deflection and the rate of change of sound frequency was varied across trials to uncouple sound frequency from elapsed time.
About 40% of the CA1 cells fired in discrete fields that, across the population, spanned all phases of the behavioral task – including individual “locations” within the frequency space, as well as periods before and after joystick deflection. Neurons active during the joystick deflection fired in the same ranges of frequencies across trials of different durations, indicating that they did

not simply represent elapsed time. Cells engaged by the acoustic task partially overlapped with place cells, identified by recording during random foraging in a separate spatial behavioral arena. MEC neurons, including grid, border, and head direction cells, also formed firing fields in the acoustic navigation task, indicating that a variety of known cell types in the hippocampal/entorhinal circuit may be repurposed between spatial and non-spatial tasks.
Our results show that an abstract, non-spatial axis may be represented by hippocampal and entorhinal activity in a fashion similar to the known representations of location. They suggest a model in which discrete firing fields are flexibly used by the hippocampal/entorhinal circuit to represent arbitrary abstract spaces, supporting not only spatial navigation, but cognition in general.

Disclosures: D. Aronov: None. R. Nevers: None. D.W. Tank: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.01/III43

Topic: I.06. Computation, Modeling, and Simulation

Title: Learning on multiple timescales with metaplasticity

Authors: *P. KHORSAND[1], S. FARASHAHI[2], A. SOLTANI[2];
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Abstract: Adaptive decision making not only requires learning from reward feedback but also necessitates adjusting this learning based on how often reward contingencies change (i.e. reward uncertainty) in a given environment. In the context of reinforcement learning, the latter is accomplished by adjusting the learning rate according to reward uncertainty in the environment. Generally, finding the proper learning rate requires optimization but neural mechanisms underlying computations necessary for finding the optimal learning rates are unknown. Alternatively, Bayesian models that incorporate uncertainty into learning can achieve optimal behavior, but they require knowledge of the environment. Recently, we have extended a cascade model of synaptic plasticity for memory (Fusi et al., 2005) to a reward-dependent domain, and showed that the ensuing model provides a plausible solution for choice during a probabilistic reversal learning (PRL) task. In this task, the subject chooses between two options with assigned reward probabilities, but these probabilities change between blocks of trials. Importantly, the difference in reward probabilities and the block length determine reward uncertainty in a given environment. Here, we examined alternative models of reward-dependent metaplasticity (RDMP) to further study how such models can achieve robust and near-optimal performance, in

the absence of any explicit optimization or knowledge of the environment. To this end, we considered three general models based on cascades of meta-states with monotonically decreasing transition probabilities. Using mean-field approaches and Monte Carlo simulations, we found a generalized cascade model of RDMP that provides a near-optimal and robust solution during the PRL task. In this model, synapses mostly occupy meta-states with transition probabilities that match the timescale of changes in a given environment (i.e. block length) and moreover, fractions of synapses in different meta-states are influenced by reward probabilities. Overall, our results show the ability of the cascade architecture to solve very different problems faced by the brain, such as preserving memories over long timescales and integrating reward under uncertainty.

Disclosures: P. Khorsand: None. S. Farashahi: None. A. Soltani: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.02/III44

Topic: I.06. Computation, Modeling, and Simulation

Title: Taming synaptic heterogeneity for adaptive learning

Authors: *M. MORADI SPITMAAN[1], P. KHORSAND[2], A. SOLTANI[2];
[1]Psychology and Brain Sci., [2]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Most of our knowledge of the world is acquired through learning from reward feedback. However, because reward contingencies can change unpredictably, this learning should be adjusted depending on the frequency of those changes (i.e. reward uncertainty) in a given environment. In the context of reinforcement learning (RL), this adjustment could happen via optimization to fine-tune the learning rate according to the reward uncertainty. Alternatively, considering the heterogeneity in reward-dependent plasticity (which underlies learning from reward feedback) throughout the brain, one could assume that adaptive learning could be achieved by combining the output of a population of synapses each with a different learning rate. In the absence of any additional mechanisms, however, signals generated by synapses with disparate learning rates might cancel each other out. Therefore, we hypothesized that specific neural mechanisms should be in place so that reward uncertainty in the environment could structure heterogeneity to make it beneficial for adaptive behavior. We have recently proposed that reward-dependent metaplasticity (RDMP) can enable synapses to adapt to reward statistics in a given environment. Therefore, here we aimed to test whether RDMP can provide a neural

substrate for structuring heterogeneity for adaptive choice. To do so, we used a heterogeneous population of metaplastic synapses with a wide range of transition probabilities and simulated a generalized probabilistic reversal learning task which allows us to control the level of uncertainty in the environment. Furthermore, we compared the results with those using a heterogeneous population of RL models. Specifically, we applied a combination of decoding (linear support vector machine, SVM) and encoding (mutual information, MI) methods to estimate the information content of these populations in different environments. Results based on the SVM classifier showed a superior performance and higher dimensionality for RDMP over RL populations. Moreover, we found a larger width of SVM weights for RDMP than RL, indicating that a larger number of synapses were used for discrimination in the former model. Critically, the increase in the width of SVM weights between RL and RDMP was predictive of the improvement in performance from RL to RDMP. The MI analysis revealed that information content of pairs of RDMP synapses overlaps less than that of RL synapses, further expanding upon the findings based on the SVM classifier. Overall, our results show that metaplasticity can provide a plausible mechanism for structuring synaptic heterogeneity in order to make it beneficial for adaptive learning.

Disclosures: M. Moradi Spitmaan: None. P. Khorsand: None. A. Soltani: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.03/III45

Topic: H.01. Animal Cognition and Behavior

Title: Chronic taurine administration from gestational day E15 to adulthood causes damage in the cerebral cortex hippocampus of male rats

Authors: *R. GÓMEZ-DÍAZ[1], G. GARCIA-ROBLES[2], C. TOMAS-SANCHEZ[2], V. BLANCO-ALVAREZ[2], D. MARTINEZ-FONG[4], J. EGUIBAR CUENCA[3], J. GONZALEZ-BARRIOS[5], D. LIMON-PEREZ[2], A. UGARTE[3], B. LEON-CHAVEZ[2];
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Abstract: Theory of aging is based on increased oxidative/nitrosative stress that occurs over time and can cause neurodegenerative diseases and cognitive impairment. Taurine has neuroprotective and antioxidant effect in the central nervous system. This study aimed to

evaluate the effect of taurine chronic administration from day E15 to 8 months of age on oxidative/nitrosative stress, learning process and memory. Pregnant Sprague-Dawley rats were separated into two groups: (1) control rats without treatment; (2) rats with taurine chronic administration (50 ppm in the drinking water). Four brain regions (cortex, brainstem, cerebellum and subcortical nuclei) were dissected out to measure lipid peroxidation by Gerard-Monnier and nitrites by Griess method at different ages: 15 day old, 1 and 8 months old. Reference spatial learning in Morris water maze was trained daily during 5 days and the long-term memory was evaluated 7 days after learning. In addition, novel objects recognition (NOR) was evaluated at 2 months old; habituation was made in the first day, familiarization in the second day; retention was evaluated 3 h and 24 h later. Our results showed an increase in lipid peroxidation and nitrite production dependent on age in the control group. Chronic administration of taurine caused damage only in the cerebral cortex-hippocampus in male rats, comprising of 300% increase in lipid peroxidation at 15 day old, 100% at 1 month old and 231% at 8 months old, as compared with the control group. Nitrite production was also increased by 70% at 15 days old, 81% at 1 month old and 137% at 8 months old, when compared with the control values. Interestingly, no significant changes were determined in female rats. Memory in treated infant rats was not different from the controls when evaluated by the Morris water maze, but NOR test performed at 2 months of age showed a decrease by 60% in the acquisition, by 53% in short-term retention and by 39% in long-term retention in male. We can conclude that the chronic administration of taurine from gestational day E15 caused damage of the cerebral cortex-hippocampus with consequent alterations of cognitive processes, such as learning and memory, in male rats, but not in female rats. These results show for the first time that exists a sexual dimorphism to the susceptibility to taurine supplementation in the cerebral cortex of the rat.

Disclosures: R. Gómez-Díaz: None. G. Garcia-Robles: None. C. Tomas-Sanchez: None. V. Blanco-Alvarez: None. D. Martinez-Fong: None. J. Eguibar Cuenca: None. J. Gonzalez-Barrios: None. D. Limon-Perez: None. A. Ugarte: None. B. Leon-Chavez: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.04/III46

Topic: H.01. Animal Cognition and Behavior

Title: Sexual dimorphism to zinc requirements of pubertal rats

Authors: *V. M. BLANCO ALVAREZ[1], C. TOMAS-SANCHEZ[2], J. GONZALEZ-BARRIOS[3], D. MARTINEZ-FONG[4], G. GARCIA-ROBLES[2], G. SOTO-RODRIGUEZ[4], E.

BRAMBILA[2], I. LIMON[2], S. RUIZ-PASTRANA[2], A. DIAZ RUIZ[2], B.-A. LEON-CHAVEZ[2]; [1]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [3]Inst. De Seguridad Y Servicios Sociales De Los Trabajadores Del Estado, Ciudad de Mexico, Mexico; [4]Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico Nacional, Ciudad de Mexico, Mexico

Abstract: Zinc is the second most abundant metal in the human body and is crucial for life. Zinc availability in neonatal and early postnatal period is required for adequate growth, normal birth weight, low morbidity, mental development and effective behavior. Zinc is highly regulated in the body, so its deficiency leads to multiple pathological conditions, whereas its excess can cause toxicity in the brain. Zinc is also known to exert a dual effect; cytotoxicity, increasing oxidative/nitrosative stress, lysosomal dysfunction, cell death and autophagy, or cytoprotection as a neuromodulator in excitatory synapses, stress response and neuronal functionality. Recent reports show that zinc subacute administration prevents the loss of long-term memory in male adult rats. However, the effect of zinc chronic administration on nitrosative stress and long-term memory is still unknown during the pubertal period of male and female rats. Male and female Sprague-Dawley rats (P30) were treated with different doses of $ZnCl_2$ (0, 0.1, 0.5 and 1 mg/Kg), every 24 h, during 14 days. Learning (P45) was evaluated by a daily training for 5 days in a Morris water maze. The long-term memory was evaluated on day 7 after learning (P57). The cerebral cortex and hippocampus were dissected out (P57) to evaluate levels of nitrites by Griess method and lipoperoxidation by Gerard-Monnier assay. Our results showed that repetitive zinc doses of 0.1 mg/kg improved learning and did not trigger nitrosative stress. Loss of memory and increase in nitrosative stress occurred in male rats treated with repetitive zinc doses of 0.5 mg/kg. Interestingly, female rats showed tolerability to all repetitive zinc doses; even the dose as high as 1 mg/Kg was unable to activate nitrosative stress although could improve long-term spatial memory. These results show for the first time that exists a sexual dimorphism to requirements of zinc in pubertal rats. The molecular and cell mechanisms remain to be elucidated.

Disclosures: V.M. Blanco Alvarez: None. C. Tomas-Sanchez: None. J. Gonzalez-Barrios: None. D. Martinez-Fong: None. G. Garcia-Robles: None. G. Soto-Rodriguez: None. E. Brambila: None. I. Limon: None. S. Ruiz-Pastrana: None. A. Diaz Ruiz: None. B. Leon-Chavez: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.05/III47

Topic: H.01. Animal Cognition and Behavior

Support: Conacyt 277701

Title: Coadministration of 5-HT7 receptor antagonist and agonist on forgetting

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Abstract: Evidence indicates that 5-HT_7 receptor antagonist have antiamnesic effects (Gasbarri et al, 2008), while its stimulation improves spatial memory and attention (Ruocco et al 2014). It is unclear the reasons the paradoxical effects; hence we are asking if these drugs have effects on forgetting, which has been little explored as therapeutic target for dysfunctional memory. Certainly forgetting and amnesia differ in neuroanatomical and pharmacological terms. In this work, SB-269970 (SB, 5-HT_7 receptor antagonist) & LP-211 (LP, 5-HT_7 agonist) are examine during short- (STM) & long-term memory (MLP), as well as forgetting protocol (Tellez et al, 2012) in an autoshaping learning task. Initial results show that SB (1 amd 10 mg/kg) and LP (0.5 and 1.0 mg/kg) have no effect on forgetting; however, LP211 (at 2.5 and 5 mg/kg) partially inhibits forgetting. Importantly, coadministration of SB 1 mg/kg plus LP 0.5 mg/kg and SB 10 mg/kg plus 2.5 mg/kg eliminated forgetting; indicating that 5-HT_7 receptor might be a useful target for altered memory.

Disclosures: R. Solís Guillén: None. A. Meneses: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.06/III48

Topic: H.01. Animal Cognition and Behavior

Title: Effect of environmental enrichment as adjunctive therapy to administration of EMD386088 5-HT_6 receptor agonist on memory formation & amnesia

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Abstract: Environmental enrichment (EE) may potentiate the promnesic effect of 5-HT_6 receptor drugs; hence, we are examining the combined effect of EE and 5-HT_6 receptor agonist EMD 386088 (EMD, 0.5 mg /kg, i.p. sub-effective dose) during authoshaping short- (STM1.5 h) and long-term memory (LTM 24 &48 h) or dizocilpine-induced (0.2 mg / kg, i.p) amnesia. EMD had no effect and dizocilpine reduces conditioned responses (CR%) in the STM and EE reverses

it. EE and EMD (sub-effective dose) combination significantly increases CR% and eliminated dizocilpine-effect. Conclusions: EE reversed memory impairment induced by a deficit in glutamatergic associative learning. Importantly, EE in combination with a sub-effective dose of EMD produce a synergistic effect on memory. These results support the therapeutic potential of environmental enrichment as adjunctive therapy and 5-HT_6 receptor on memory and its dysfunctions

Disclosures: F. Aparicio Nava: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.07/III49

Topic: H.01. Animal Cognition and Behavior

Title: Preclinical characterization of SUVN-I4035- a novel and selective muscarinic m1 positive allosteric modulator

Authors: *R. ABRAHAM[1], R. MEDAPATI[2], R. BADANGE[3], V. REBALLI[3], A. SHINDE[3], V. GOYAL[4], S. PANDEY[4], S. YATHAVAKILLA[2], V. GOURA[2], S. EDULA[5], R. SUBRAMANIAN[5], V. MEKALA[5], N. MUDDANA[6], V. UTHUKAM[6], S. DARIPELLI[6], B. DEVARAPALLI[6], R. EEDA[6], R. NIROGI[7];
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Abstract: Memory decline in the elderly due to dementia and Alzheimer's disease (AD) are widely known causes. Currently, there are only symptomatic treatments available for these conditions. Though muscarinic acetylcholine subtype 1 (M_1) receptor agonists showed cognitive improvement, their clinical utility was limited by insufficient selectivity leading to cholinergic side effects. The conservative nature of the orthosteric site makes it difficult to identify selective M_1 receptor agonists. As allosteric receptor modulation leads to high selectivity and minimal side effects, the positive allosteric modulators (PAM) of the M_1 receptor were designed and explored. SUVN-I4035 is one of our lead M_1 positive allosteric modulator. It was evaluated for its binding potential at the orthosteric site of muscarinic receptors and its ability to potentiate the effect of acetylcholine in the reporter gene assay. The pharmacokinetics and brain penetration of SUVN-I4035 was evaluated in rodents. The efficacy of SUVN-I4035 was evaluated in animal models of cognition. Cardiovascular safety was assessed using the patch clamp technique. General safety was assessed in rodents and mutagenicity was assessed using the bacterial reverse mutation assay. The physiochemical properties were studied as well. The results indicate that SUVN-

I4035 did not bind to the orthosteric site up to the tested concentration of 10μM. It significantly potentiated the effect of acetylcholine. It also showed good selectivity against M_2 - M_5 receptors. It has adequate water solubility, and was found to be orally bioavailable (76 ± 7%) in rats with adequate brain penetration and free fractions (fu, 17%). SUVN-I4035 reversed memory deficit in object recognition task and T-maze task. SUVN-I4035 potentiated the pro-cognitive effect of donepezil and promotes non-amyloidogenic APP processing in rats. SUVN-I4035 also enhanced the cerebral blood flow in rats. In rats SUVN-I4035 did not increase salivation induced by acetylcholine esterase inhibitors. SUVN-I4035 was found to be safe when tested in hERG patch clamp assay and in early stage animal toxicity studies. SUVN-I4035 is non-mutagenic in the bacterial reverse mutation assay. Hence, SUVN-I4035 was characterized as a M_1-PAM demonstrating robust efficacy in animal models of cognition.

Disclosures: **R. Abraham:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Medapati:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Badange:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Reballi:** A. Employment/Salary (full or part-time): Suven Life Sciences. **A. Shinde:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Goyal:** A. Employment/Salary (full or part-time): Suven Life Sciences. **S. Pandey:** A. Employment/Salary (full or part-time): Suven Life Sciences. **S. Yathavakilla:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Goura:** A. Employment/Salary (full or part-time): Suven Life Sciences. **S. Edula:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Subramanian:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Mekala:** A. Employment/Salary (full or part-time): Suven Life Sciences. **N. Muddana:** A. Employment/Salary (full or part-time): Suven Life Sciences. **V. Uthukam:** A. Employment/Salary (full or part-time): Suven Life Sciences. **S. Daripelli:** A. Employment/Salary (full or part-time): Suven Life Sciences. **B. Devarapalli:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Eeda:** A. Employment/Salary (full or part-time): Suven Life Sciences. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.08/III50

Topic: H.01. Animal Cognition and Behavior

Title: Esculetin attenuates sleep deprivation-induced cognitive dysfunction by modulating neuroinflammation and TrkB-BDNF signaling pathway in mice

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Abstract: Converging line of evidences suggest that insomnia is a predisposing factor for the cognitive dysfunction and about 45% of Alzheimer's disease (AD) patients show sleep disturbances. Neuroinflammatory and TrkB-Brain derived neurotrophic factor (BDNF) signaling pathway involved in the pathogenesis of AD and associated cognitive impairments. BDNF plays an important role in the synaptic plasticity and memory function. Total sleep deprivation is an inflammatory process which further leads to neurobehavioural changes and alteration of TrkB-BDNF signaling process. Esculetin (ESC), a coumarin derived potent antioxidant, also possessing anti-inflammatory and neuroprotective activity. The present study investigated the effect of ESC pre-treatment on SD-induced cognitive impairment and neuroinflammattory as well as TrkB-BDNF signaling pathway in mice. Mice (N=8) were pre-treated with ESC (50 mg/kg BW, p.o) for 14 days and subjected for 5 days of sleep deprivation protocol. After SD protocol animals were subjected to Morris water maze (MWM) and novel object recognition test to evaluate cognition and memory. Hippocampal cytokines, TrkB, BDNF, MDA and GSH level were analyzed. Results showed that 5 days sleep deprivation (SD) significantly increased the escape latency in MWM ($P<0.001$) and decreased recognition index ($P<0.001$) in mice which was significantly ($P<0.01$) attenuated by 14 days pre-treatment of ESC. Moreover, pro-inflammatory cytokines (IL-1β, IL-6 & TNF-α), MDA & GSH level increased significantly ($P<0.001$) after SD in mice which was reversed by the ESC pre-treatment. Furthermore, hippocampal TrkB & BDNF level were decreased after SD protocol which was attenuated by chronic pre-treatment of ESC. In conclusion, results suggested that ESC provided protective effect against SD-induced cognitive dysfunction by modulating neuroinflammation and TrkB-BDNF signaling pathway. Thus, ESC may be potential therapeutic agent for the treatment of insomnia and cognitive impairments associated with Alzheimer's disease.
Keywords: Esculetin; Neuroinflammation; Sleep deprivation; BDNF; Cognitive dysfunction

Disclosures: K. Sulakhiya: None. C.C. Barua: None. V. Paramanik: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.09/III51

Topic: H.01. Animal Cognition and Behavior

Title: Positive effect of biflorin: memory amelioration in cholinergic blockade-induced memory deficit in mice

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Abstract: To examine the effect of biflorin, a component of *Syzygium aromaticum,* on memory deficit, we introduced a scopolamine-induced cognitive deficit mouse model. A single administration of biflorin increased latency time in the passive avoidance task, ameliorated alternation behavior in the Y-maze, and increased exploration time in the Morris water maze task, indicating the improvement of cognitive behaviors against cholinergic dysfunction. The biflorin-induced reverse of latency in the scopolamine-treated group was attenuated by MK-801, an NMDA receptor antagonist. Biflorin also enhanced cognitive function in a naïve mouse model. To understand the mechanism of biflorin for memory amelioration, we performed Western blot. Biflorin increased the activation of protein kinase C-ζ and its downstream signaling molecules in the hippocampus. These results suggest that biflorin ameliorates drug-induced memory impairment by modulation of protein kinase C-ζ signaling in mice, implying that biflorin could function as a possible therapeutic agent for the treatment of cognitive problems.
Keywords: Biflorin; N-methyl D-aspartate receptor; Cognition; Protein kinase C-ζ

Disclosures: Y. Gwon: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.10/III52

Topic: H.01. Animal Cognition and Behavior

Support: ES022831

EPA 83543701

Title: Sex differences in ventral hippocampal muscarinic acetylcholine antagonist scopolamine effects on spatial memory in rats

Authors: *B. J. HALL[1], Y. ABREU-VILLAÇA[2], M. CAULEY[2], A. KIANY[2], S. JUNIAD[2], H. WHITE[2], E. D. LEVIN[2];
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Abstract: Sex differences in cognitive function have been previously documented in experimental animal studies. One key difference that has been shown is that male rats tend to perform more accurately than females during spatial working memory tasks. The neural sources for these sex-related differences in cognition are not well understood. In the current study, we examined the roles of muscarinic and nicotinic acetylcholine receptors in the hippocampus and frontal cortex on spatial working memory in rats performing the radial-arm maze task. It was hypothesized that cholinergic projections to these areas played roles in sex differences in spatial memory performance. Young adult male and female Sprague-Dawley rats were trained for 18 sessions on a 16-arm radial maze. Twelve arms of the maze were baited at the beginning of each session to assess working memory, and the remaining four arms were left unbaited to assess reference memory. After training, the rats were implanted with bilateral cannulae for local infusions of muscarinic (scopolamine, 0, 1, 3 and 10 µg/side) and nicotinic (mecamylamine, 0, 1, 3 and 10 µg/side) antagonists into the ventral hippocampus or medial frontal cortex. Scopolamine infusions into the ventral hippocampus showed a differential effect on male and female rats. Females showed significant spatial working memory impairments in the task, whereas males did not show impairments under this dose range of scopolamine. In the frontal cortex, the highest test dose of scopolamine caused spatial working memory impairment regardless of sex. No differential effects of mecamylamine were observed in the task when infused in the hippocampus or frontal cortex of male and female rats. These studies show that muscarinic acetylcholine receptors in the ventral hippocampus are differentially vulnerable to cause spatial memory impairments in females. Ventral hippocampal muscarinic acetylcholine receptors may play a key role in male-female differences in spatial memory.

Disclosures: B.J. Hall: None. Y. Abreu-Villaça: None. M. Cauley: None. A. Kiany: None. S. Juniad: None. H. White: None. E.D. Levin: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.11/JJJ1

Topic: H.01. Animal Cognition and Behavior

Support: FAPESP

CAPES

PIBIC/Universidade Presbiteriana Mackenzie

Title: Prolonged exposure to varenicline did not altered the memory of male rats

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Abstract: Varenicline is a synthetic chemical used for the treatment of smoking. Its mechanism of action is by binding to nicotinic cholinergic receptors as a partial agonist of the receptors α4β2 and α3β4 and a full agonist of the receptor α7. Varenicline has some effects on memory and cognition. Pre-clinical trials show that this substance can improve attention and labor memory in patients with only three days with absence of nicotine. This mechanism is still not clear, but the hypothesis is that these effects are related with the pre-synaptic neurotransmitters released, including gamma-aminobutyric acid (GABA). Considering that there is a tendency to expand the clinical use of varenicline and that are few studies related to behavioral, cognitive and motor effects, become necessary more studies on their pharmacological effects. The aim of this study is to evaluate the behavior of male rats exposed to different doses of varenicline for 28 days in the open field, Barnes maze and passive avoidance. Forty male rats received different doses of varenicline or tap water by gavage for 28 days: control group (A) - 1mL/kg of water; group B - 0.03 mg/kg of varenicline (therapeutic human dose); group C - 0.1 mg/kg of varenicline; group D - 0.3 mg/kg of varenicline. n=10 male rats/group. Varenicline was obtained from the commercial product Champix® - Pfizer, diluted on tap water. It was evaluated the general activity of these animals in the open field and the memory using the Barnes maze and passive avoidance. The parameters evaluated on the open field were: frequency of locomotion, rearing, grooming and defecation, and time of grooming and immobility. The parameters evaluated on the Barnes maze were the number of errors and the latency to find the escape box. The parameter evaluated on the passive avoidance was the latency to enter on the black box. The analysis of the behavior of male rats in the open field showed alterations on only two parameters, when compared to the control group: the rats from group B had an increase of the locomotion frequency on the 14º day of treatment ($p<0,01$) and a decreased of the time of immobility on the same day of treatment ($p<0,05$). Regard the Barnes maze and the passive avoidance, the statistical analysis did not showed significant differences between the groups. The results indicate that although there were some alterations on the open field parameters, the exposure to different doses of varenicline did not altered the general activity and the exploratory behavior of male rats. This exposure also did not alter the space and the short term memory of male rats.

Disclosures: **J.Z. Magalhães:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **D.P. Figueiredo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); PIBIC. **G.R. Abreu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); PIBIC. **E.L. Ricci:** A. Employment/Salary (full or part-time): Mackenzie Presbiterian University. **M.S.B. Udo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **H.S. Spinosa:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CAPES.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.12/JJJ2

Topic: H.01. Animal Cognition and Behavior

Support: CIHR 10677

CIHR 227397

Title: The t-type calcium channel blocker, z944, reduces conditioned fear in genetic absence epilepsy rats from strasbourg and the non-epileptic control.

Authors: *W. N. MARKS[1], N. K. ZABDER[1], Q. GREBA[1], S. M. CAIN[2], T. P. SNUTCH[2], J. G. HOWLAND[1];
[1]Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: T-type calcium channels exhibit unique biophysical properties implicated in the generation of low-threshold spikes that can lead not only to the oscillatory behavior in the brain observed during sleep, but also the burst-firing observed during pathological events such as absence seizures. Although the electrophysiological properties of T-type calcium channels have been studied extensively, less is known about the contribution of T-type calcium channels to cognition and behaviour. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are a well-described rodent model of childhood absence epilepsy that display a gain-of-function missense mutation in the *Cacna1h* gene encoding the Cav3.2 T-type calcium channel. GAERS have previously been demonstrated to have heightened learning and delayed extinction of Pavlovian fear conditioning. Our objective was to examine the effects of the highly potent and selective pan-T-type calcium channel blocker, Z944, on conditioned fear behaviour in GAERS and the Non-Epileptic Control (NEC) strain. Z944 (10 mg/kg; ip) was administered 15 min prior to either the conditioning or recall (24 hrs later) of delay-conditioned fear. Extinction of conditioned fear was examined 48 hrs after conditioning. Z944 treatment prior to conditioning enhanced freezing during conditioning, but reduced freezing to tone cues during recall in both strains. In GAERS, Z944 treatment prior to conditioning reduced freezing during initial exposure to context cues during recall and reduced freezing to both tone and context cues during extinction. Z944 treatment prior to recall produced similar behaviour to vehicle treated animals during recall of tone cues, but increased freezing during initial exposure to context cues during recall in both strains. Z944 treatment prior to recall had no effect on extinction. Regardless of treatment, GAERS showed increased freezing during conditioning, recall, and extinction of conditioned fear relative to NEC animals. Overall, Z944 treatment prior to conditioning normalized fear behaviour in GAERS whereas treatment in NEC animals impaired recall of

conditioned fear. The effects of Z944 on Wistar rats is currently in progress. These results demonstrate that T-type calcium channels contribute to the neural systems that mediate the learning and memory of conditioned fear. Continued research into the therapeutic potential of T-type calcium channel regulation may be particularly fruitful for the treatment of disorders characterized by enhanced memory of negative experiences.

Disclosures: W.N. Marks: None. N.K. Zabder: None. Q. Greba: None. S.M. Cain: None. T.P. Snutch: None. J.G. Howland: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.13/JJJ3

Topic: H.01. Animal Cognition and Behavior

Support: NIH RO1 NS050465

NIH RO1 DK104363

Title: Impact of short-term fructose ingestion on brain plasticity and function

Authors: *A. JIMENEZ MALDONADO[1], Z. YING[1], H. BYUN[1], F. GOMEZ-PINILLA[1,2,3]; [1]Dept. of Integrative Biol. and Physiol., [2]Dept. of Neurosurg., UCLA, Los Angeles, CA; [3]Brain Injury Res. Ctr., UCLA, Los angeles, CA

Abstract: The monosaccharide fructose is abundant in the Western diet and it is becoming a major cause of metabolic syndrome (MetS), diabetes, and obesity. Even more recently, clinical evidence has started to accumulate pointing to the pervasive effects of fructose for a range of neurological and psychiatric disorders. Previous studies have found that long-term high fructose ingestion associated with MetS induces lower cognitive performance and protracted brain plasticity in rodents (Agrawal et al, J. Physiol., 2012). The aim of this study is to evaluate the effect of short-term fructose ingestion on the brain before establishment of the MetS condition. Male Sprague-Dawley rats were supplemented with fructose in water (8% w/v) or pure water (control) for one (1W) or three weeks (3W), under standard chow feeding. Results of the glucose tolerance (GTT) and insulin tolerance (ITT) tests showed no disruption in glucose homeostasis or insulin tolerance, respectively. There was a reduction in the hippocampal dry weight in the 1W, which was accompanied by a reduction in the neuronal marker NeuN and Myelin Protein Basic (MBP). The same 1W time point showed an increase in the fructose transporter Glut5 in the hippocampal region. Levels of the peroxisome proliferator activated-receptor gamma,

coactivator 1 alpha (PGC-1α) and Cytochrome c oxidase subunit II (COX2) were lower for 1W group, suggesting a reduction in mitochondrial function in the hippocampus. There also was a trend to reduce the spatial memory in the1W group. The liver weight and the PGC-1α levels were higher in the 3W group. The data suggest that short-term fructose ingestion affects the brain directly before the metabolic syndrome condition is established. The results of these studies are particularly significant on the light of recent studies showing the pervasive effects of fructose on many genes associated with important neurological and psychiatric disorders (Meng et al., eBiomedicine, 2016)

Disclosures: A. Jimenez Maldonado: None. Z. Ying: None. H. Byun: None. F. Gomez-Pinilla: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.14/JJJ4

Topic: B.07. Synaptic Transmission

Support: HKU 761812M

Title: Antidepressant reactivates the plasticity of vestibular-dependent navigation in the adult

Authors: *Q. JIANG, D. MA, D. SHUM, Y. CHAN;
The Univ. of Hong Kong, Hong Kong, China

Abstract: Antidepressant is known to reactivate the critical period for ocular dominance in adult rats. In the vestibular system, we have previously revealed an early critical period during which neuronal activities in the vestibular nucleus (VN) shaped adult navigation behavior. It is known that one key factor that determines the critical period is the ratio of excitation to inhibition (E/I ratio), which declines from birth to adulthood. We therefore hypothesize that antidepressant can restore the plasticity of navigation behavior by switching E/I ratio of the adult VN to a neonatal state. In the present study, we treated juvenile rats with antidepressant for one week, and found that perturbation of neurotransmission in the VN even after the postnatal critical period could lead to deficits in spatial navigation at adult stage. To determine whether the observed restoration of navigation plasticity is accompanied by an increase in E/I ratio within the VN after antidepressant treatment, we used *in vitro* whole-cell patch-clamp technique to measure excitatory postsynaptic current (EPSC) and inhibitory postsynaptic current (IPSC) of VN neurons. We found that the E/I ratio was increased to a level comparable to that observed within the postnatal critical period. Notably, a decrease in the frequency of mIPSC but not mEPSC was

observed in the VN of juvenile rats treated with antidepressant for one week, suggesting that reduction of inhibitory neurotransmission is the major cause of the increase in E/I ratio. In addition, immunohistochemical results from these juvenile rats revealed a significant increase in the number of proliferating GABAergic VN interneurons following antidepressant treatment. This suggests that antidepressant accelerates neurogenesis of interneurons within the VN. Taken together, our findings suggest that antidepressant restores navigation plasticity in adulthood by increasing E/I ratio in the VN. The mechanism by which antidepressant-induced neurogenesis of interneurons influences the excitation-inhibition balance of the VN circuitry awaits further investigations.

Disclosures: Q. Jiang: None. D. Ma: None. D. Shum: None. Y. Chan: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.15/JJJ5

Topic: H.01. Animal Cognition and Behavior

Support: National Institute of Psychobiology in Israel

Title: In quest for finding the key regulators of the molecular mechanisms of long-term memory

Authors: *I. MICHAELEVSKI[1], N. BOROVOK[1], E. NESHER[2], A. SHEININ[1], M. REICHENSTEIN[1], A. PINHASOV[2];
[1]Tel Aviv Univ., Tel Aviv, Israel; [2]Ariel Univ., Ariel, Israel

Abstract: Contemporary understanding of memory formation relies on the concept of long-term synaptic plasticity, for which protein synthesis is a vital requirement. Activity-dependent changes occurring in synapses upon memory acquisition initiate multiple signal transductions enhancing protein turnover, facilitating *de novo* synthesis of plasticity related proteins, crucial factors of memory engram. The best understanding of the synaptic plasticity comes from the studies of hippocampus, playing the central role in memory formation. Deterioration of hippocampal activity is associated with multiple forms of cognitive impairments and neurodegeneration. Despite extensive studies to elucidate molecular mechanisms of memory traces formation and the role of their impact on cognitive impairment, identity of the involved molecular factors is still elusive. Using the advantage of radial arm maze long-term spatial memory paradigm, we investigated protein turnover in mouse hippocampus during the learning process. Utilizing quantitative proteomics, we identified 1592 proteins, exhibiting a complex picture of expression changes during spatial memory formation. Variable linear decomposition

enriched factors responsible for memory-related protein levels' variance at: the initial phase (167 proteins), during the steep learning improvement (150 proteins) and final phase (123 proteins). Gene ontology and signaling pathways analysis revealed correlation between memory improvement and learning phase-curbed expression profiles of proteins belonging to specific functional categories. We found differential enrichment of: a) neurotrophic factors signaling pathways, proteins regulating synaptic transmission, microfilament assembly during the first day of learning curve; b) transcription and translation machinery, protein trafficking, metabolic activity and Wnt pathway during the steep phase of learning; c) cytoskeleton organization proteins at the final step. Network analysis of protein expression profiles revealed candidate key regulators of memory formation. Further the role of two selected candidates was confirmed in synaptic plasticity. Summarizing, identification of the key regulators opens new horizons in understanding of memory formation molecular mechanisms, as well as in therapeutic targeting of specific protein-protein interaction networks to shut down pathogenetic pathways of memory impairment.

Disclosures: I. Michaelevski: None. N. Borovok: None. E. Nesher: None. A. Sheinin: None. M. Reichenstein: None. A. Pinhasov: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.16/JJJ6

Topic: H.01. Animal Cognition and Behavior

Support: NWO Grant ALW2PJ12095

ERCadvanced grant #339490

Title: Attentional gating of learning in monkey visual cortex

Authors: *U. H. SCHNABEL, X. CHEN, P. R. ROELFSEMA;
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Abstract: Theories have proposed that attentional feedback is important for the gating of synaptic plasticity so that attended items are learnt and non-attended items are not (Roelfsema et al., 2010). To examine the influence of attention on learning, we used a redundant cue paradigm (Wagner et al. 1968) in which one item is attended during learning and another item is not, although both items are equally informative about the required behavioral response. A macaque monkey learned to choose between two responses while he saw an attended shape and an equally

informative, but unattended, second shape. We then used a morphing strategy in which familiar shapes were gradually morphed into new shapes, until the new shapes bore no resemblance to the originals. Shapes were presented on top of a moving texture pattern, allowing us to examine neuronal activity in area V1 that was elicited by texture elements, throughout the learning process. The monkey consistently selected one of the to-be-attended shapes (the "special" shape) and selected the behavioral response based on the presence or absence of this shape. The special shape elicited stronger multi-unit activity in area V1 than any other shape, in accordance with the hypothesis that it was attended. As the special shape morphed into a novel shape, the morphed shape continued to elicit an increased neural response, whereas the other shapes elicited weaker responses. After this morphing procedure, the monkey learned to map the attended shape onto the correct response but he failed to correctly map the unattended shape, although it had been consistently paired with the attended shape and was equally informative about the required response. Our results demonstrate that attention gates the learning of new shapes in the visual cortex.

Roelfsema, P. R., van Ooyen, A. & Watanabe, T. Perceptual learning rules based on reinforcers and attention. *Trends Cogn.Sci.* 14**,** 64-71 (2010).
Wagner AR, Logan FA, Haberlandt K, Price T. Stimulus selection in animal discrimination learning. *Journal of Experimental Psychology*. 76:171-180. (1968)

Disclosures: U.H. Schnabel: None. X. Chen: None. P.R. Roelfsema: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.17/JJJ7

Topic: H.01. Animal Cognition and Behavior

Support: NIH-NINDS Grant, NS48156.

Title: Conditioned inhibition and extinction of conditioned excitation in Hermissenda: similarity of cellular and intracellular signaling mechanisms.

Authors: *J. FARLEY;
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Abstract: Exposure of *Hermissenda (H.c.)* to explicitly-unpaired (EU) presentations of light (L) and rotation (R), separated by many mins, results in persistent enhancement of phototaxis and complementary changes in photoresponses and excitability of Type B and A photoreceptors (conditioned inhibition learning and memory, CI). EU-produced decreases in photoresponses and

excitability of B cells (and increased photoresponses and spiking of A cells) reflect intrinsic somatic ionic conductance changes. Voltage clamp analysis of B cells revealed increases in I_A and $I_{K\text{-}Ca}$ K^+ currents for days after CI training. Elimination of $I_{K\text{-}Ca}$ abolished CI-produced difference in photoresponses; 4-AP block of I_A abolished differences in spiking. Computational models of B cells incorporating the EU-changes in I_A and $I_{K\text{-}Ca}$ reproduced the physiological results in photoresponses and spiking. Protein phosphatase 1 (PP1) and the 12-lipoxygenase (12-LOX) metabolite, 12(S)-HPETE, are implicated in EU-increases in B cell K^+ currents and decreases in spiking (Walker et al. 2010). Using *in vitro* conditioning protocols and pharmacology, we've found these same signaling pathways to underlie both CI learning as well as extinction of conditioned-excitation (CE) associative memory (i.e., effects of L-R pairings; Cavallo et al. 2012). After intact *H.c.* received either L-R pairings (Paired), or control training (Random or Untrained preparations), B cells were recorded from during *in vitro* extinction stimulation (two series of 15 consecutive light-steps, LSs). When extinction was administered shortly after Paired training, B cells showed robust declines in spiking while control cells did not. Selective inhibitors of PP1 (Calyculin A), PP2B (cyclosporine A), and 12(S)-HPETE (baicalein, a 12-LOX inhibitor) indicated that all three molecules contributed to the spiking decreases produced by extinction training. Conversely, injection of catalytically-active PP1 (caPP1) or PP2B (caPP2B) into Untrained B cells partially mimicked the spike frequency declines observed in Paired cells, as did bath-applied arachadonic acid (AA), and occluded additional LS-produced reductions in spiking in Paired cells. Similar results have now been observed for the decreased spiking and hyperpolarization produced by *in vitro* CI stimulation.

Disclosures: J. Farley: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.18/JJJ8

Topic: H.01. Animal Cognition and Behavior

Support: Neuralstem, Inc

Title: NSI-189, a neurogenic compound enhances short-term and long-term potentiation in C57BL/6 mice and reverses LTP impairment in a mouse model of Angelman Syndrome.

Authors: *Y. LIU[1], M. P. HEFFERAN[2], K. JOHE[2], X. BI[1], M. BAUDRY[1];
[1]Western Univ. of Hlth. Sci., Pomona, CA; [2]Neuralstem, Inc., Germantown, MD

Abstract: Previous studies have examined the tolerability and efficacy of NSI-189, a benzylpiperizine-aminopyridine neurogenic compound for treating major depressive disorder (MDD). NSI-189 has shown significant potential as a treatment for MDD, with concurrent improvement of cognition in a small double-blind, placebo-controlled study. NSI-189 is a novel chemical entity, which enhances neurogenesis from human hippocampus-derived neural stem cells in vitro, stimulates neurogenesis in normal young adult mouse hippocampus, and increases hippocampal volume of normal young adult mice. However, the precise mechanism of NSI-189 action is still unknown. To directly test the effect of NSI-189 on hippocampal synaptic plasticity, we incubated hippocampal slices from wild-type adult mice with various concentrations of NSI-189 (100 nM to 10 μM) for various periods of times, from 1 h to 3.5 h, before applying theta burst stimulation (TBS) to elicit long-term potentiation (LTP) in field CA1. NSI-189 had no effect on baseline synaptic transmission or on paired-pulse facilitation. On the other hand, NSI-189 produced a time- and concentration-dependent enhancement of LTP magnitude. It also produced a time- and concentration-dependent enhancement of short-term potentiation (STP), and the concentration dependency for STP was shifted to the left as compared to LTP. NSI-189 had no effect on burst responses, indicating that it does not affect NMDA receptor properties. In addition, NSI-189 restored normal LTP in hippocampal slices from a mouse model of Angelman Syndrome, $Ube3a^{m-/p+}$ mice, which exhibit a deficit in LTP and learning and memory. Further studies will delineate the mechanisms underlying the enhancement of both STP and LTP, as well as the possibility of using NSI-189 for reversing learning and memory impairment in AS mice.
Keywords: NSI-189, hippocampus, LTP, STP, AS mice. Supported by Neuralstem, Inc.

Disclosures: **Y. Liu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Neuralstem, Inc. **M.P. Hefferan:** A. Employment/Salary (full or part-time): Neuralstem, Inc. **K. Johe:** A. Employment/Salary (full or part-time): Neuralstem, Inc. **X. Bi:** None. **M. Baudry:** None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.19/JJJ9

Topic: H.01. Animal Cognition and Behavior

Support: CNPq

FAPEMIG

CAPES

Title: Social isolation decreases social memory persistence and induces a depressive-like phenotype in Swiss mice via distinct mechanism.

Authors: *L. D. GUARNIERI[1], A. R. PEREIRA-CAIXETA[1], N. S. S. AQUINO[2], R. E. SZAWKA[2], M. F. D. MORAES[1], G. S. PEREIRA[1];
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Abstract: Memories tasks involving perceived connection between response and reinforce are affected by mood fluctuations. However, there are few data regarding the influence of depressive states in non-conditioned memories. Social recognition memory task relies in the olfactory ability of mice to recognize co-specifics and is based on their natural interest for novelty. We showed previously that seven days of social isolation (SI) limits to few hours the persistence of social recognition memory (SRM), while enriched environment (EE) rescued that deficit. Here we tested the hypothesis that SI is affecting animal's mood, which in turn impairs SRM persistence. Adult male Swiss mice were maintained in groups of 5 (Standard Grouped, SG) or alone (Isolated, IS) for seven days (7d). After, mice were submitted to different behavioral tests to measure mood alterations. We also verify whether EE would block SI effect on mood behaviors, as did with social memory. SI mice presented a depressive-like state measured in tail suspension, forced swimming (FST) and sucrose preference test. Furthermore, food and water intake, as well as weight gain were decreased along the 7d of SI. EE prevented all the behavioral and metabolic deleterious effects of SI. We also verified if animals under SI would present morphological and biochemical features related with depression. SI mice showed a smaller olfactory bulb (OB) as well as lower levels of norepinefrine, dopamine and serotonin in the dorsal hippocampus and OB, with no changes in BDNF expression. Afterward, we treated animals with Fluoxetin (30 mg/kg) or Desipramine (30 mg/kg) i.p., 30 minutes before FST (acute) or once a day during 7d of social isolation (chronic). Both drugs, similar to EE, were effective in recover depressive-behavior caused by SI. Finally, we predicted that if SI animal's mood is limiting SRM duration, antidepressant drugs should also recover the SRM deficit. Only chronic fluoxetin was effective. Our group already showed that EE rescued SRM of SI mice via a neurogenesis-dependent mechanism. Therefore, our next step is to evaluate neurogenesis levels in the SI mice treated with antidepressants. Altogether, our results show that 7d of SI prone Swiss mice to mood disorders; at the same time it impairs SRM. As only chronic treatments with fluoxetin or EE were able to rescue SRM deficit, it is possible that the social memory deficit observed in SI mice are at least partially unrelated to mood disorder.

Disclosures: L.D. Guarnieri: None. A.R. Pereira-Caixeta: None. N.S.S. Aquino: None. R.E. Szawka: None. M.F.D. Moraes: None. G.S. Pereira: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.20/JJJ10

Topic: H.01. Animal Cognition and Behavior

Support: SATT AxLR maturation project

Title: Topographical memory impairments in the Hamlet Test

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Abstract: An alert sign for aged people suspected of developing Alzheimer's type dementia is spatio-temporal disorientation, particularly in a familiar environment. No behavioral procedure in animal studies addresses currently this alert sign of the pathology. We developed a learning task allowing mice to familiarize with a complex environment mimicking a small village, by decidedly adopting an anthropomorphic approach, named The Hamlet Test®. The apparatus comprises a central agora and 20 streets expanding from it in a 5-branch shape leading to functionalized houses. The agora served as a gathering area and as a start box for training and test trials. The functionalized houses contained food (physiological function encoded: Eat), water (Drink), an assortment of toys (Play), a running whell (Run) or a stranger/unknown mouse (Interact). Animals were trained in groups by spending 4 h/day during 2 weeks in the Hamlet Test and their ability to orientate can be tested after short or long time periods, in normal or pathological conditions. Topographical memory was tested in a probe trial after a 20 h water deprivation. Animals were placed in the agora and their latency and number of errors spent to reach the Drink house measured. Water-deprived animals showed significantly decreased latencies and numbers of errors or increased entries into the Drink house, showing that topographical memory is activated in the test. Animals administered with scopolamine 5 mg/kg SC, but not 0.5 mg/kg SC, before the probe test showed a significant impairment of topographical memory. In order to address the topographical disorientation in Alzheimer's disease, animals were trained during 2 weeks and then intracerebroventricularly injected with oligomeric Amyloid-$ß_{25-35}$ peptide (9 nmol) or a scrambled control peptide 3 days after training. When retested in the Hamlet Test after 7 days, $Aß_{25-35}$-treated animals showed a significant memory impairment to find the Drink house. Analyses are in progress with senescence-accelerated mice and several transgenic mouse lines, but the Hamlet Test appears as a novel test that can address specifically topographical disorientation, an alert sign in Alzheimer's disease.

Disclosures: T. Maurice: None. D. Gilabert: None. L. Crouzier: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.21/JJJ11

Topic: H.01. Animal Cognition and Behavior

Title: Adolescent cannabinoid exposure and novelty-seeking: Effects on spatial memory and hippocampal neurogenesis in female Long-Evans rats

Authors: K. L. STANSAK[1], A. L. RIGDON[1], F. E. GRIFFEY[1], *P. A. JACKSON[2], D. M. HAYES[1];
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Abstract: In a national survey on drug use, about 7.4% of persons aged 12-17 years reported using marijuana in the past month. Additionally, use is on the rise despite knowledge that chronic adolescent marijuana exposure leads to a host of neurobiological and behavioral alterations in adulthood. Thus, the current study examined the effects of chronic adolescent cannabinoid exposure in rats on behavior and hippocampal neurogenesis in adulthood. Neurogenesis is the 4-stage process of proliferation, differentiation, maturation, and survival in the brain through which new cells are born and ultimately integrated into existing circuitry. One of the regions in which this occurs is the hippocampus. Abboussi et al. (2013) found that chronic adolescent exposure to cannabinoids significantly decreased hippocampal neurogenesis and impaired spatial memory in adulthood. However, Jiang et al. (2005) showed that chronic cannabinoid exposure in adult rats actually promoted cell proliferation. Other research has suggested that adolescent cannabinoid exposure has no effect on neurogenesis in females (Lee et al., 2013) resulting in discrepancies amongst cannabinoid studies. Due to the variability in results for cannabinoid studies, novelty-seeking phenotype was investigated as a potential mediating variable. Novelty seeking defines an animal's tendency to explore novel environments; high responders (HR) appear less anxious and have greater activity while low responders (LR) have more anxiety and less activity. Dellu et al. (1996) has also shown that HR animals have a prolonged increase in corticosterone levels after exposure to novelty, and chronic high levels of stress have also been shown to suppress neurogenesis in rats (Brummelte & Galea, 2010). In the current study, adolescent female Long-Evans rats were injected with CP 55,940 (0.35 mg/kg) or saline for 14 days during adolescence and then tested on a spatial memory task during adulthood. All rats were screened for phenotype before the injection period and were classified as HR or LR using a median split for the time spent with a novel object. Results from an object location task (recognition of spatial placement in an open-field) revealed that LR drug-exposed animals performed better than control animals. At the end of behavioral data collection, rats were perfused and the brains collected for analysis of cell proliferation. Sections of brain revealing ki67-positive cells were counted in hippocampal tissue using an Olympus BX-43 microscope.

Neurogenesis analyses are ongoing but the behavior suggests that the LR drug animals may show increased levels of cell proliferation in the hippocampus.

Disclosures: K.L. Stansak: None. A.L. Rigdon: None. F.E. Griffey: None. P.A. Jackson: None. D.M. Hayes: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.22/JJJ12

Topic: H.01. Animal Cognition and Behavior

Title: Genetic deletion of neurexin-1 in rats impairs learning and memory.

Authors: *L. VER DONCK[1], S. EMBRECHTS[2], M. MAHIEU[2], H. VAN CRAENENDONCK[2], H. DUYTSCHAEVER[2], P. DE HAES[2], R. WILLEMS[2]; [1]Janssen Res. & Development, A Div. of Jans, Beerse, Belgium; [2]Neurosci., Janssen Res. & Development, A Div. of Janssen Pharmaceutica NV, Beerse, Belgium

Abstract: Neurexins (NRXNs) are presynaptic proteins that help to connect neurons at the synapse by interaction with postsynaptic neuroligins. They mediate signaling across the synapse, and influence the properties of neural networks by synapse specificity. In humans, alterations in genes encoding NRXNs are implicated in cognitive disabilities and neurological disorders such as autism. The objective was to evaluate behavioral and cognitive performance of rats with homozygous knock-out of NRXN1. Adult male and female NRXN1-KO rats and wild type (WT) littermates (n=7-10/group; Horizon, Cambridge MA) for evaluation in a battery of behavioral tests involving motor activity, sensory processing and learning and memory. Prepulse inhibition and acoustic startle response were not different between genotypes, but a gender effect was observed in PPI and a trend effect in startle (2w-ANOVA). Spontaneous locomotor activity was not different between male and female WT and male NRXN1-KO, but was significantly higher in female KO ($p<0.001$), indicating a hyperactive phenotype. Working memory was assessed in a 2-arm V-shaped maze as the preference to spend more time in exploring the novel arm vs the alternate familiar arm that was explored just before. Female, but not male NRXN1-KO showed trend for reduced preference for the novel arm compared to WT. Spatial learning and memory was evaluated in the Morris water maze test. Rats did 3 training sessions of maximally 60 sec each per day for 4 days to learn the location of an escape platform hidden under the water surface. Latency to find the platform decreased over the training days in WT to 18±3 sec (female) and 13±2 sec (male) on day 4 vs 53±3 (female) and 59±1 (male) sec in KO, indicating that NRXN1-KO did not learn the location of the platform ($p<0.001$). During a probe trial 3 days

after the last training day, the platform was removed to evaluate memory consolidation. WT rats showed a strong preference for the quadrant where the platform was located during the training sessions, while NRXN1-KO rats did not. Further testing in other paradigms is in progress. It is concluded that deletion of NRXN1 results in a hyperactive phenotype and impaired working memory in female rats, while spatial learning was impaired in both sexes.

Disclosures: L. Ver Donck: None. S. Embrechts: None. M. Mahieu: None. H. Van Craenendonck: None. H. Duytschaever: None. P. De Haes: None. R. Willems: None.

Poster

264. Learning and Memory

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 264.23/JJJ13

Topic: H.01. Animal Cognition and Behavior

Support: Supported by a grant from the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University in Korea (2014050477) funded by the Ministry of Science, ICT and future Planning

Title: Learning and memory of sweet taste in rats with bilateral transection of chorda tympani nerves

Authors: *J. JAHNG, S. CHUNG, J.-H. LEE;
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Abstract: Some patients in dental clinics appeal not only altered taste perception but also negative emotion after lingual nerve damages. We have previously reported that bilateral transections of the lingual and chorda tympani nerves (Nx) lead to the development of depression-like behaviors in rats. Furthermore, the retrieval of hedonic taste memory was blunted in Nx rats. In this study, we have examined if the disrupted sweet taste memory is mainly due to the loss of chorda tympani nerves rather than the loss of lingual nerves. In the experiment 1, male SD rats underwent 7 days of drinking training with 3-bottles (one sucrose bottle with visual cue, two water bottles without visual cues) and then received bilateral transections of chorda tympani nerves (CTx) or sham surgery. CTx and sham rats were subjected to drinking test with three water bottles (one with visual cue, two without visual cues) after a week of post-operational recovery. Water consumption from cued bottle was significantly increased in sham rats, but not in CTx, compared to non-cued bottles. In the experiment 2, drinking training was performed for 9 consecutive days after a week of recovery from CTx or sham surgery, and then the drinking test was done on the following day. Sham rats consistently drank more sucrose than water during

the whole training period. However, sucrose consumption of CTx rats did not differ from water during the first two days of training and thereafter it was increased for the rest of period. On the test day, water consumption from the cued bottle was increased in sham rats compared to both the non-cued bottles, but in CTx only to one of them. Results suggest that taste sensory loss from the anterior 2/3 of tongue may affect both the acquisition and retention of hedonic taste memory.

Disclosures: J. Jahng: None. S. Chung: None. J. Lee: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.01/JJJ14

Topic: H.02. Human Cognition and Behavior

Support: Fondecyt 1150289

Title: Stimulus viewing duration prompts divergent learning trajectories in a same-different visual discrimination task

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Abstract: ”Sameness” and “difference” judgments (SDJ) are basic cognitive processes that underlie perceptual discrimination. SDJ have been characterized with simple visual stimuli in same-different tasks (SDT) and asymmetries in performance for same and different stimuli have been observed. We studied whether the time to view the stimuli modified the performance asymmetries in a SDT with a complex stimuli category. Performance was obtained in six daily sessions of SDT with checker-like stimuli (30 exemplars). Two groups of participants, performed a SDT (360 trials/session, 4 blocks, 90 trials each) stimuli presented sequentially with short (SSE, 100 ms, n = 16) or long (LSE) 1000 ms, n=17) stimulus exposures and obtained d’, criterion, percentage of correct responses (PCR) for same and different responses. Statistical significance was evaluated by ANOVA and paired comparisons with Bonferroni correction. SDT practice increased d’ across sessions with SSE (2.2$\pm$.8 to 4.1$\pm$.5) and LSE (3.1$\pm$.6 to 5.4$\pm$.8). In contrast, LSE produced a greater increase in the criterion (1.6$\pm$.3-2.5$\pm$.5) compared to SSE (1.3$\pm$.4-1.8$\pm$.4; $p<0.01$). These results are consistent with a similar practice dependent improvement on discrimination with both stimuli durations, although there is a smaller performance across sessions and a smaller reduction in the probability of false alarms for same stimuli with SSE. PCRs increased with same (.72$\pm$.10 to .91$\pm$.06) and different (.85$\pm$.09 to

.97±.02) stimuli pairs with LSEs, and there was no interaction between session and duration. Similarly, PCRs increased with same (.63±.14 to .78±.09) and different (.72±.14 to .94±.03) pairs with SSEs, but there was an interaction between session and pair (p=0.05), suggesting a different effect of sessions in same and different performance. Pairwise comparisons showed similar performance in sessions 1 and 2, but greater difference detection in sessions 3 to 6. Overall, performance with same pairs was consistently smaller than with different pairs, with both stimulus exposures. Moreover, there was a similar improvement in performance with same pairs across sessions, but an overall greater performance with LSEs. On the contrary, there was a greater performance improvement with different pairs with SSEs. Taken together, these results indicate that SDJs with a complex stimulus category are determined by the time subjects have to view the stimuli. Specifically, with LSEs both, sameness and differences, detections are easier compared to SSEs, and with SSEs it is easier to detect the differences compared to sameness. Finally, irrespective of stimulus exposure, there was an advantage of difference over sameness detection.

Disclosures: M. Aylwin: None. M.S.M. Quiñones: None. D.M. Gomez: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.02/JJJ15

Topic: H.02. Human Cognition and Behavior

Support: DFG

SFB 874

International Graduate School of Neuroscience, Ruhr-Universität Bochum

Title: Feature-unspecific repetitive sensory stimulation induces visual perceptual learning

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Abstract: Past studies showed that repetitive sensory stimulation could induce improvements or impairments of perceptual abilities in touch (e.g. Pleger et al., 2003; Ragert et al., 2008) and in vision (Beste et al., 2011) which depended on stimulation frequency. Here, we investigated whether intermittent high-frequency stimulation is able to improve orientation discrimination

(OD) ability in human observers. In a pre-post design, subjects performed a fine-OD task (Schoups et al., 1995), where a central circular grating pattern's orientation had to be judged to either deviate clockwise or counter-clockwise from an oblique target orientation. Between sessions, subjects randomly received one of four possible treatments: stimulation with a phase-alternating sinusoidal grating (SG) of either the same or the orthogonal target orientation used during the OD task, stimulation with an orientation-alternating bar or sham stimulation with only a static fixation cross present on the screen. In each case, stimulation lasted for about 42 minutes, was presented centrally and flickered intermittently at 20 Hz (on/off for 5 sec/5 sec). There was a significant between-subject effect of treatment on performance (repeated-measures ANOVA: $p = .004$), with Bonferroni-corrected post-hoc analysis revealing a significant lowering of OD thresholds post-stimulation for same-orientation SG (threshold-change: -6.4%, paired-samples t-test: $p = .001$), orthogonal-orientation SG (-3.4%, $p = .002$), orientation-alternating bar (-3.8%, $p = .0002$) but not for sham stimulation (-2.6%, $p = .22$). These results show that fine-OD ability can be improved by peripheral high-frequency stimulation. Unlike previous studies in the visual modality, which investigated the influence of stimulation on attentional processes (Beste et al., 2011), here we could show improved discrimination ability for a low-level visual feature. Furthermore, this effect does not require the stimulation features to be congruent to those of the behavioral task (orientation, shape), which suggests a different degree of specificity than that of classical perceptual learning, which is usually highly specific to stimulus features.

Disclosures: A. Marzoll: None. H.R. Dinse: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.03/JJJ16

Topic: H.02. Human Cognition and Behavior

Title: Refocusing mental fixation and visual one-shot learning

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Abstract: The fixating and defixating (or focusing and defocusing) of salient features are one of the important functions in our visual systems. The same is also true of the cognitive counterpart, i.e., mental fixation. Mental fixation refers to an inability to complete certain types of cognitive operations (e.g., remembering, problem solving, and creative idea thinking) as the result of interference from inappropriate knowledge and experience (Smith, 2003). In the context of

creative problem solving, the mental fixation is thought to be a nuisance, which prevents us from getting the right solution. For instance, in the verbal domain, inappropriate associate prior cues generate additional mental fixation (Storm, 2011), resulting in distracted and impaired remote associate task performance (Smith and Blankenship, 1991). In the visual domain, the ease of perceiving alternative interpretations of multistable ambiguous figure (e.g., Duck-Rabbit figure), indicating the degree of freedom from mental fixation, predicts creative divergent thinking task performance (Wiseman, et al. 2011). As a special case of the ambiguous figure, two-tone black and white hidden figures such as "the Cow" (Dallenbach, 1951) and "the Dalmatian" (Gregory, 1970) are difficult to interpret properly for naïve viewers. The only perception in the state of mental fixation or impasse, is meaningless blob pattern (Ishizu, 2013) or "false alarm", i.e., spurious perception (Ludmer, et al. 2011). Once holistic perception is acquired appropriately for these hidden figures, however, it seems almost impossible to return to the previous ignorant state (mental fixation): one-shot learning (Giovannelli, et al. 2010; Ishikawa and Mogi, 2011) sometimes accompanying positive emotional reaction, or "aha!" experience (Gick and Lockhart 1995; Topolinski and Reber 2010). Although the mentally fixated state before creative insight may be an inevitable waypoint, the role of mental fixation in the visual one-shot learning and "aha!" experience is not yet well known. Here we employ the dynamic hidden figure presentation method using morphing paradigm (Ishikawa and Mogi, 2011) to investigate variation, irresistibility ("attractiveness"), and duration of mental fixation in the hidden figure perception and its association with total reaction time and positive surprise measured by subjective "aha!" rating. We discuss and shed light on the sunny side of the mental fixation preceding one-shot learning: that is, it may not be merely a certain kind of cognitive failure viewed in the short-term standpoint, but "desirable undesirable state" (Jarman, 2016) leading to long-term cognitive benefit.

Disclosures: T. Ishikawa: None. M. Toshima: None. K. Mogi: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.04/JJJ17

Topic: H.02. Human Cognition and Behavior

Title: Anodal but not cathodal stimulation (tDCS) over the human right occipital cortex modulates visual cognition

Authors: *D. RIVOLTA[1], M. BARBIERI[1], M. NEGRINI[2], M. A. NITSCHE[3];
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[3]Leibniz Res. Ctr. for Working Envrn. and Human Factors (IfADo), Dortmund, Germany

Abstract: Non-invasive techniques that affect neural plasticity, such as Transcranial direct current stimulation (tDCS), have been successfully adopted to modulate human cognition. It is typically believed that "depolarization" (which induces performance improvement) is induced by an anodal stimulation (a-tDCS), whereas "hyperpolarization" (which induces performance decrease) is induced by a cathodal stimulation (c-tDCS). A cognitive domain that has, so far, received little attention is "face processing". This is surprising given that effective neuromodulation protocols may be applied in clinical conditions characterised by face processing aberrations (e.g., autism, schizophrenia and prosopagnosia). Thus, we here examined whether the administration of anodal-tDCS (a-tDCS) and cathodal-tDCS (c-tDCS) over the right occipital cortex modulates the performance on four tasks tapping memory and perception for faces and non-face stimuli. In this single-blind, sham-controlled study, tDCS has been delivered at 1.5 mA via a pair of surface sponge electrodes (25 cm^2) soaked in a saline solution (0.9% NaCl). Subjects received tDCS stimulation for 20 minutes before task execution. The sites of stimulation were identified using the Electroencephalography 10-20 system, with the active electrode (i.e., anodal/cathodal) over PO8 (i.e., right occipito-temporal cortex) and the reference electrode over FP1 (left prefrontal cortex). A total of sixty healthy adult volunteers (i.e., twenty in each of the three conditions) took part in the experiment. Results showed that, compared to the sham condition, offline a-tDCS causally enhanced the perception and memory performance of both faces and objects. There was no effect of c-tDCS on behaviour. Overall, our findings showed a polarity-specific effect of tDCS on visual cognition. In particular, we showed that a-tDCS causally enhances cognitive skills, whereas c-tDCS has no effect. Our results add relevant information about the breadth of cognitive processes that can be modulated by tDCS, and about the design of effective neuromodulation protocols, which have implications for advancing theories in cognitive neuroscience and clinical applications.

Disclosures: D. Rivolta: None. M. Barbieri: None. M. Negrini: None. M.A. Nitsche: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.05/JJJ18

Topic: H.02. Human Cognition and Behavior

Support: NSERC

Title: Orienting to probable stimuli affects early perception: Modulating the 'C1' visual evoked potential in an orientation estimation task

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Abstract: Probable stimuli are detected faster and more accurately. What is the source of this perceptual benefit? Electrophysiological studies of stimulus probability find modulation of the P3, a relatively late component thought to reflect fronto-parietal activity and suggesting that probability effects are top-down and attentional. However, there is also evidence for effects earlier in visual processing. For example, single-unit recordings in macaques have found that orientation training changes the widths and preferences of V1 neurons. Behaviorally, we find precision differences that are strongest for cardinal orientations and an interaction with obliquity, effects thought to be V1 mediated. We have therefore hypothesized that the probability-driven changes in perceptual processing are mediated early, possibly at the level of V1, with P3 changes reflecting secondary, follow-on effects.
To test this hypothesis we recorded EEG while participants performed a modified orientation estimation task in which probable orientations were location-contingent. While we did find probability effects on the magnitude of 'P3' potentials, we also demonstrated robust earlier modulations. Probability modulations at parieto-occipital electrodes were detectable within the first 100 ms. The polarity of the potentials at this time depended on whether the stimuli were presented in the upper or lower visual fields suggesting that they were an early, 'C1' visual response.
At the level of the individual participant the size of the probability-based C1 modulation correlated with the size of the probability-based precision difference measured behaviorally. C1 magnitude also correlated with the probability-based P3 modulation. On a trial-by-trial basis, the magnitude of the C1 amplitude predicted the magnitude of the later P3 amplitude. However the magnitude of the P3 potential did not predict the subsequent, next trial, C1 amplitude.
The results of this study support our hypothesis that probability effects are at least partially mediated by changes local to V1. This suggests that, like orientation training, probability 'learning' is the consequence of acquired local changes in neuronal tuning that are driven by stimulus frequency and that do not require expectation or top-down 'attentional' influences.

Disclosures: S. Jabar: None. A. Filipowicz: None. B. Anderson: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.06/JJJ19

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant SFB779/A04

Title: FMRI evidence for an intrinsic positive prediction error signal in the putamen for memory guided visual search

Authors: *S. POLLMANN, S. SOMMER;
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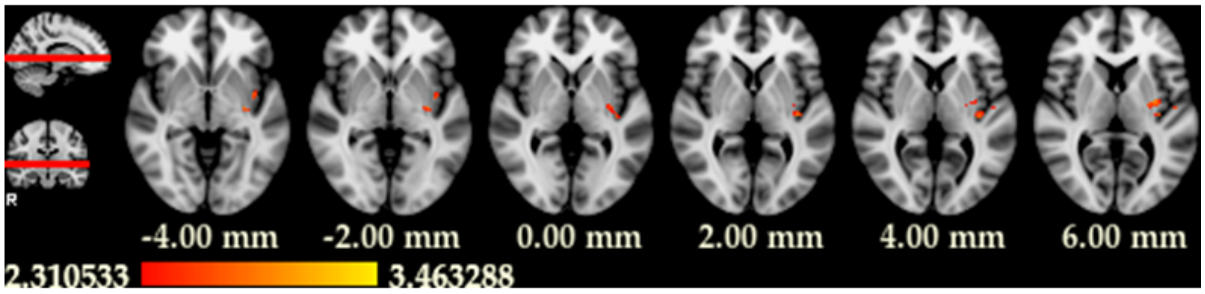
Abstract: We investigated fMRI responses to visual search targets appearing at locations that were predicted by the search context. Based on previous work in visual category learning (Daniel and Pollmann, *NeuroImage*, 59, 3457-3467, 2012) we expected an intrinsic reward prediction error signal in the putamen whenever the target appeared at a location that was predicted with some degree of uncertainty.
We ran an event-related fMRI-experiment (Siemens Trio 3T, 8-channel head coil, T2*-weighted EPI, TE = 30ms, TR = 2000ms, slice thickness = 3mm, slice gap thickness = 0.3 mm, FOV = 192mm, matrix size = 64 x 64, flip angle = 80°, 34 AC-PC oriented slices) with 18 participants (age: mean=25.7, sd=3.93; 8 female). Participants searched for a T among L-shaped distractors. During learning, 16 displays were repeated across 16 blocks, along with 8 novel displays with randomly arranged - non-predictive - distractor configurations. In the subsequent fMRI session containing 16 blocks, part of the repeated displays remained the same as during training. In another part, the target occured in the learnt or a novel location in 50% of repetitions, respectively. In this way, part of the repeated contexts predicted the target location with probability p=1, respectively p=0.5.
Comparing target appearance at locations predicted with probability p=0.5 to either locations predicted with p=1 or unpredicted locations, increased activation was observed in left posterior putamen and adjacent left posterior insula. Thus, our hypothesis of an intrinsic prediction error-like signal was confirmed. This extends the observation of intrinsic prediction error-like signals, driven by intrinsic rather than extrinsic reward, to memory-driven visual search.



The figure shows the overlap of activation obtained in the following contrasts: (1) target occurence at the expected location with p=0.5 - target occurence with p=1 respectively (2) target occurence with p=0.5 - target occurence at an unpredicted location.

Disclosures: S. Pollmann: None. S. Sommer: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.07/JJJ20

Topic: H.02. Human Cognition and Behavior

Title: Timing of the lateralized readiness potential is consistent with analyses of behavioral reaction times: Application to perceptual learning

Authors: *M. J. WENGER[1], S. E. RHOTEN[2];
[1]Ctr. for Applied Social Res., [2]Psychology, The Univ. of Oklahoma, Norman, OK

Abstract: The present effort tests the hypothesis that improvements in perceptual performance as a function of practice with multi-element stimuli may lead to the subjective organization of the stimulus information into a meaningful perceptual object. This has been referred to as ``unitization," “holism,” or the learning of a Gestalt. The goal of this study was to determine whether timings on a feature of electroencephalography (EEG)---specifically, the lateralized readiness potential (LRP)---could provide neural evidence consistent with the behavioral evidence. Specifically, we investigated the extent to which analysis of timings on this EEG feature would be consistent with analysis of behavioral reaction times (RTs). The LRP is a negative-going waveform, measured in central electrodes contralateral to the motor response that it precedes. Our concern was with the start time for the LRP, here operationalized as the earliest time at which the LRP became reliably less than 0. Four undergraduate students (three females, ages 19-21 years) were paid to participate in 12-15 sessions of practice. All participants were all right-handed and reported having normal to corrected-normal vision. Stimuli consisted of either 0, 1, or 2 contrast-defined target pattern features at varying contrast levels. Stimuli were constructed at levels of contrast (Michaelson ratio) running from 0.1% to 60.0%, in steps of 0.1%. Analyses were performed on RTs and start times for LRPs for correct responses. Examination of the RTs and the start times for the LRPs revealed a modest but reliable correlation. Analysis of the RTs supported the conclusions that observers began practice by processing the stimulus features in a serial exhaustive manner and that, after practice, observers processed the stimulus features in a parallel self-terminating manner. Critically, when the start time for the LRPs were analyzed in the same way, the same conclusions were supported. These results suggest that timings on brain dynamics have the potential to support the timings on behavioral responses as a source of converging evidence.

Disclosures: M.J. Wenger: None. S.E. Rhoten: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.08/JJJ21

Topic: H.02. Human Cognition and Behavior

Support: NARSAD Young Investigator Award to WKS

NIH Grant K01MH096175-01

Title: The neural bases of interoceptive recall.

Authors: D. C. DEVILLE, K. BURROWS, K. L. KERR, J. A. AVERY, C. MULLINS, J. BODURKA, M. PAULUS, *W. K. SIMMONS;
Laureate Inst. For Brain Res., Tulsa, OK

Abstract: Recent theories have proposed that the brain's interoceptive network continuously monitors the state of the body, associating external stimuli with their interoceptive consequences, thereby allowing later recall of these associations to guide behavior. While previous studies have identified the neural circuitry involved in interoceptive stimulation, it is unknown whether these brain regions are involved in the recall of interoceptive sensations. We thus examined the neural basis of interoceptive recall using a paired associates learning task. Fifteen right-handed healthy adults (9 female; mean age = 26 years) completed the Interoceptive Encoding and Recall (IER) task. During the IER task, subjects were intermittently exposed to an inspiratory breathing load (loads ranging from 10 to 50 cmH2O/L/sec), an unconditioned aversive interoceptive stimulus, while viewing one of 3 abstract geometric symbols. As an exteroceptive control, subjects viewed 3 different geometric symbols while an aversive auditory scream played at varying volumes through headphones. Geometric symbols were counterbalanced across conditions and subjects. Later, while undergoing fMRI scanning, subjects performed an incidental (i.e., unexpected) recall task, in which they were presented with the geometric symbols they saw during the encoding phase and were instructed to focus on the intensity of the stimulus (i.e., load or scream) associated with each shape. Recall of breathing loads, relative to recall of auditory screams, resulted in activation within a broad network of regions previously implicated in visceral interoception, including the dorsal mid-insula, anterior cingulate, and thalamic nuclei known to relay afferent visceral interoceptive signals from the body. Thus, simply recalling stimuli associated with a previous interoceptive challenge was sufficient to activate the brain's interoceptive network; at the time of the fMRI scan, subjects were no longer experiencing actual interoceptive stimulation. By providing a better understanding of how the brain constructs and retrieves interoceptive associations, these findings may eventually help to shed light on the interoceptive dysfunctions reported in multiple psychiatric disorders and inform the development of novel psychiatric treatments.

Disclosures: D.C. DeVille: None. K. Burrows: None. K.L. Kerr: None. J.A. Avery: None. C. Mullins: None. J. Bodurka: None. M. Paulus: None. W.K. Simmons: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.09/JJJ22

Topic: H.02. Human Cognition and Behavior

Title: You look like an expert: eye movement dynamics and performance in cognitive tasks

Authors: *J. PERRICONE[1], B. HELFER[2], D. FORD[3], T. QUATIERI[2];
[1]Bioengineering Systems and Technologies, [2]MIT Lincoln Lab., Lexington, MA; [3]NCSU, Raleigh, NC

Abstract: Previous studies have shown that experts and novices have distinctive eye movement dynamics; however, the nature of these characteristics and how they are learned remain poorly understood. This study provides evidence that there exist favorable eye movement dynamics which may be learned and applied to novel tasks resulting in superior performance.
Ten healthy subjects (4 female, 6 male) performed two novel visual tasks (120 trials/task) that test pattern recognition and spatial reasoning. In the first task, subjects were presented three cards with objects of varying shape, number, color, and shading. They were then asked to determine if the cards adhered to a pattern defined by the rules of the game SET. In the second task, subjects were presented a shape and a clockwise rotated version, where the rotation angle was drawn randomly from 45°, 90°, 180°, or 270°. They were then asked to identify if the presented angle of rotation was set to 90°. In both tasks, subjects gave time-constrained responses via keypad and received immediate feedback after each trial (correct/incorrect). Saccades, blinks, fixations, position of gaze, and pupil diameter were measured using eye-tracking glasses (SMI, Boston, MA). Both accuracy and mean response time were strongly correlated for subjects between the two tasks ($R = 0.8$, $R = 0.95$). Features derived from fixations and saccades proved to have strong predictive power of subjects' accuracies for both tasks. In particular, the average number of fixations per trial showed a strong negative correlation ($R = -0.6$) with accuracy, while the average duration of fixations showed a strong positive correlation ($R = 0.6$). Furthermore, the standard deviation of number of fixations across trials produced even stronger negative correlations with accuracy ($R = -0.7$). Together these results suggest that top-performing subjects tend to produce fewer fixations of longer duration in each trial, efficiently extracting task-relevant information, as has been shown in other eye-tracking studies. Additionally, subjects who perform well show less variability in the number of

fixations/saccades from one trial to the next.The presented results suggest that eye dynamics can be used as an indicator of relative expertise. Future work will build upon these findings to identify if these features can be used to predict proficiency and learning in untested visual tasks. [1] This work is sponsored by the Assistant Secretary of Defense for Research & Engineering under Air Force contract #FA8721-05-C-0002. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the United States Government.

Disclosures: J. Perricone: None. B. Helfer: None. D. Ford: None. T. Quatieri: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.10/JJJ23

Topic: H.02. Human Cognition and Behavior

Title: Human EEG exhibits task related changes in fractal scaling and dimensionality, congruent with a soft-assembled system

Authors: *T. L. MCKINNEY[1], T. J. WILTSHIRE[2], M. J. EULER[2], J. E. BUTNER[2]; [1]Psychology, Univ. of Utah, Salt Lake City, UT; [2]Psychology, Universioty of Utah, Salt Lake City, UT

Abstract: Soft-assembled dynamical systems are comprised of components that are temporarily formed and have flexible dynamics where the system self-organizes to functionally, and often efficiently, meet the demands of a task or situation. In prior work, the primary evidence that a system is softly-assembled has taken the form of fractal scaling relations, though we argue this case can be strengthened by changes in fractal scaling, as well as dimensionality. We conducted a priming experiment in which we recorded electroencephalography (EEG) for 14 participants as they made an arbitrary semantic decision about visually depicted objects that were each seen six times. Event related potentials were calculated for each of the six stimulus presentations and we used a multi-level modeling approach to compare changes in fractal scaling and the correlation dimension from pre to post stimulus and as a function of familiarity. Overall, we found that the dimensionality decreased from pre-to-post stimulus and the fractal scaling exponent increased from pre-to-post stimulus. The correlation dimension was also sensitive to the repetition of stimuli, although increased as a function of familiarity. The change in dimensionality suggests that following the stimulus, cortical activity overall is more coordinated, given that it required a lower dimensional structure to respond to the stimuli. The increase in fractal scaling following stimulus onset interacted with the effect of familiarity, which was associated with a decrease in

fractal scaling. This suggests the task induced transition to a non-stationary state with more long range temporal correlations was mitigated with increasing familiarity. Taken together, these task-related changes in both the fractal scaling exponents and the correlation dimension provide a more nuanced account of human EEG reflecting a softly-assembled dynamical system.

Disclosures: T.L. McKinney: None. T.J. Wiltshire: None. M.J. Euler: None. J.E. Butner: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.11/JJJ24

Topic: H.02. Human Cognition and Behavior

Support: NSF DGE-1068871

Army Research Office W911NF-15-1-0390-002

Title: Sensorimotor orienting in immersive virtual reality: Psychophysics of skill learning.

Authors: *H. M. RAO[1], R. KHANNA[7], D. J. ZIELINSKI[2], Y. LU[7], N. D. POTTER[3,4], R. KOPPER[5], M. A. SOMMER[1,8,6], L. G. APPELBAUM[7];
[1]Biomed. Enginnering, [2]Duke Immersive Virtual Envrn., [3]Athletic Dept., [4]Dept. of Physical Therapy, [5]Dept. of Mechanical Engin. and material Sci., [6]Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC; [7]Dept. of Psychiatry and Behavioral Sci., [8]Dept. of Neurobio., Duke Univ. Sch. of Med., Durham, NC

Abstract: There is a tight interplay between perception and action. Interacting with a dynamic world requires rapid integration of information from the environment that leads to the generation of movement. A prime example of perception-action coordination is the performance in a marksmanship paradigm, and our goal was to study the sensorimotor processes driving the acquisition of skill in this visually-guided task. Moving beyond conventional laboratory experimentation, we implemented a novel marksmanship simulation task, modeled after Olympic Trap Shooting standards in the Duke immersive Virtual Environment. During the task, a subject is surrounded on all sides by an interactive, dynamic, 3-dimensional virtual reality. We examined the movement dynamics and end-point performance during marksmanship training in twenty naïve human subjects. The task was to point and “shoot” a ray at a flying target that simulated the physics of an arcing clay pigeon. The population showed consistent improvements in shot accuracy during a training session that consisted of 7 repeated blocks of 50 trials. Through precise tracking of hand movement patterns that were split into ballistic and refinement phases

based on velocity, we observed systematic changes in dynamics that correlated with the improvement of performance. In particular, while reaction times and shot response times did not change over the course of practice, individuals exhibited longer and slower movements during the ballistic phase. At the end of the ballistic phase, the angular error between the ray direction and target direction decreased with training, indicating a more precise and directed initial phase of movement. The changes in rotational movements of the head mimicked those observed with the hand in that peak velocities decreased through training. The total angular rotation of the head decreased, as well. In order to evaluate the relative contribution of task parameters, head and hand movement dynamics, and trial history on the shot accuracy, we used regularized regression analysis. In addition to task parameters such as trial number and target trajectory that were consistently informative, the inclusion of movement related parameters significantly improved the model fit. On a trial-by-trial basis, movement reaction times, peak ballistic velocities, velocities at the time the shots were taken, and shot times were highly predictive of success. The results provide novel insight into the behavioral changes that accompany full-body skill learning, demonstrating the potential for studying the interplay of human perception and action in a naturalistic, yet tightly quantifiable, virtual reality environment.

Disclosures: H.M. Rao: None. R. Khanna: None. D.J. Zielinski: None. Y. Lu: None. N.D. Potter: None. R. Kopper: None. M.A. Sommer: None. L.G. Appelbaum: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.12/JJJ25

Topic: H.02. Human Cognition and Behavior

Support: Army Research Office W911NF-15-1-039

NSF DGE-1068871

Title: Sensorimotor orienting in immersive virtual reality: EEG correlates of skill learning

Authors: *L. G. APPELBAUM[1,2], J. CLEMENTS[3], Y. LU[2], H. M. RAO[4], R. KHANNA[2], D. J. ZIELINSKI[5], K. VITTETOE[2], N. D. POTTER[6], R. KOPPER[5], M. A. SOMMER[4]; [2]Psychiatry and Behavioral Sci., [3]Electrical and Computer Engin., [4]Biomed. Engin., [5]Duke Immersive Virtual Envrn., [6]Athletic Dept., [1]Duke Univ., Durham, NC

Abstract: Sensorimotor learning refers to improvements that occur through practice in the performance of sensory-guided motor behaviors. Psychophysiological studies, in which

performance is measured through quantitative biometric analysis, have revealed regularities at the neural, biomechanical, and behavioral level suggesting organizational principles for learning. Despite this growing body of research, there is still a very incomplete picture of the full cascade of component neuro-behavioral processes that underlie complex visual-motor coordination and a growing demand to utilize comprehensive technologies to implement this knowledge in applied setting. In order to gain a more complete picture of the processes and mechanisms that underlie skill learning and to improve scientific translation to real-world applications, we developed a novel marksmanship simulation performed in an immersive virtual reality environment (see accompanying abstract by Rao et al.). During this training simulation, modeled after Olympic Trap Shooting standards, eleven participants performed 7 blocks of 50 trials each, with the goal of shooting (using a pistol grip) a flying target that simulated the physics of an arcing clay pigeon. During this task, 16-channel electroencephalography (EEG) was recorded while end-point precision and head/hand movement dynamics were monitored with 3D tracking. From these data we observe systematic changes in movement dynamics that underlie improved shot accuracy over the training protocol. In particular, while reaction times and shot response times did not change over the course of practice, individuals exhibited longer, slower and more precise ballistic hand movements, leading to less need for refinement and better shooting accuracy. Analysis of head tracking and horizontal EOG suggests a tradeoff wherein head movements reduce in magnitude (peak velocity and total degrees) while eye movement increase in magnitude over the course of practice. EEG analyses reveal visual evoked potentials over the occipital cortex that index sensory processing for the different launch conditions prior to the onset of eye, head, and hand movements, while shot-related ERPs exhibit central distributions peaking around 400 ms that distinguish hits from misses in the shooting task. These findings illustrate the capacity to perform precision monitoring of movement dynamics and neural activity while individuals perform end-point full body orienting in virtual environments. Future work will explore the possibility that these biomarkers may predict performance accuracy in our simulation and may be utilized to provide real-time feedback for skill training.

Disclosures: L.G. Appelbaum: None. J. Clements: None. Y. Lu: None. H.M. Rao: None. R. Khanna: None. D.J. Zielinski: None. K. Vittetoe: None. N.D. Potter: None. R. Kopper: None. M.A. Sommer: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.13/JJJ26

Topic: H.02. Human Cognition and Behavior

Support: US Army Research Office through the Institute for Collaborative Biotechnologies under Grant W911NF-07-1- 0072

Title: Novel representations that support rule-based categorization are acquired on-the-fly during category learning

Authors: *F. A. SOTO[1], F. G. ASHBY[2];
[1]Dept. of Psychology, Florida Intl. Univ., Miami, FL; [2]Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: A body of evidence suggests that some stimulus dimensions have a "preferred" status: they are represented independently and can be selectively attended. In the cognitive psychology literature, these are usually called "separable" dimensions. Humans learn categorization rules that are aligned with separable dimensions through a rule-based learning system implemented in prefrontal cortex, hippocampus, and basal ganglia. Behaviorally, such categorization rules are learned faster and they generalize more easily than categorization rules that require integration of information from different dimensions. Recent research suggests that learning to categorize objects along a new dimension changes the perceptual representation of such dimension, making it more separable and discriminable. In this study, we asked whether such newly learned dimensions could support rule-based category learning. One group received extensive categorization training and a second group did not receive such training. Later, both groups were trained in a task that made use of the category-relevant dimension, and then tested in an analogical transfer task (Experiment 1, testing generalization to new stimuli sharing the relevant dimension) and a button-switch interference task (Experiment 2, testing whether the category-response assignments could be easily switched under verbal instructions). Based on the previous literature, we expected that only the group with extensive pre-training (with well-learned dimensional representations) would perform well in these tasks, showing evidence of rule-based behavior. Surprisingly, both groups performed as expected from rule-based learning. A third experiment tested whether a single session (less than one hour) of training in a categorization task would facilitate learning in a task requiring executive function. There was a substantial learning advantage for a group with brief pre-training with the relevant dimension. We hypothesize that extensive experience with separable dimensions is not required for rule-based category learning. Instead, the rule-based system seems to learn representations "on the fly" that allow rule application. We discuss what kind of neurocomputational model might explain these data best, given the speed of acquisition of novel representations and their immediate pronounced effect on rule-based behavior.

Disclosures: F.A. Soto: None. F.G. Ashby: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.14/JJJ27

Topic: H.02. Human Cognition and Behavior

Title: The role of frontal polar cortex in category learning: rule integration or switching?

Authors: *D. PANIUKOV, T. DAVIS;
Psychological Sci., Texas Tech. Univ., Lubbock, TX

Abstract: Category learning is a critical neurobiological function that allows organisms to simplify a complex world. Recent research on the neurobiology of category learning has focused on the role that different learning systems, such as the striatum and medial temporal lobes, play in acquiring new categories. However, there are a number of additional underexplored brain regions that are critical for categorization. One underexplored brain region that is often active in neurobiological studies of category learning is the frontal polar cortex (FPC). A number of functions have been ascribed to the FPC including theories that emphasize its role in switching between representations and theories that emphasize its role in representational integration. The goal of the present study was to clarify the role of FPC in category learning by developing a task in which representational switching and integration could be dissociated. To this end, we utilized two common types of category learning tasks, matching and classification. The matching task involved matching a multidimensional reference stimulus to target stimuli that matched the reference image on a single dimension. Participants would learn a rule about how to match the reference stimulus to a particular target via trial and error. After a number of correct trials, the rule would switch. In the classification task, participants were shown a single stimulus and learned to classify it into one or two categories based on trial and error. Like in the matching task, the rule that determined category membership was unidimensional and was switched after participants completed a number of correct trials. Although nearly identical, matching and classification place differential demands on switching and integration. In matching, a rule can be known with certainty after a single correct answer. In classification, participants may need to integrate evidence for a rule even after an initial correct response. This critical difference allows isolation of integrative functions from switching functions by comparing activation during asymptotic performance in matching and categorization. If the FPC is primarily involved in switching between representations, it should cease to be active once participants settle on a given rule in both tasks. If the FPC is involved in integration, its activation should persist in the classification task, but not matching, as additional evidence in support of a rule is integrated. The results revealed that FPC activation persisted into correct trials in classification, but not matching, suggesting that it continues to integrate information even after a rule has been settled upon.

Disclosures: D. Paniukov: None. T. Davis: None.

Poster

265. Human Learning: Perceptual, Motor, and Category

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 265.15/JJJ28

Topic: H.02. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada (RGPIN-2014-04465)

Title: Lengthening of circuit memory via mechanisms of synaptic plasticity

Authors: *K. HIMBERGER, A. FINN, C. J. HONEY;
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Abstract: How do neural circuits integrate past information with input that may arrive seconds later? The ability to integrate information over time is fundamental to cognition. For example, we need to be able to understand the words at the end of a sentence in terms of the words that came before. Currently popular models (e.g. liquid and echo state machines) suggest that reverberating activity enables information to persist over seconds of time in local circuits. But why does information about some stimuli (e.g. meaningful sequences) persist for longer than others (e.g. random sequences)?
Inspired by models of birdsong learning, we hypothesized that a combination of spike-timing dependent plasticity (STDP) and heterosynaptic plasticity (HP) mechanisms can reshape circuit reverberations, lengthening the circuit memory of common input patterns. We tested this idea computationally by implementing a spiking model of a localized cortical circuit, incorporating both STDP and HP. Consistent with our hypothesis, we observed that circuit memory was lengthened specifically for stimuli that were repeatedly pre-exposed to the network, and only when timing dependent plasticity was in operation. In ongoing work, we are (i) analyzing the topology of synaptic changes that support the lengthening circuit memory, and (ii) behaviorally testing predictions of this model for visual and auditory statistical learning.

Disclosures: K. Himberger: None. A. Finn: None. C.J. Honey: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.01/JJJ29

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R43AG047722

Title: Can adaptive cognitive training improve efficiency of attentional control in the aging brain?

Authors: *J. KENT[1], H. LEE[4], E. SCHULTZ[1], E. FOSTER[2], F. WOLINSKY[1], M. MERZENICH[4], M. VOSS[3];
[2]Biostatistics, [3]Psychology, [1]Univ. of Iowa, Iowa City, IA; [4]Brain Plasticity Inst., San Francisco, CA

Abstract: Evidence supporting the efficacy of cognitive training programs in older adults is mixed. We tested a rigorous and novel cognitive training program to provide clearer evidence for potential outcomes. The Plasticity-based Adaptive Cognitive Remediation (PACR) is a computerized adaptive training program designed to improve cognition by challenging participants with a variety of tasks that require attention and inhibitory control. Here, participants were randomized to either the PACR or a control program of non-adaptive games (e.g. checkers). Both groups completed five cognitive training sessions weekly for ten weeks. We asked whether the PACR training improves performance on the Flanker task which was not a part of the training program. The flanker task tests higher order cognitive functioning such as response competition and attentional focus. We measured performance using reaction time and functional neuroimaging before and after training. Sixteen participants have completed the trial thus far (Age=69.38, 56% female). Behaviorally, PACR (N=8) and control (N=8) groups significantly decreased their reaction time ($p < .05$) from pre-training to post-training, but there was no group-by-time interaction ($p = .912, .147$). However, the functional neuroimaging did reveal a significant group-by-time interaction selective to the flanker condition requiring the most attentional control in the Superior Parietal Lobule ($p < 0.05$, cluster corrected, max MNI coord: -36 -50 48). This area is important for attentional control and response competition suggesting that adaptive training may lead to more efficient stimulus processing relative to non-adaptive training.

Disclosures: J. Kent: None. H. Lee: None. E. Schultz: None. E. Foster: None. F. Wolinsky: None. M. Merzenich: None. M. Voss: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.02/JJJ30

Topic: H.02. Human Cognition and Behavior

Title: Perceptual and motor contributions to spatial neglect following parietal damage

Authors: *T. D. PUNT[1], G. W. HUMPHREYS[2];
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Abstract: Patients with spatial neglect following stroke typically fail to respond to stimuli presented on the side of space contralateral to their lesion. This complex syndrome may be generated by a number of different deficits, including some that are perceptual in nature (e.g. poor uptake of information on the affected side of space) and others that are more motoric in nature (e.g. a failure to programme movement to the affected side). In order to disentangle perceptual from motoric aspects of the syndrome, Mattingley et al. (1998) had patients make directional responses to targets on either the left or right of fixation and found that response times to contralesional targets were only slowed when the response required a contralesional movement. Using a similar procedure, we too found an interaction between perceptual and motor processing in parietal patients, though the perceptual deficits were more pronounced and eliminated effects of movement direction to contralesional targets. For some patients, these deficits were only evident when a directional response was programmed. We discuss implications for understanding the role of the parietal lobe as a sensorimotor interface, and the relations to prior studies.
Mattingley, J.B., Husain, M., Rorden, C., Kennard, C., & Driver, J. (1998). Motor role of human inferior parietal lobe revealed in unilateral neglect patients. *Nature, 392*(6672), 179-182.

Disclosures: T.D. Punt: None. G.W. Humphreys: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.03/JJJ31

Topic: H.02. Human Cognition and Behavior

Support: DFG grant EXC307

The Max Planck Society, Germany

Title: Attention changes connectivity strength in a hierarchical manner across the visual network

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Abstract: Attention improves behavioral performance by selectively enhancing neural responses to attended task-relevant stimuli. Several models have been suggested that explain modulation of neural responses by attention. These models are typically limited to effects of attention on single regions, and do not take into account the hierarchical organization of regions involved in processing. Here, we introduce a model that quantifies the connectivity changes across the visual hierarchy encompassing regions V1, V2, V3, up to hV4 and V5/MT+. We measured fMRI activity in humans performing a demanding visual attention task during ultra-long blocks lasting 2 minutes, alternating with passive viewing blocks involving the same stimuli. This paradigm allowed for high-quality functional connectivity measurements free of confounds related to on- and offset effects of stimulus blocks. Functional connectivity was measured between regions of the dorsal attention network (DAN) and visual regions, as well as between default mode network (DMN) regions and visual regions. We then quantified the slope and baseline of connectivity strength of a given DAN or DMN region with the visual hierarchy, as a function of attention. The results revealed that each of the DAN regions had a gradient in its connectivity strength along the visual processing hierarchy: the DAN regions showed stronger connectivity with high-level areas that decreased towards low-level areas, revealing a descending gradient from V5/MT+ and V4 towards V1. Attention enhanced this baseline connectivity pattern in additive manner for IPS, whereas right FEF additionally increased the slope, thus showing also multiplicative effects along the visual hierarchy. DMN regions had an inversed gradient of connectivity with the visual hierarchy. Attention tended to have multiplicative effects for all regions, but (negative) additive effects only in left lateral parietal cortex. The current study provides a first quantitative model of attention induced changes in connectivity between attention and default mode networks and the hierarchy of early visual areas.

Disclosures: S. Kwon: None. A. Bartels: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.04/JJJ32

Topic: H.02. Human Cognition and Behavior

Title: Effects of top-down attention to a polygon on N2pc in a visual search task

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Abstract: In a visual search task, N2pc (Negative 2nd peak Posterior Contralateral), an ERP component, is thought to reflect the attentional capture when we search a specific target. The N2pc has been reported to appear when participants search not only a specific target but also targets defined by a category (category target) such as letters or digits. (Nako et al., 2014a; Wu et al., 2013) Since it is easy to detect familiar letters and digits, they would be special cases as the category targets in the searching tasks. In a similar experiment that used pictures of clothing and kitchen items as category targets, N2pc was also observed (Nako et al., 2014b). Although they showed the attentional capture by the categorical top-down process, it remains unclear whether N2pc is observed when we search more unfamiliar and abstract category target. Here, using the N2pc component, we attempted to clarify whether or not the attentional capture would occur when plane figures with physical features such as number of vertices were category targets in a visual search task. The figures were not so familiar as letters and digits. Moreover, the category target was distinguished from a specific target by changing orientation and length of sides. After the target cue was presented, six figures arranged in a circle were presented. Subjects were asked to answer whether the target was present in the left or right side, or absent in the search array. First, one of three kinds of targets is indicated: (1) exactly the same figure as the target cue (item-based task), (2) a figure belongs to the indicated category, e.g. triangle and rectangle (category-based task), (3) the form singleton (control task). The mean reaction time was as follows: item (586ms), category (771ms), and control (932ms). This shows that the item and category based searching are promoted by the top-down attention, and item-based attention is more effective than that of category-based one. ERP analysis exhibited that there was clear N2pc in the item-based task ($p<0.001$). On the other hand, in the category-based and control tasks, no N2pc was observed. These results suggest that the attentional capture does not occur because of the vague attentional template when we search a category of polygon, which is more abstract than letters and kitchen items.

Disclosures: A. Kitami: None. T. Urakawa: None. H. Azetaka: None. A. Sayama: None. O. Araki: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.05/JJJ33

Topic: H.02. Human Cognition and Behavior

Support: ARC Centre Grant CE140100007

ARC Australian Laureate Fellowship FL110100103

ARC Future Fellowship FT120100033

Title: Theta and alpha oscillations play dissociable roles in goal-directed attention

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Abstract: Recent studies have characterized distinct oscillatory frequencies associated with feedforward and feedback visual information flow. Theta and gamma oscillations have been associated with feedforward signaling, while alpha/beta oscillations have been associated with feedback processes. It remains unclear, however, whether such oscillatory activity is recruited for task-based processing. Here we investigated the roles of theta (4-8 Hz) and alpha (8-14 Hz) oscillations in human goal-directed visual attention. We had participants respond to a brief target of a particular color among heterogeneously colored distractors. Prior to target onset, we cued one location with a lateralized, non-predictive cue that was either target- or non-target-colored. During the behavioral task, we recorded brain activity using electroencephalography (EEG), with the aim of analyzing cue-elicited oscillatory activity. We found that theta oscillations lateralized early and in response to all cues, and this lateralization was stronger if the cue matched the target color, consistent with a feedforward signal enhanced by feature-based prioritization. Alpha oscillations lateralized later, and only in response to target-colored cues, consistent with a feedback signal involved in the allocation of spatial attention. Our findings suggest that changes in theta and alpha amplitude reflect the task-based modulation of feedforward and feedback signaling, respectively.

Disclosures: A.M. Harris: None. P.E. Dux: None. C.N. Jones: None. J.B. Mattingley: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.06/JJJ34

Topic: H.02. Human Cognition and Behavior

Support: BAA-AFOSR-2013-0001

Title: Mental fatigue in a prolonged multiple object tracking task

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Abstract: Prolonged cognitive activities associated with considerable mental fatigue are becoming increasingly common in modern life. Mental fatigue can lead to lack of attention and hence difficulties in performing tasks that require concentration and sustained effort. Yet the psychophysiological, behavioral aspects, and neural signatures of this phenomenon are not well understood.

To study the mechanisms of mental fatigue, we performed a MEG experiment using an attention paradigm that demands sustained spatial attention. The multiple object tracking (MOT) task involved tracking several objects (target dots) moving on a screen while ignoring other distractor dots. The number of target dots varied randomly in trials, enabling us to investigate the relationship between task difficulty, attention, and mental fatigue. We further collected data from an eye tracker and a high speed camera, aiming to develop a computational model predictive of neuro-motor representations of fatigue. The task duration was 3 hours and there were no rest periods. Every 30 minutes the subjects were prompted to report their resistance in continuing the task, which is a self-report measure previously shown to highly correlate with mental fatigue. Analysis of MEG data showed a progressive increment in alpha band power over the duration of the task, which signifies lack of attention towards the end of the experiment. Alpha power was overall inversely related to task difficulty. The eye-tracker data revealed the pupil diameter increased with task difficulty, corroborating previous studies on pupil size as a measure of individual's attentional efforts (Alnaes et al. 2014). Moreover, our data demonstrated that pupil diameter consistently increased over time, indicating the task required increased mental efforts to maintain performance towards the end of the experiment.

This work was funded in part by grant BAA-AFOSR-2013-0001 (to D.M., D.P. and D.M.) and was conducted at the Athinoula A. Martinos Imaging Center at the McGovern Institute at MIT.

Disclosures: Y. Mohsenzadeh: None. K. Michmizos: None. D. Metaxas: None. D. Pantazis: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.07/JJJ35

Topic: H.02. Human Cognition and Behavior

Title: Flow: The application of psychophysiology to understand user experience in video games

Authors: M. ROSAZZA, *M. M. NIEDZIELA, B. THAU;
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Abstract: Usability research involves understanding human limits of perception, attention, and memory in ease of use and learnability of human-made objects such as websites or video games. Cognitive modeling involves creating a computational model to estimate usability based on psychological principles and experimental studies. Cognitive models can be used to improve user interfaces or predict problems, errors, and pitfalls during the design process. Flow is the mental state of operation in which a person performing an activity is fully immersed in a feeling of energized focus, full involvement, and enjoyment in the process of the activity, a theory pioneered by psychologist Csikszentmihalyi. The aim of our study was to use electrophysiology (fEMG, HRV, GSR) and eye tracking to identify flow events in physiological responses linked to in-game events. Twelve male participants (18-35 yrs) were measured with electrophysiology (fEMG, HRV, GSR), eye tracking while playing a novel video game, Darksiders 2, and given a post-game survey. Flow was calculated by amount greater than average arousal (GSR) generated and attention/motivation experienced (HRV) and then compared with performance in video game play at that time interval. On average, we found gamers entered a flow state 20.3% of the time coinciding with intense moments during the game with 24.6% of this time being a positive experience. Our study revealed that it is possible to predict the occurrence of flow during video game play. The ability to model flow using physiological measures may help game designers create better games and help researchers better understand game play.

Disclosures: M. Rosazza: None. M.M. Niedziela: None. B. Thau: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.08/JJJ36

Topic: H.02. Human Cognition and Behavior

Support: National Nature Science Foundation of China Grants to H. L. (31522027, 31571115)

Title: Rhythmic sampling of visual objects is mediated by inhibitory alpha activity

Authors: *J. JIA[1,2,3], F. FANG[1,2,3], H. LUO[2,3];
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Abstract: To deal with a crowded visual scene, it is important that attention is allocated over time and space efficiently. Previous studies suggest that attention acts as a moving spot light dwelling on each location serially, whereas other studies reveal that attention can stay on multiple locations simultaneously. Interestingly, recent behavioral findings demonstrate rapid temporal fluctuations in attentional behavior, suggesting that attention shifts between two spatial locations rhythmically. However, the neural mechanism underlying the space-time distribution of attention remain largely unexplored. In the present study, we combined covert attentional paradigm and temporal response function techniques (TRF) to address the issue. EEG was recorded from fifteen human subjects as they were presented with 5-sec dynamic sequences at two spatial locations and were asked to attend to one of them. Notably, the visual sequences at the two locations were randomly modulated in luminance and independently manipulated, so that we can estimate the TRFs for attended and unattended visual sequences separately (Att vs. Unatt) from the same EEG response. First, compared to Unatt condition, TRFs for Att condition showed an alpha-band (~10 Hz) power inhibition around 100ms, commensurate with previous findings that alpha activities represent inhibitory processes during attention. Second, the alpha inhibition did not display spatial specificity as found before (e.g., decrease on contralateral side and increase on ipsilateral side), suggesting that it may represent an object-level attention independent of space. We further examined TRFs when both visual sequences are in motion and revealed similar alpha inhibition, confirming its nature of object-based attention. Finally, the alpha inhibition was followed by a subsequent alpha enhancement, indicating attentional switching from attended to unattended object. Interestingly, this alpha switching pattern was modulated by task context. Specifically, the Att-Unatt alpha switching pattern became stronger with the decrease in attentional cuing validity (from 100% to 75% and 50%). Our findings suggest that attention efficiently and flexibly distributes over space and time to accommodate changing task demands. We propose that attention samples multiple visual objects in a rhythmic manner, by modulating and coordinating inhibitory alpha-band neuronal activities.

Disclosures: J. Jia: None. F. Fang: None. H. Luo: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.09/JJJ37

Topic: H.02. Human Cognition and Behavior

Title: Neurophysiological signatures of proactive versus reactive control in young adults

Authors: *V. EXPOSITO, T. HAGEN, J. HALVORSEN, S. AMINIHAJIBASHI, M. STAVRINOU, M. FOLDAL, T. ESPESETH;
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Abstract: During the execution of a cognitive task, processing and maintaining context information is necessary for guiding behavior and achieving desired goals. The AX-Continuous Performance Task (AXCPT) has been developed to study cognitive control and has proven successful to demonstrate differences in context processing between young vs. old people or healthy adults vs. patients with schizophrenia. The task requires to provide a specific response (eg pressing on right key) to the target trials in which the valid cue (A) is followed by the valid probe (X) . All other cue-probe pairs are considered as non-target trials and require a non-target response (eg pressing the left key). While in some non-target trials, the invalid cue (all non-A letters) has a predictive value to trigger the correct response, in others, attending to the invalid probes (all non-X letters) is determining for accuracy. Here we used pupillometry to study the temporal dynamics of attention during the delay period between the presentation of the cue and the probe, and also after the presentation of the probe. During the delay period the context information should be maintained, and previous studies using physiological and neurophysiological measures suggest different patterns of neural processing for the different cue types (A, non-A). We recorded the pupillary changes in a sample of 170 participants during performance of the AXCP task. We hypothesized that individual differences in the processing of the cues, and therefore of context information, would be reflected in different patterns of pupil dilations, and that these would be correlated with behavioral performance. The results suggest that individual behavioral differences can be tracked by physiological signatures. The results are explored within the framework of theories of behavioral performance and cognitive control.

Disclosures: V. Exposito: None. T. Hagen: None. J. Halvorsen: None. S. Aminihajibashi: None. M. Stavrinou: None. M. Foldal: None. T. Espeseth: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.10/JJJ38

Topic: H.02. Human Cognition and Behavior

Support: NSERC

National Institutes of Health Grant R21-EY021644

National Science Foundation BCS-1534823

Title: Neural correlates of perceptual grouping during a visual illusion

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Abstract: Grouping local elements of our visual environment together is crucial for meaningful perception. Since perceptual grouping is generally thought to occur before attentional selection, the neural mechanisms involved have been mainly studied at early and mid-levels of the visual processing stream. However, less is known about how higher-level brain regions may contribute to these responses. While one previous study found involvement of the parietal cortex in mediating the perception of dynamic illusory Gestalts (Zaretskaya et al., 2013), the neural mechanisms involved in perceptual grouping across the whole brain have been largely unexplored. Here we use functional magnetic resonance imaging (fMRI) to examine the brain networks involved in perceptual grouping using a whole-brain analysis. Healthy human adults completed a line discrimination task in which two horizontal lines were superimposed on a background of black and white dots organized so that, on occasion, the dots induced the Ponzo illusion if perceptually grouped together. This task was completed under conditions of divided-attention, in which participants were asked to complete the line discrimination task while paying attention to the pattern that on some trials would be formed in the background. Results of the behavioural analysis revealed that at the end of the task all participants were able to correctly identify the background pattern and made line judgments consistent with an illusion based response. Group whole-brain analysis clearly revealed increased activation in response to the perception of the illusory Gestalts compared to perception of the ungrouped local elements in early visual areas of the right occipital lobe and the postcentral gyrus of the right parietal lobe. While activation in the occipital and parietal regions is consistent with previous research, we also found activation in the right middle frontal gyrus of the frontal lobe, suggesting that this region

may be driving top-down feedback in perceptual grouping under divided-attention conditions. This is supported by electrophysiological evidence showing that the fronto-parietal network facilitates perceptual grouping processes (Han & Humphreys, 2007), refining these findings to a more specific region of the frontal lobe. A recent case study has also suggested that the right middle frontal gyrus plays an important role in reorienting attention (Japee et al., 2015). These results suggest that while lower and mid-level regions are an important part of perceptual grouping under conditions of divided-attention, activity in the frontal lobe may also function to mediate perceptual grouping.

Disclosures: T.A. Carther-Krone: None. J. Lawrence-Dewar: None. S. Shomstein: None. J.J. Marotta: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.11/JJJ39

Topic: H.02. Human Cognition and Behavior

Title: Variability in performance during perceptual decision making is related to attentional filtering

Authors: *M. D. NUNEZ, A. GOSAI, J. VANDEKERCKHOVE, R. SRINIVASAN;
Univ. of California, Irvine, Irvine, CA

Abstract: The aim of the current study was to determine whether variation in subjects' performance (accuracy and RT) during training could be explained by changes in attentional strategies. The Perceptual Template Model (PTM; Dosher and Lu; 2000) predicts that attentional filtering in perceptual decision making either occurs by 1) reducing internal noise to enhance visual input from the entire visual field or 2) reducing external noise and boosting external signal by tuning neural-population responses. The PTM also predicts that reducing internal noise is optimal in low-noise conditions and reducing external noise is optimal in high-noise conditions. In previous studies (e.g. Nunez et al., 2015), we have made use of the steady-state visual evoked potentials (SSVEPs) to measure attention during perceptual decision making tasks. SSVEPs are narrow band EEG responses evoked at the flicker frequencies of visual stimuli that reflect cortical processing of specific visual stimuli. By assuming a hierarchical cognitive model of the decision making process, the drift-diffusion model (Ratcliff 1978; Nunez et al., 2015), we have shown evidence that individual differences in attention predict differences in perceptual encoding and evidence accumulation. In the present experiment, SSVEPs were used as measures of attention deployment to three different visual noise conditions of low-, medium-, and high-

contrast noise in a two-alternative forced choice task in which subjects had to choose Gabors of high (2.6 degrees visual angle) or low (2.4 degrees visual angle) spatial frequency embedded in a broadband masking noise (bandpass filtered between 2 and 3 degrees visual angle). Each subject repeated the experiment over 7 experimental sessions, in order to measure variability in performance and potential improvements due to training. SSVEP responses to the external visual signal (flickering at 30 Hz) and external visual noise (changing at 40 Hz) were detected on each trial using 128-channel EEG recordings. These SSVEP responses were used to index the attentional filtering applied on each trial. In order to explore the effects of attention on specific components of decision making process (encoding time, decision time, and motor response time) for all conditions and subjects, a hierarchical drift-diffusion model was assumed that accounts for trial-by-trial and session-to-session variability. We found that signal enhancement (internal noise reduction) and external noise reduction both lead to improved accuracy within subjects, and can account for the improvement in performance during repeated experiments, and the variability in performance from trial-to-trial.

Disclosures: M.D. Nunez: None. A. Gosai: None. J. Vandekerckhove: None. R. Srinivasan: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.12/JJJ40

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01NR014181

NSF Grant BCS-1344285

Title: cholinergic modulation of eyes-closed to eyes-open alpha reactivity

Authors: *L. WAN, N. LAM, A. RAJAN, H. HUANG, N. SCHWAB, C. PRICE, M. DING; Univ. of Florida, Gainesville, FL

Abstract: Alpha oscillations (8 to 12 Hz) are prominent in the human brain during eyes-closed (EC) resting. Opening of the eyes (EO) significantly attenuates alpha activity. The ratio between EC alpha and EO alpha is referred to alpha reactivity. Alpha reactivity has found applications in both basic and clinical neuroscience. Its neural underpinning, however, remains unclear. Past work has implicated acetylcholine in alpha modulation. To further examine this issue we recorded EEG and fMRI separately from twenty participants undergoing two sessions of resting-

state scanning: eyes-closed resting and eyes-open resting. Treating the BOLD signal from basal nucleus of Meynert (BNM) as a proxy of cholinergic activity, functional connectivity between BNM and the rest of brain were computed separately for EO and EC, and the difference was correlated with alpha reactivity. The following results were found. First, BNM connectivity to the visual cortex increased from EC to EO. Second, BNM connectivity to the anterior cingulate cortex, supplementary motor area and inferior parietal lobule decreased from EC to EO. Third, alpha reactivity was negatively correlated with the EC to EO difference of the BNM-visual cortex connectivity, namely, the stronger the EC to EO BNM-visual cortex functional connectivity increase, the larger the alpha reactivity. These findings support a role of acetylcholine in alpha reactivity.

Disclosures: L. Wan: None. N. Lam: None. A. Rajan: None. H. Huang: None. N. Schwab: None. C. Price: None. M. Ding: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.13/JJJ41

Topic: H.02. Human Cognition and Behavior

Support: Darrel K Royal Foundation for Alzheimer's Disease

Faculty Research Initiative award

Title: Illusory conjunctions in visual short-term memory: individual differences in Corpus Callosum connectivity and splitting attention between the two hemifields

Authors: *S. QIN, N. RAY, M. O'CONNELL, C. BASAK;
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Abstract: Studies on split-brain patients have found independent operations in separated hemispheres during attentional scanning suggesting that the two hemispheres can independently process visual information by splitting attention. Overloading visual attentional capacity would likely result in mistakenly combining the various features of an object, i.e. illusory conjunctions. We hypothesize that connectivity of corpus callosum would predict the degree of illusory conjunctions. In the current study, illusory conjunctions from features presented either at the opposite hemifield or at the same hemifield were assessed using a memory-scanning paradigm. DTI scans were obtained for all participants. This study is the first to investigate the role of corpus callosum in splitting attention between the opposite hemifield vs. within the same

hemifield. Results indicate that successful recollection for illusory conjunctions from opposite hemifield was better than that from same hemifield, lending support to inter-hemispheric independence of two hemispheres in visual short-term memory in healthy adults. Moreover, the individual variations in posterior corpus callosum white matter integrity were more predictive of across hemifield illusory conjunctions than within hemifield illusory conjunctions. That is, individuals with lower posterior corpus callosum white matter integrity had higher recollection when features were recombined from two hemifields than when recombined from the same hemifield.

Figure 1

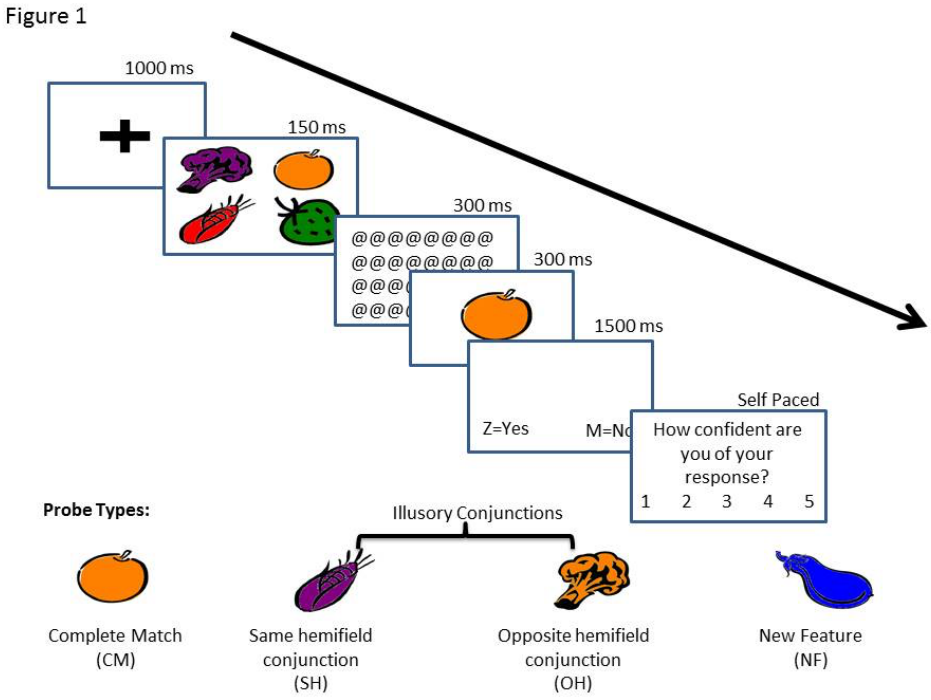
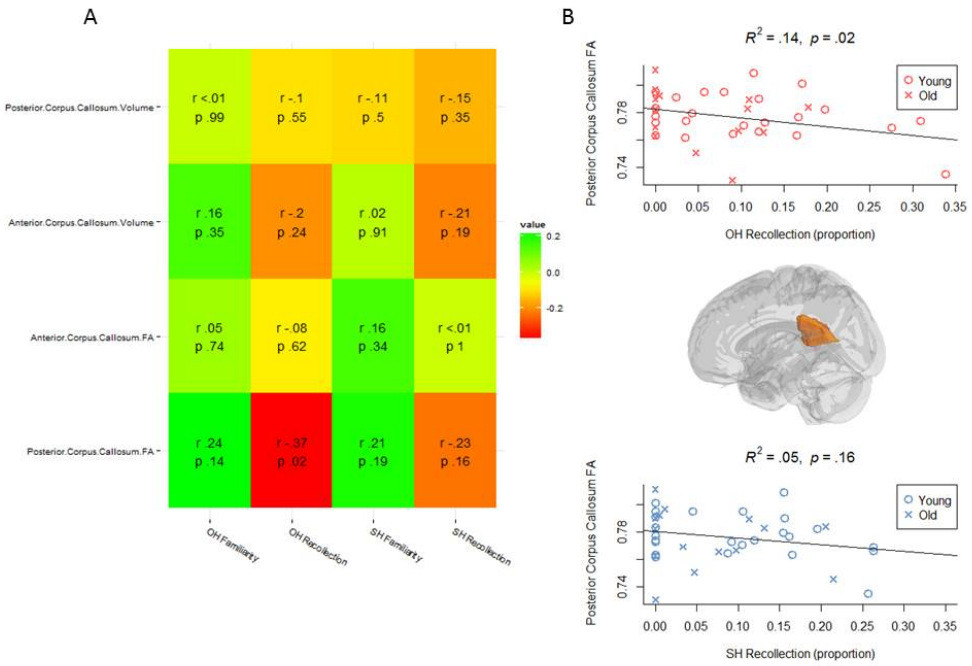


Figure 2



Disclosures: S. Qin: None. N. Ray: None. M. O'Connell: None. C. Basak: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.14/JJJ42

Topic: H.02. Human Cognition and Behavior

Title: Top-down adjustment of alpha phase in anticipation of predictable relevant stimuli

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Abstract: Oscillatory alpha activity (8-12Hz) has been proposed to represent an active mechanism of pulsed, functional inhibition over neural processing, due to the finding that its power is reduced in anticipation of incoming relevant information at sensory regions. In line with this assumption, the alpha cycle includes a phase of inhibition and a phase of excitability.

Whether alpha phase can be top-down adjusted in order to improve information processing under appropriate experimental conditions remains unclear, with evidence for and against that possibility. Also, the source of such top-down control is unknown. We carried out a cross-modal (visual/auditory) attention experiment with magnetoencephalography in thirty-four healthy subjects. In each trial, a somatosensory cue indicated to the participants whether to attend the visual or auditory domain, and ignore the simultaneous information from the other domain. The time onset of all stimuli was predictable across trials. We found that when visual information was attended, anticipatory alpha power was reduced in visual areas, while its phase adjustment was increased. Alpha phase adjustment optimized the processing of relevant stimuli, as assessed by performance speed and stimulus-induced gamma activity. In addition, such adjustment predicted effective inter-sensory interference avoidance across participants. We further found that phase distribution for optimal performance between domains was different in visual areas, indicating specific phases for information processing depending on its relevance. Finally, alpha oscillations in the left prefrontal cortex controlled the adjustment of alpha phase in visual areas. Our results confirm that alpha phase can be adjusted in anticipation of predictable stimuli and improve their processing and related behaviour. Modulation of anticipatory power and phase adds to the computational versatility of the alpha rhythm, since it allows for adjustment of the processing capabilities of the visual system on a fine temporal scale. These findings also call for further research about possible changes of such modulation in attentional disorders.

Disclosures: R. Solis-Vivanco: None. M. Bonnefond: None. O. Jensen: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.15/JJJ43

Topic: H.02. Human Cognition and Behavior

Title: Inattentional deafness is signified by activity in brain regions involved with attentional bottleneck

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Abstract: Inattentional deafness is the failure to hear otherwise audible sounds (usually alarms) that may occur under high workload conditions and has been cited as the source of many aviation related accidents. One potential cause for the occurrence of inattentional deafness could be an

attentional bottleneck that occurs when task demands are high, resulting in a lack of resources for processing of additional tasks. In this fMRI experiment we explore the brain regions active during the occurrence of inattentional deafness using a flight simulation task in which the participants (n=15) fly through a Red Bull air race course (using a joystick to control elevator and aileron deflections: pitch and roll) passing through a number of gates and at the same time push a button on the joystick to the presence of audio and visual alarms. A first person view was presented from within the cockpit such that the bottom half of the display was the instrument panel of the plane and the top part of the display was the view of the world through the cockpit canopy. In real flight, pilots must maintain visual attention to both the instruments and to the world outside the plane. In order to simulate these perceptual demands we presented a light on the instrument panel that would inform the orientation (either horizontal or vertical) in which they were to fly through the gates. The auditory alarm was a short beep sound played loud enough to be audible even within the fMRI scanner. The visual alarm was an additional light on the instrument panel. Subjects were instructed to focus on the difficult piloting task and to try not to crash and to press the button on the joystick quickly when they noticed an auditory or visual alarm. The results of the fMRI analyses revealed that auditory misses relative to auditory hits (as well as the interaction of auditory misses relative to hits compared to visual misses relative to hits) had significantly greater activity ($p < 0.05$ cluster level corrected) in the pre-SMA, ACC, the right IFG and right insula. Consistent with our hypotheses these regions have been implicated in several studies as being involved with an attentional bottleneck. Activity in these brain regions could be used as a neural signature of the probability of occurrence of inattentional deafness. Using this information it may be possible to develop neuroadaptive automation that attempts to reduce workload when these regions are highly active and/or present more attention grabbing alarms in these cases.

Disclosures: D.E. Callan: None. F. Dehais: None. N. Gonthier: None. G. Durantin: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.16/JJJ44

Topic: H.02. Human Cognition and Behavior

Support: Korea Reasearch Institute of Standard and Sciense

Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Title: Gamma band analysis in DLPFC during stroop task : MEG Study

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Abstract: BackGround & Purpose : Magnetoencephalography is a functional neuroimaging technique for mapping brain activity by recording magnetic fields produced by electrical currents occurring naturally in the brain. MEG is a very effective way of spatial and temporal analysis with the number of nerve activity in the brain. While performing the cognitive task we want to find area of the brain which regions are activated and the reaction by the analysis of neural activity. Stroop task is a cognitive task (When the name and color are matched, we called 'congruent', is not 'incongruent) that we will find the difference between those 2 situation. Methods : Stroop effect is a demonstration interference in the reaction time of a task. When the name of a color (e.g., "blue", "green", or "red") is printed in a color not denoted by the name (e.g., the word "red" printed in blue ink instead of red ink), naming the color of the word takes longer and is more prone to errors than when the color of the ink matches the name of the color. When the name and color are matched we called 'congruent', aren't matched 'incongruent'. 8 people were included in the analysis. Recording the MEG data during the stroop task and make the ERP and apply Fourier transform to analysis time-frequency spectrum . Results : In the behavior response, Congruent and incongruent reaction time showed a difference. Congruent reaction time is more faster than incongruent. Percentage of correct answers in the case of some falling trend seems incongruent. But not the trend seems to be common from all subjects. We focused on dorsolateral prefrontal cortexSpecially in gamma range, we can find significant difference between 'congruent' and 'incongruent'. In 40-50Hz, a 100~250ms before tapped right butten, there is a big difference in ERSP(event-related spectral perturbation). In that case, one of the subjects 'Congruent' tapping ERSP power is -3.8794 dB and 'Incongruent' tapping ERSP power is -.4753. These 2 data analyzed using paried t-test, p-value comes out 0.00. The same result comes out when it applies to all subject. That shows significant different between congruent and incongruent in gamma band activity(40-50Hz). Discussion: According to a popular theory, gamma band waves may be implicated in creating the unity of conscious perception. In cognition task, several research in gamma-bank oscillations may explain the heightened sense of consciousness and its cognitive functions. In this experiment, as the gamma band is more active in a difficult situation, when subject encountered in two different situations to found solving problems.

Disclosures: S. Hwang: None. W. Chang: None. B. Kim: None. J. Chang: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.17/JJJ45

Topic: H.02. Human Cognition and Behavior

Support: NRF-2015M3C7A1031969

NRF-2015R1A5A7037676

Title: The effects of task irrelevant information on the dynamics of selective attention

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Abstract: We investigated how task irrelevant information influences target processing using a flanker task. Participants (N = 20) performed a gender discrimination task for a centrally presented face stimulus (target), which was crowded by four distractor faces. We manipulated the information congruency in the *task-relevant* dimension by matching or not matching the gender of the target to that of the flankers. The information in the *task irrelevant* dimension was manipulated by varying the facial expression of the target and flankers independently (happy or fearful). In addition, to probe the dynamics of flanker effects, we varied the stimulus onset asynchrony (SOA) between the target and flankers: the target appeared simultaneously with the flankers or advanced the target (SOA = 0ms, 100ms, or 300ms). In overall, consistent with previous studies, gender discrimination was slower when the gender of the target and flankers was incongruent than when congruent (‘*flanker effect’*). Intriguingly, the temporal dynamics of flanker effects was affected by the task-irrelevant information: the flanker effect increased with an increasing SOA when the emotional expression of the target and flankers were matched; but when not matched, the flanker effect was found only at 100-ms SOA. This implies that selective attention failed to suppress the features in the task irrelevant dimension (emotional expression) to some degrees. Because the task irrelevant features were manipulated both in the target and in the flankers, the suppression could have failed either in the target, or in the flankers, or both. To resolve this issue, we carried out another experiment. Here again participants (N=20) performed the same gender discrimination task with varying SOA but, unlike the first experiment, the features in the task-irrelevant dimension were manipulated either only in the target (‘*target-only’* condition) or only in the flankers (‘*flanker-only*’ condition). In the ‘target-only’ condition, the facial expression was varied over trials in the target (happy, neutral and fearful) but remained neutral in the flankers, and vice versa in the ‘flanker-only’ condition. The influence of the task-irrelevant information on flanker effects was more pronounced in the ‘flanker-only’ condition than in the ‘target-only’ condition. Taken together, our findings indicate that the task-irrelevant

features survive feature-selective suppression in the flankers, where spatial attention gain was weak, producing sustained influence in task-relevant information processing over both the target and flanking regions.

Disclosures: J. Kim: None. M. Kang: None. S. Lee: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.18/JJJ46

Topic: H.02. Human Cognition and Behavior

Support: This work was supported by the ARC Centre of Excellence for Integrative Brain Function (ARC Centre Grant CE140100007).

JBM was supported by an ARC Australian Laureate Fellowship (FL110100103).

PED was supported by an ARC Future Fellowship (FT120100033).

Title: Neural correlates of goal directed attentional capture in the absence of conscious perception

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Abstract: An observer's current goals can influence the efficiency with which visual stimuli are processed. This is demonstrated in the 'contingent capture' effect, in which spatial attention is captured to the location of a briefly presented cue that shares its features (e.g., color) with the observer's current task set. Here we combined behavioral testing and electroencephalography (EEG) to test whether a set-matching cue can capture attention to its location even when that cue is masked from conscious awareness. We used a variant of the classic contingent capture paradigm, in which participants searched arrays of four letters for a single target in a specific, cued color (e.g., red). Immediately prior to the letter array, a non-predictive cue display was presented in which one item matched the searched-for color, and appeared either at the location of the subsequent target (valid trials) or at another location (invalid trials). In separate trials, cue displays were either fully visible or were masked from awareness using continuous flash suppression (CFS). Behaviourally, target-colored cues yielded significantly faster and more accurate responses when these were spatially valid (at the same location as the target) than spatially invalid (at another location). Critically, these cueing effects occurred for both conscious and unconscious cue displays, and were roughly equivalent in their magnitude. In line with the

behavioral results, the EEG data showed that target-colored cues produced a robust N2pc response – a well known signature of spatial orienting – for both conscious and unconscious cues, although the amplitude was somewhat reduced for the latter. Our findings suggest that top-down control settings for relevant features elicit attentional orienting even in the absence of conscious perception.

Disclosures: S.L. Travis: None. P.E. Dux: None. J. Mattingley: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.19/JJJ47

Topic: H.02. Human Cognition and Behavior

Support: NSERC of Canada Discovery Grant to RMK

Title: Exploring the neural signatures of multimodal inhibition of return

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Abstract: When the interval between a spatially uninformative cue and subsequent spatial target is long enough response times to targets presented in the vicinity of the cue are delayed. This behavioral effect, called Inhibition of Return (IOR), has been been observed cross-modally between all pairs of vision, audition and touch. It has also been subjected to electroencephalographic examination using event-related potentials (ERPs). Here we use ERPs to explore, for the first time, cross-modal IOR. Early work looking at sensory-related ERP components (e.g., P1 in visual studies) suggested that P1 reductions might be an electrophysiological marker of IOR. This suggestion is challenged, however, by the observation of P1 reductions with and without IOR, and vice versa. Based on recent behavioral and electrophysiological evidence, we hypothesized that early sensory-related ERP component reductions, are the result of repetitive sensory stimulation and not IOR. We tested this hypothesis by exploring cross-modal IOR using ERPs. We found behavioral IOR between all four possible pairings of touch and vision as cues and targets. Cue-related sensory ERP (P1) reductions were not observed with visual targets. Supporting our hypothesis, a cue-related sensory ERP reduction was observed for tactile targets, but only when preceded by a tactile cue.

Disclosures: R.M. Klein: None. G. d'Entremont: None. A. Jones: None. M.A. Lawrence: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.20/JJJ48

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI 15K21414

Title: The difficulty of response pattern-dependent tasks to affect attention

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Abstract: Depending on changes in the environment, it is important to inhibit and/or change responses to produce an appropriate behavior. Attention during the preparatory period affects the inhibition and change to an accurate response. To elucidate the relationship between attention and performance of an accurate response, contingent negative variation (CNV) was examined in tasks with different response patterns.
In the tasks, participants were required to press a switch when a go-signal was presented. However, participants were occasionally required to withhold the response when the go-signal was followed by a stop-signal during a stop-signal task (SST), and were occasionally required to change the response when the go-signal was followed by a change-signal during a stop-change task (SCT). In the multi-SCT (m-SCT), the participants were occasionally required to withhold and/or change the response when the go-signal was followed by a stop- or change-signal. To obtain the CNV, EEG data were recorded from the scalp during the tasks.
The reaction time (RT) increased in the order SST < SCT < m-SCT which suggests the relative degree of difficulty of these tasks. The late CNV amplitude was larger during SCT than during the other tasks. There was no difference in the late CNV amplitudes during SST and m-SCT. The results of CNV were not completely in accord with RT. In the m-SCT, the motivation of the participant might be decreased because of the difficulty of the task. These findings suggest that with the degree of difficulty of the task, the attention of the preparatory period, but attention decreases when the degree of difficulty is too high.

Disclosures: M. Takayose: None. R. Koshizawa: None. K. Oki: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.21/JJJ49

Topic: H.02. Human Cognition and Behavior

Title: The N2pc and localization of visual information

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Abstract: Traditional conceptualizations of the N2pc component have characterized this event related potential as reflecting the deployment of visual attention to task relevant stimuli appearing in lateral portions of ones visual field (Luck & Hillyard, 1994a, Luck & Hillyard, 1994b). However, more recent work has begun to suggest that this component may instead reflect the localization of, or orientation to, target information (Tan & Wyble, 2015; Drew, Koch, Chrobak & Karst, 2015). Specifically, Tan & Wyble (2015) demonstrated that when two targets were sequentially presented in the same lateralized location, no prolonged N2pc component was observed. Further, Drew, Koch, Chrobak, & Karst (2015) demonstrated that when a non-target spatial cue preceded a target, the cue elicited an N2pc, and not the target. This suggests that the localization process initiated by the cue, a stimulus that did not require selection, was sufficient to elicit the N2pc. However, the aforementioned study used a red cue and target stimulus among black distractors. The color properties of the cue in this case may have inadvertently caused attentional selection to occur because of its contextually salient nature. The current study seeks to address this concern by using black distractor, cue, and target stimuli. These stimuli were used to further disambiguate the distinction between attentional processes of localization and selection, as they eliminated the confounding nature of overly salient cue features. Trials consisted of two rapidly presented stimulus streams comprised of black distractors and the potential for task relevant stimuli that were presented laterally of a fixation point. Each trial had an equal probability of containing no cue or target, only a cue, only a target, or a cue immediately followed by a target in the same location. Preliminary data suggests that when a cue and target were presented successively, the cue elicited an N2pc whereas the target did not. Further, a cue presented alone elicited an N2pc as well. These data further support the hypothesis that the N2pc may reflect the localization of target information in visual space, rather than attentional selection.

Disclosures: A. Drew: None. J. Berger: None. K. Bowe: None. R. Timmins: None. A.T. Karst: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.22/JJJ50

Topic: H.02. Human Cognition and Behavior

Title: Improving dorsal stream functioning remediates visual timing deficits, improving figure-ground discrimination, reading fluency, attention, and memory

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Abstract: The current investigation explores whether deficits in magnocellular-parvocellular integration, causing both selective and sustained attention deficits, underlies processing problems in dyslexia. Two interventions were compared, one targeting the temporal dynamics (timing) of the visual pathways, visual movement direction-discrimination, with a second reading intervention (control group) using a computer-based guided reading program. This randomized controlled-validation study, balanced on student's reading ability, was conducted by training the entire 2nd and 3rd grade classrooms (42 students) at a local elementary school for 30 minutes twice a week at the beginning of the school day. Standardized tests were administered at the beginning and end of 12 weeks of intervention training to evaluate improvements in academic skills. The direction-discrimination training group significantly improved both the dyslexic and typically developing (TD) students' selective and sustained attention, visual motion timing and sensitivity, reading fluency, working memory, as well as improving figure-ground discrimination. These improvements were not found for the computer-based guided reading group. This study suggests that improving visual dorsal stream function by training on figure-ground discrimination of a test pattern moving left or right relative to a stationary background pattern is the key for reading acquisition to happen at an efficient speed for dyslexic and at-risk TD students. We suggest this occurred because the direction-discrimination training acted to increase the temporal precision and neuronal sensitivity of magnocellular neurons in the dorsal stream relative to linked parvocellular neurons, increasing the activity of inhibitory circuits. Remediating visual timing deficits in the dorsal stream revealed the causal role of visual motion discrimination and attention in reading acquisition. This study supports the hypothesis that faulty timing in synchronizing the activity of magnocellular with parvocellular visual pathways is a fundamental cause of dyslexia. Moreover, this study suggests that visual movement direction discrimination can be used to not only diagnose dyslexia early, but also for its successful treatment, so that reading problems do not prevent children from readily learning. Furthermore, this study provides more evidence that a short amount of visual movement direction-discrimination training improved attention, thereby improving sequential processing, reading fluency, memory, and multitasking, enabling reading and learning to be much easier.

Disclosures: **T.A. Lawton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Perception Dynamics Institute. **J. Shelley-Tremblay:** None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.23/JJJ51

Topic: H.02. Human Cognition and Behavior

Support: CIHR Operating Grant #491746

CIHR Michael Smith New Investigator Salary Prize

OMHF New Investigator Fellowship

CIHR Operating Grant #142192

Title: Neurogenetically altered norepinephrine availability affects behavioral and electrocortical indices of affect-biased attention

Authors: ***M. R. EHLERS**[1], K. H. ROBERTS[1], M. G. M. MANALIGOD[1], D. J. MUELLER[2], L. J. FÜRST[3], M. J. WIESER[3], R. M. TODD[1];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Psychology, Univ. Würzburg, Würzburg, Germany

Abstract: It is well known that we continually filter incoming information allocating attentional resources to what is emotionally or motivationally salient or relevant to our current goals. Depending on the context, when emotionally salient and task relevant stimuli are both present, the salient stimuli can either enhance or distract from task performance (Wieser, McTeague & Keil, 2012, Schmitz, De Rosa & Anderson, 2009). In humans, the influence of neuromodulators on cortical processes underlying competition and facilitation of biased attention remains poorly understood. A common genetic variation in the ADRA2b gene coding for α2b adrenoceptors has been observed in over 50% of the populations studied. This variation, which inhibits autoreceptor activity, has been related to increased extracellular norepinephrine (NE) levels, providing a naturally occurring means of probing the influence of NE activity on brain and behavior. The goal of the current study was to employ genotyping with use of EEG steady state visual evoked potentials (ssVEPs) to examine the role of norepinephrine in allocation of cortical resources to competing emotionally salient and task relevant stimuli.
209 East Asian and Caucasian participants (mean age = 21 ± 3 yrs, 128 female) were genotyped

for the ADRA2b polymorphism. EEG was recorded while participants performed a change-detection task. In each trial participants were asked to detect phase shifts in a target Gabor patch that was overlaid over an emotionally expressive face (happy, angry, neutral). Faces and targets were flickered at competing driving frequencies of 15 and 20 Hz (counterbalanced within the task). The amplitudes of the steady state potentials over visual cortex linked to the frequency of faces and targets were examined to measure allocation of cortical resources to distinct stimuli occupying the same area of space.
Results revealed an interaction between genotype and facial expression. The presence of angry faces improved accuracy on the change-detection task and evoked larger ssVEP amplitudes for targets overlaid over angry faces, indicating biased facilitation by negative stimuli only for those who did not carry the deletion variant. These results remained robust after controlling for ethnic group and similar patterns were observed in Caucasians and East Asians. Consistent with previous research showing that high levels of tonic norepinephrine lead to reduced task performance, the current study suggests that putatively lower levels of norepinephrine present in carriers of the ADRA2b wild type are associated with the often-reported "weapon focus," whereby attention is sharpened in the presence of negative affect.

Disclosures: M.R. Ehlers: None. K.H. Roberts: None. M.G.M. Manaligod: None. D.J. Mueller: None. L.J. Fürst: None. M.J. Wieser: None. R.M. Todd: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.24/JJJ52

Topic: H.02. Human Cognition and Behavior

Support: Reed College Science Research Fellowship

Title: Does spatial attention modulate afferent activity in primary visual cortex?

Authors: *M. A. PITTS, H. BAUMGARTNER;
Psychology, Reed Col., Portland, OR

Abstract: Whether visual spatial attention can modulate feedforward input to human primary visual cortex (V1) is debated. In a recent book entitled "Controversies in Cognitive Neuroscience", Slotnick (2013) designates the nature of attentional modulation in V1 as one of the top seven controversies in the field. One prominent and long-standing hypothesis is that visual spatial attention can influence processing in V1, but only at delayed latencies suggesting a feedback-mediated mechanism and a lack of modulation during the initial afferent volley

(Mangun, 1995; Clark & Hillyard 1996; Martinez et al., 1999; Di Russo et al. 2003; Ding et al. 2014). The most promising challenge to this hypothesis comes from an event-related potential (ERP) study that showed an amplitude enhancement of the earliest visual ERP, called the "C1" component, for spatially-attended relative to spatially-unattended stimuli (Kelly et al., 2008). In the Kelly et al. (2008) study, several potentially important experimental design modifications were introduced, including tailoring the stimulus locations and recording electrodes to each individual subject, cueing endogenous attention on a trial-by-trial basis, and flexibly adapting the difficulty of the target detection task. In the current study, we employed all of these methodological procedures, and tested for attentional enhancements of the C1 component for left and right upper visual field stimuli and left and right lower visual field stimuli, separately. The comparison between ERPs to stimuli in the upper and lower visual fields capitalizes on the polarity inversion property of the C1 component. Using the same analysis strategies as Kelly et al. (2008), we found no evidence for an attention-based modulation of the C1 (50-80ms). In other words, C1 amplitudes were statistically identical for stimuli that were attended versus unattended. Attention-based amplitude enhancements were clear and robust for the subsequent P1 (90-140ms) and N1 (150-180ms) components, which were localized to extrastriate neural generators. Thus, despite using methods specifically designed to reveal C1 attention effects, the current study provided no confirmatory evidence that such effects exist.

Disclosures: M.A. Pitts: None. H. Baumgartner: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.25/JJJ53

Topic: H.02. Human Cognition and Behavior

Title: Distractor expectation modulates proactive control mechanisms in visual search

Authors: *M. A. PETILLI[1], F. MARINI[2], R. DAINI[1];
[1]Psychology, Univ. of Milano-Bicocca, Milano, Italy; [2]Dept. of Psychology, UCSD, San Diego, CA

Abstract: Visual search literature has long investigated the contributions of top-down and bottom-up processes in guiding search behavior. It is well-established that top-down attentional processes support the efficient discrimination of a target stimulus presented among distractors in serial search tasks. However, whether any proactive control mechanisms guide visual selection during parallel pop-out search remains unresolved. Here, we combined two attention paradigms: Covert Visual Search and Distraction Context Manipulation. While the former has been widely

used to study serial and parallel attention displacement, the latter was recently introduced to investigate proactive and reactive control mechanisms that minimize visual distraction (Marini et al., 2016, *Journal of Neuroscience*). We hypothesized that top-down processes intervene in parallel search and leveraged on the Distraction Context Manipulation to help characterizing the recruitment of top-down control processes in serial and parallel search. Accordingly, blocks of trials were of three types: Pure blocks, Parallel Search Mixed blocks, and Serial Search Mixed blocks. Pure blocks included 100% distractor-absent trials while Mixed blocks (Parallel and Serial Search) included 33% distractor-absent and 66% distractor-present trials each. Moreover, in Parallel Search Mixed blocks the target differed from distractors along a single feature (local contrast), while in Serial Search Mixed blocks the target differed from distractors based on the conjunction of two features (local contrast and orientation). Participants' task was to detect the target's presence or absence on each trial. The comparison of distractor-absent trials of Mixed versus Pure blocks allows detecting proactive control mechanisms. Results showed an enhancement of detection sensitivity and a slowing-down of reaction time (RT) when distractors were expected, yet not presented (distractor-absent trials of Mixed versus Pure blocks). This RT slowing-down was larger in Serial versus Parallel Search Mixed blocks. Thus, the expectation of distractors recruited a proactive process that improved detection sensitivity and entailed a RT cost in both types of search tasks, although its magnitude was modulated by the type of search. Finally, the magnitudes of RT-costs in Parallel and Serial Search Mixed blocks were positively correlated across-subjects (after controlling for other individual differences), thus suggesting a common underlying process. In conclusion, proactive control mechanisms appear to be recruited in both serial and parallel visual search and to be modulated by the type of search demanded by the task.

Disclosures: M.A. Petilli: None. F. Marini: None. R. Daini: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.26/JJJ54

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-MH071916

Veterans Administration VISN 22 MIRECC

Title: Amphetamine effects on a traditional and a cross-species variant of the continuous performance task

Authors: *D. A. MACQUEEN[1,2], J. W. YOUNG[1,2], M. A. GEYER[1,2], B. L. HENRY[2], A. MINASSIAN[2], W. PERRY[2];
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Abstract: Introduction: Cognitive impairment is a core component of numerous psychiatric conditions and is associated with poor functional outcome. At present, non-human studies are required to fully evaluate the neurobiological underpinnings of impaired cognitive processing. Yet, such efforts are hampered by a discontinuity of testing methods across species. The 5-choice Continuous Performance Task (5C-CPT) has been developed to allow for analogous assessment of attention in both rodents and humans. As with alternative CPT variants, impairments in 5C-CPT performance have been detected in psychiatric populations. To provide additional validation, the present study sought to evaluate the consistency of amphetamine (AMP) effects on 5C-CPT and Conner's CPT (CPT-II) performance.
Methods: Separate samples of healthy participants aged 18-35 were randomized to receive placebo, 10, or 20 mg of AMP before completing either the 5C-CPT (n = 54) or the CPT-II (n = 73).
Results: On the 5C-CPT, AMP impacted hit rate [$F(2,51) = 4.0$, $p < .05$] with both doses improving accuracy relative to placebo ($p < .05$). AMP also tended to improve D' on the 5C-CPT [$F(2,51) = 2.8$, $p = 0.072$], with 10 mg significantly improving performance compared to placebo ($p < .05$) and marginal improvements observed after 20 mg ($p < .1$). AMP did not impact hit rate on the CPT-II ($p > .05$) but, produced an effect on the ADHD confidence index [$F(2,51) = 4.0$, $p < .05$] with significant reductions observed at the 20 mg dose ($p < .05$). A significant interaction of dose x trial block was detected on D' for the CPT-II [$F(4,140) = 4.0$, $p < .05$]. After placebo, D' was significantly lower on the third and final block relative to the first ($p < .05$). However, this effect was not observed after either dose of AMP ($p > .05$) suggesting that the drug protected participants from vigilance deficits which emerge over time. Although no interaction between AMP and trial block was observed in the 5C-CPT for D' ($F < 1.1$, ns), only the placebo-treated subjects exhibited reduced D' in the third trial block compared with the first ($p < .05$).
Conclusion: The general consistency of amphetamine effects across these two tasks support the utility of the 5C-CPT for translational research. Critically, use of the 5C-CPT will facilitate identification of neural substrates for conditions characterized by impaired attention as captured by earlier CPT variants.
Funding Sources: Supported by VISN 22 MIRECC, and the National Institute of Mental Health (R01-MH071916)

Disclosures: D.A. MacQueen: None. J.W. Young: None. M.A. Geyer: None. B.L. Henry: None. A. Minassian: None. W. Perry: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.27/JJJ55

Topic: H.02. Human Cognition and Behavior

Support: NSF SBE Grant 1514246

NIGMS-NIH Grant P20GM103645

Title: Dissociable suppression of salient distractors during perception and goal-directed action

Authors: *J. D. MCCARTHY[1], J.-H. SONG[1,2];
[1]Dept. of Cognitive, Linguistic & Psychological Sci., [2]Brown Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: In a complex and continually changing environment, humans face the challenge of performing actions toward desired goals while ignoring other salient irrelevant stimuli. For instance, a successful hit in a baseball game requires attending the ball while tuning out the various sights and sounds on the field. While it is generally held that highly salient stimuli (e.g., camera flashes, the bright scoreboard) cause more perceptual interference than weaker ones, our lab recently demonstrated this is not the case for action. Specifically, the presence of a weakly salient singleton distractor was more detrimental to performance during goal-directed reaching compared to when a highly salient distractor was present (Moher, Anderson, & Song, 2015). The authors concluded that highly salient distractors are rapidly suppressed when action is required, leading to more efficient movements toward the target. Here, we used EEG to investigate the time course of attentional allocation during target search in the presence of highly and weakly salient distractors for perception and goal-directed action. Interestingly, we found that the amplitude of the N2pc—an ERP component in which greater negativity is observed at posterior electrode sites contralateral to the attended visual hemifield—was reduced on trials containing a high, compared to a low salient distractor during a perceptual task, suggesting increased competition in attentional selection. In contrast, the opposite pattern was observed when participants reached to the target: the presence of a highly salient distractor was associated with a larger N2pc amplitude relative to displays containing a weakly salient distractor when goal-directed action was required. These results suggest that consistent with the behavioral pattern of data, the efficiency of attentional selection is enhanced when highly salient distractions are present during action, but reduced during perceptual discrimination. Our results suggest that while high salience strongly interferes with perceptual processing, increased salience facilitates target selection during goal-directed action.

Disclosures: J.D. McCarthy: None. J. Song: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.28/JJJ56

Topic: H.02. Human Cognition and Behavior

Support: STaR investigator grant (NMRC / STaR /0015/2013)

Title: Effects of mindfulness training on behavior and electrophysiology in the Psychomotor Vigilance Test

Authors: *J. Z. LIM[1], K. WONG[2], J. TENG[2], M. W. CHEE[2], K. DOSHI[3];
[1]Neurosciences and Behavioral Disorders, Duke-Nus Med. Sch., Singapore, Singapore; [2]Duke-NUS Med. Sch., Singapore, Singapore; [3]Singapore Gen. Hosp., Singapore, Singapore

Abstract: Mindfulness, defined broadly as the awareness and non-judging acceptance of moment-to-moment thoughts and feelings, is gaining popularity as a method to improve general wellbeing and cognition. While a number of studies have shown benefits of mindfulness training on executive attention, studies of its effects on sustained attention have produced mixed results, motivating us to test the effects of an 8-week mindfulness training course on a robust and widely used assay of vigilance. 30 nurses from the Singapore General Hospital (28 female; mean (SD) age = 28.77 (7.74)) fully or partially completed an 8-week (1.5 hours/session) mindfulness-based training protocol (MBSR) based on the program created by Jon Kabat-Zinn. Participants came for 2 EEG recording sessions, before and after the program (S1 and S2 respectively). As most participants were not able to attend the entire program (mean sessions (SD) = 4.16 (2.42); range = 0-8), we used attendance as a moderator for the behavioral and EEG analysis. During EEG recording, participants performed the Psychomotor Vigilance Test (PVT), a 20-minute test of sustained attention, and rated their energy and mood before and after this test. As a manipulation check, trait mindfulness showed a marginally significant increase from S1 to S2 (t_{22} = -2.06. p = .052). Median response speed and slope of response speed on the PVT were compared between S1 and S2. Using attendance as a moderator, we found that median response speed was significantly altered post-program ($F_{1,28}$ = 25.07 ,p = 0.00003), with higher attendance associated with more improvement. No effects were found on slope of response speed. For subjective variables, we found a significant effect of session ($F_{1,28}$= 4.59, p = 0.04), as well as a marginally significant time-by-session interaction ($F_{1,28}$= 4.13, p = 0.052) on subjective sensations of energy. No effects on mood were found. P300 amplitude in response to PVT targets was calculated and compared across task (first 4 vs. last 4 minutes of the PVT) and session. There was a significant reduction in P300 amplitude with time ($F_{1,28}$= 8.32, p = 0.007), and a significant time-by-session interaction ($F_{1,28}$= 6.70, p = 0.02). Similar results were found for event-related desynchronization (ERD) to PVT events in the alpha band, with a significant

reduction in ERD over time ($F_{1,28}$= 6.27, p = 0.02), and a time-by-session interaction ($F_{1,28}$= 4.67, p = 0.04). Overall, these results suggest that mindfulness training benefited sustained attention by sharpening attention to relevant stimuli via suppression of background neural noise, particularly for participants who attended a majority of the training program.

Disclosures: J.Z. Lim: None. K. Wong: None. J. Teng: None. M.W. Chee: None. K. Doshi: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.29/JJJ57

Topic: H.02. Human Cognition and Behavior

Support: National Science Foundation BCS-1534823

Title: Intrusive effects of task-irrelevant semantic relationships between real-world objects

Authors: *J. C. NAH[1], G. L. MALCOLM[2], S. SHOMSTEIN[1];
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Abstract: Every object encountered in the environment consists of a combination of low-level properties (e.g., colors and shapes) as well as high-level semantic relationships. Past research has provided evidence emphasizing the importance of semantic information when it benefits the current goal (Moores, Laiti, & Chelazzi, 2003). However, the pervasive nature of semantic information suggests a continuous semantic bias of attentional allocation independent of task relevance. Accordingly, recent data from our lab provides evidence that task-irrelevant semantic properties influence attentional allocation (Malcolm & Shomstein, 2016). Here, we utilized univariate and multivariate methods (pattern classification) to assess the degree to which semantic relatedness influences sensory responses in the early visual cortex (EVC: V1-V3), object-sensitive lateral occipital complex (LOC), and the inferior parietal sulcus (IPS0-2). If task-irrelevant semantic relationships modulate neural activity via object selective regions, then semantic-based modulation should be observed not only in spatially selective IPS0-2, but also in LOC. If, on the other hand, semantic relationships influence spatial allocation of attention exclusively, then modulatory effects will be observed only in IPS0-2. For the multivariate analyses, decoding of object identity in the EVC, LOC and IPS was examined with a hypothesis that increased attentional allocation leads to an increased pattern classification accuracy. Participants viewed three objects, with one just above the central fixation and one in each

periphery, below the midline. In one condition, one of the peripheral objects was semantically related to the central object. In the control condition, all three objects were semantically unrelated. After the objects were presented, a target and two distractors were superimposed on top of each object. Most importantly, the target appeared on all three objects equally, rendering semantic relatedness task-irrelevant. Behavioral performance supported the conclusion that task-irrelevant semantic relatedness influenced attentional allocation represented by faster RTs and greater accuracy for targets positioned on the semantically related objects. A semantic-based modulation throughout the EVC as well as IPS was observed. EVC and IPS also showed significantly higher decoding accuracy of object identity when objects were semantically related than when semantically non-related, while no difference was seen in the LOC. Combined, these results demonstrate that semantic relationships between objects modulate visual cortical activity via the parietal cortex.

Disclosures: J.C. Nah: None. G.L. Malcolm: None. S. Shomstein: None.

Poster

266. Human Cognition: Attention I

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 266.30/JJJ58

Topic: H.02. Human Cognition and Behavior

Title: Decrease in the visual search & spatial working memory function accompanying Long-term Consecutive Visual Search

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Abstract: We investigated the influence of a long-term consecutive visual search (LTCVS) on a cerebral activity during visual search and spatial working memory (VSSWM). 10 subjects performed VSSWM tasks before and after LTCVS tasks. As for the LTCVS and VSSWM, the Advanced Trail Making Test Random Task (ATMT Task R) and the Advanced Trail Making Test Fixed Task (ATMT Task F) was introduced respectively, in which a subject, using a computer mouse, clicked a circle with the numbers from 11 to 40 in serial order. Once a numbered target was clicked, it was to disappear and another circle with the clicked number plus 30 showed up at the same time. In the Task R all the circles were rearranged at random, while the circles were not rearranged in the Task F; 30 circles were on the screen at any time in both tasks. Each subject performed Task R 40 times and Task F 10 times. The performance time, the error frequency and the electroencephalogram power values of the 13-30 Hz (beta power) before

and after the LTCVS are compared. It was clarified that the performance of the Task F impaired and the beta power of F3 and F7 electrodes more significantly increased after the LTCVS than before the LTVS. These results suggested that the LTCVS increased the activities of distraction and impaired the activities of the area.

Disclosures: K. Oki: None. R. Koshizawa: None. M. Takayose: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.01/JJJ59

Topic: H.02. Human Cognition and Behavior

Title: Removing the curse of dimensionality: a trade-off between adaptability and precision

Authors: *S. GHAANIFARASHAHI, K. ROWE, Z. ASLAMI, A. SOLTANI;
Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Learning from reward feedback is essential for survival but can become very challenging in naturalistic conditions where choice options have many features (e.g. color, shape, texture) and each feature can take many values, resulting in a large number of options whose reward values have to be learned. This challenge is referred to as the "curse of dimensionality," since many value-based learning models (e.g. reinforcement learning) do not scale up as the dimensionality of the environment and thus the number of possible options increase. We hypothesize that humans tackle this problem by adopting models of the environments that reduce the dimensionality and allow faster learning (model-based approach). We therefore designed a series of experiments to identify factors that influence such model adoption over the slower, but more precise model-free approach, which learns individual values for options. Moreover, we constructed different computational models and tested alternative learning architectures for solving the curse of dimensionality. During these experiments, subjects selected between pairs of options that had different features and would yield reward with different probabilities. We found that when faced with a volatile environment in which reward contingencies changed frequently, most subjects adopted a model-based approach, enabling them to learn quickly. However, when we introduced inconsistencies that prevented reward probabilities for all options to be determined based on their feature values, subjects adopted a model-free approach. The presence of inconsistencies prompted a model-free approach even when we increased the dimensionality of the environment. Finally, we found that these results are compatible with a hierarchical decision-making and learning process where the best sources of information (in alternative models of the environment) were used to make decisions and successively updated according to

the reward outcome. Overall, our results reveal that a novel trade-off between adaptability (dealing with the volatility in the environment) and precision (dealing with inconsistencies in the reward structure) determines how humans adopt a model of the environment to reverse the curse of dimensionality.

Disclosures: S. Ghaanifarashahi: None. K. Rowe: None. Z. Aslami: None. A. Soltani: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.02/JJJ60

Topic: H.02. Human Cognition and Behavior

Title: Timing the onset of the decision to move in arbitrary and deliberate decisions

Authors: *N. ZIARI[1], S. WONG[2], M. SAMAD[2], U. MAOZ[2];
[1]UCLA, Los Angeles, CA; [2]UCLA, LOS ANGELES, CA

Abstract: In its most general form, the Libet paradigm instructs subjects to arbitrarily move their left or their right hand, at a time of their choice, and report the time of the onset of their decision to move--henceforth W time--using a clock. It was shown that predictive information about upcoming action exists in the brain before W time. This led some to assert that conscious intentions play no part in the causal chain leading to action, rendering free will and moral responsibility illusory. However, free will and moral responsibility focus on deliberate decisions. So it is crucial to understand to what extent the Libet results extend to deliberate decisions. This was the focus of our investigation.
In the first part of the experiment, subjects tasted drinks and rated their favorability. In the second part of the experiment, subjects were presented with two randomly selected drinks and a rapidly changing stream of letters. The subjects then select a drink using the keyboard and reported the letter that was on the screen at the moment they decided which drink to select. The experiment included 3 types of decisions, in a randomly counterbalanced blocked design, with 10 trials per block. Subjects were informed that one trial per block would be selected and they would have to drink from the drinks presented in that trial, depending on the trial type. In deliberate-decision blocks, subjects were instructed to select the drink they preferred. To motivate them to deliberate, they were informed that they would only need to drink from the drink they chose on the selected trial at the end of the block. In arbitrary-different subjects were told that, regardless of their selection, they would have to drink both drinks in the selected trial, at the end of the block. In the arbitrary-same blocks, subjects were presented with the same drink twice, again motivating arbitrary selection. To ensure that subjects were paying attention

throughout the experiment, we randomly introduced catch trials after some trials. There, subjects were required to identify which of the presented drinks was presented in the previous trial. If they answered incorrectly, they had to taste one of their least favorite drinks.
We observed clear and notable differences among the W time distributions for the 3 types of decisions, where the deliberate-decision block consistently resulted in earlier W time values compared to the other 2 arbitrary block types. This challenges the generalizability of the Libet results for arbitrary and deliberate decisions and, with that, the validity of the claims that free will and moral responsibility are illusory.

Disclosures: N. Ziari: None. S. Wong: None. M. Samad: None. U. Maoz: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.03/JJJ61

Topic: H.02. Human Cognition and Behavior

Support: Hermann and Lilly Schilling foundation

Graduate School for Neurosciences, Biophysics, and Molecular Biosciences (GGNB)

Title: Modality specific neural signals of evidence accumulation for spatial decisions

Authors: *A. NAZZAL[1], C. SCHMIDT-SAMOA[1], M. HOLZGRAEFE[2], M. BÄHR[1], J. ERLICH[3], M. WILKE[1];
[1]Cognitive Neurol., Univ. Med. Ctr. Göttingen, Goettingen, Germany; [2]ASKLEPIOS Hosp. Schildautal, Seesen, Germany; [3]Inst. of Brain and Cognitive Sci., Shanghai, China

Abstract: To successfully navigate through the environment, organisms need to be able to receive, accumulate, and integrate spatial information. It is proposed that sensory information is processed in modality-specific sensory regions under the influence of fronto-parietal cortices leading to the formation of spatial decisions. However, since most decision studies in humans have been performed in a single sensory modality and with speeded motor responses, it remains unclear whether brain regions accumulate modality-specific sensory evidence and/or evidence for an action. The goal of the study was to 1) identify brain regions that are involved in modality-specific accumulation of sensory evidence and 2) test the causal contribution of parietal cortices to evidence accumulation in the visual and auditory modality. To this end, we performed an event-related fMRI study in 17 healthy human subjects and tested patients with lesions in parietal cortices using a spatial perceptual accumulation task in both sensory modalities. Stimuli were

presented discretely over time and space and we asked subjects to respond with a button press, deciding whether a trial had more stimuli on the right or on the left side. The discreetness of the stimuli allowed us to fit a quantitative model that captures the dynamics of evidence accumulation during the decision formation (Brunton et al., 2013). For fMRI analysis, for each trial we used the predicted signed evidence to create a parametric regressor representing the decision variable of interest and the absolute of predicted evidence to create a parametric regressor representing the task difficulty regardless of space. In patients' behavioral analysis, we estimated spatial bias and slope by fitting a sigmoidal curve to the patients' ipsilesional choices. Regions correlating with the signed evidence were occipital cortex for the visual task, and superior temporal gyrus for the auditory task. In contrast fronto-parietal cortices did not correlate with the signed evidence but with the absolute evidence in both modalities, i.e. increased activity as the decision became harder irrespective of spatial location in both modalities. Consistent with our fMRI predictions, patients with unilateral lesions in parietal cortices exhibited a slope (i.e. performance) decrease in the visual and auditory tasks. Patients exhibited a spatial bias towards deciding that there were more stimuli presented on the ipsilesional side, which was not predicted by the fMRI data. Our data suggests that sensory evidence accumulates in modality-specific sensory cortices, while supramodal activity in parietal cortices might reflect evidence for action choice.

Disclosures: **A. Nazzal:** None. **C. Schmidt-Samoa:** None. **M. Holzgraefe:** None. **M. Bähr:** None. **J. Erlich:** A. Employment/Salary (full or part-time): NYU-ECNU Institute of Brain and Cognitive Science, New York University Shanghai, Shanghai, China. **M. Wilke:** None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.04/KKK1

Topic: H.02. Human Cognition and Behavior

Title: Wide-eyed and wrong? Pupil dilation correlates with imperfect evidence accumulation in auditory perceptual decisions

Authors: *T. HAGEN, R. C. WILSON;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: The temporal integration of evidence is crucial for effective decision making. For simple perceptual decisions, a large body of work suggests that humans and animals are able to perform such temporal integration in a near-optimal manner (e.g. Brunton et al., 2013). Yet other work suggests that such evidence integration is subject to neuromodulation (Cavanagh et al.,

2014). In this work we set out to investigate this modulation in more detail by measuring pupil dilation (a putative correlate of norepinephrine) in 68 humans making perceptual decisions.
In particular, we used a modified version of the Poisson Clicks Task in which participants hear a sequence of clicks in their left and right ears and must decide which ear received the most clicks. Optimal decision making in this task requires the temporal integration of evidence and prior work has shown that both humans and rats can perform this task remarkably well (Brunton et al., 2013).
Using a simple regression analysis we were able to compute the relative contribution of each click to the decision and show how this contribution was modulated by pupil diameter from trial to trial.
Our results show that, on average, people did not perfectly integrate information in this task. We found that participants showed a pronounced primacy effect, with earlier clicks receiving about 50% more weight than later clicks. Moreover, the weights of later clicks were negatively correlated with pupil diameter: high pupil diameter was associated with lower weights and greater primacy effect, while low pupil diameter was associated with higher weights and a weaker primacy effect. These findings suggest a role for norepinephrine in modulating the temporal integration of information, with near-perfect integration only possible when norepinephrine levels are low.
Brunton, B. W., Botvinick, M. M., & Brody, C. D. (2013). Rats and Humans Can Optimally Accumulate Evidence for Decision-Making. Science, 340(6128), 95-98. http://doi.org/10.1126/science.1233912
Cavanagh, J. F., Wiecki, T. V, Kochar, A., & Frank, M. J. (2014). Eye tracking and pupillometry are indicators of dissociable latent decision processes. Journal of Experimental Psychology. General, 143(4), 1476-1488. http://doi.org/10.1037/a0035813

Disclosures: T. Hagen: None. R.C. Wilson: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.05/KKK2

Topic: H.02. Human Cognition and Behavior

Support: NSFC Grant 31571117

Funding from Peking-Tsinghua Center for Life Sciences

NSFC Grant 31322022

NSFC Grant 31371019

Title: Humans represent visuo-spatial probability distribution as k-means clusters

Authors: *J. SUN[1], J. LI[1,2], H. ZHANG[1,2,3];
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Abstract: The reward for a real-world choice is often randomly distributed on a continuum (e.g. the future price of a stock). Many behavioral and neuroimaging studies have shown that human decisions are sensitive to the statistical moments (mean, variance, etc.) of reward distributions. However, little is known about how reward distributions—or, probability distributions in general—are represented in the human brain. When the possible values of a probability distribution is numerous (infinite for a continuous distribution), it would be unrealistic or at least cognitively costly to maintain the probability for each possible value. Here we explored potential heuristic representations of probability distributions and tested them on human subjects. In particular, we tested a recently developed hypothesis that human representations of probability distributions are mixtures of a small number of non-overlapping basis distributions, which can potentially reduce the computational load of probabilistic calculations as well as alleviate the cognitive load.
Methods: In two experiments, we constructed a variety of multimodal distributions of spatial positions. On each trial, 70 vertical lines—the horizontal coordinates of which were samples independently drawn from the distributions—were briefly presented, one at a time on the computer screen. Human subjects were asked to locate (on the axis where stimuli were presented) the mean and the mode of the samples. A total of 19 naïve subjects participated and completed 144-162 trials each.
Results: All subjects' mean and mode responses were highly correlated with the true means and modes of the samples. Interestingly, all subjects' mean and mode responses had systematic deviations from the true means and modes. The deviation patterns could be well predicted by computational models that assume a division of samples into a small number of clusters following the k-means clustering algorithm. Only the centroid and the relative weight of each cluster were necessary for the further calculation of mean and mode responses.

Disclosures: J. Sun: None. J. Li: None. H. Zhang: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.06/KKK3

Topic: H.02. Human Cognition and Behavior

Title: Discrimination of brightness biased by flicker rate in alpha frequency range

Authors: *J. K. BERTRAND[1], N. J. WISPINSKI[2], D. L. CORMIER[2], A. SINGHAL[2], K. E. MATHEWSON[2], C. S. CHAPMAN[1];
[1]Fac. of Physical Educ. and Recreation, [2]Dept. of Psychology, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Work from over a hundred years ago (Brücke, 1867) showed that a single light flickering at certain frequencies was perceived as brighter than a constant light despite having only half the actual intensity. Notably, the single light frequencies with the greatest difference between perceived and actual luminance occurred within the alpha frequency range (8 to 12 Hz). Recently, entrainment of the alpha frequency has been shown to modulate attention and bias detection of stimuli. We believe the previously reported brightness advantage for flickering stimuli may therefore be causally related to alpha oscillations. To test this, we employed electroencephalography (EEG) and behavioural analysis to investigate how the discrimination of brightness difference across two flickering stimulus may be influenced by their relative frequencies and how oscillatory activity in the alpha frequency may play a part in this biased perception.
Participants were shown two circular stimuli, flashing on either side of a fixation cross. Both stimuli could flicker at either 9.23 or 12 Hz, and both could be either lighter or darker. Before the experiment, light and dark brightness levels were established through a staircase procedure to approximate when a participant perceived one target as brighter 75% of the time. During the experiment, an arrow at fixation cued participants to make a yes/no keyboard response if the cued stimulus was brighter (or darker, counterbalanced) than the other stimulus. Based on the Brücke-effect, we hypothesized that stimuli at 9.23 Hz would appear brighter than those at 12 Hz.
Behaviorally we found a main effect of brightness and, most importantly, a main effect of frequency, with no interaction. As predicted, the slower oscillating target was perceived as brighter than the faster oscillating target more than 70% of the time, even when its true luminance was equivalent. Overall, we were able to powerfully bias brightness discrimination solely by manipulating relative stimulus frequency.
We predict EEG analysis will reveal a third main effect of alpha power where a difference in alpha power between hemispheres will correlate with biased brightness discrimination. Specifically, we predict that greater alpha power in one hemisphere will correspond to a decrease

in attention and subsequently decrease the perceived brightness of the contralateral stimulus. We believe that by uncovering how these neural oscillations affect perceptual judgements, we may be able to extend our understanding of, and ability to bias, value-based judgments.

Disclosures: J.K. Bertrand: None. N.J. Wispinski: None. D.L. Cormier: None. A. Singhal: None. K.E. Mathewson: None. C.S. Chapman: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.07/KKK4

Topic: H.02. Human Cognition and Behavior

Title: The tuning of reflexes to environmental risk

Authors: *A. DUNNING, M. BERTUCCO, T. SANGER;
USC, Los Angeles, CA

Abstract: In order to characterize and emphasize the influence of risk in the environment on human movement, we have designed a series of experiments. In previous work, we have shown that humans continuously adapt their movement trajectory to compensate for perceived environmental risk. Here we test whether they also adapt long and short-latency stretch reflexes to minimize the effect of perturbations in a risky environment. Many studies have demonstrated that humans have the ability to modulate the long latency stretch reflex based on the goal of a task, such as in a go-don't-go paradigm. It is our hypothesis that awareness to risk is so fundamental, that humans also maintain reflexes tuned specifically to the risk of the environment, even when the goal of the task remains constant.
In the experiment, subjects were positioned in front of a monitor with their arm strapped to a manipulandum designed to apply torque at the elbow joint while maintaining all other arm joints immobile. The subject's hand gripped a rigid joystick attached to the arm of the manipulandum that controlled a cursor horizontally on the screen. The monitor displayed three equidistant rectangles, two cost regions on either side of a center reward region. Subjects were instructed to maximize points by keeping the cursor within the center reward region while avoiding the cost regions. Nine cost functions were evaluated, all combinations of no penalty, low penalty, and high penalty, in order to evaluate the effect of both symmetric and asymmetric risk. Thus the goal of the task was always to remain in the center target, but the risk of hitting the penalty regions was varied. The robot generated a constant 1 N force (activating the bicep) and randomized 3 N perturbations in both directions (in addition to baseline force) at a mean rate of 5 seconds. Surface electromyography from the bicep and triceps were recorded and filtered. Only

trials in the direction that provoked the bicep stretch reflex were analyzed. Reflex response was categorized into standard epochs for baseline, short latency, long latency, and voluntary response. The filtered EMG within each epoch was averaged to a single value per trial for analysis.
Results demonstrate a difference in the long latency stretch reflex dependent on the risk of the environment. However, results were only clear for the symmetric cost conditions; subjects did not demonstrate a consistent ability to set separate reflex responses for different directions when the direction of perturbation was unplanned. Therefore, results suggest that humans do plan for error by tuning reflexes to the risk of the environment and that they do this independent of the goal of the task.

Disclosures: A. Dunning: None. M. Bertucco: None. T. Sanger: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.08/KKK5

Topic: H.02. Human Cognition and Behavior

Support: IFT-29

ISF-93/15

Title: Comparing visual and tactile Simon effects: a model-based analysis

Authors: *Y. SALZER[1,2], G. DE HOLLANDER[1], L. VAN MAANEN[1], M. SALTI[2], B. U. FORSTMANN[1];
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [2]Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel

Abstract: Quickly adapting to a changing environment is crucial for everyday behaviours. Conflict tasks such as the Simon task have been extensively used to study the temporal dynamics of conflict resolution between relevant and irrelevant stimulus-response mappings. In this task, participants discriminate between a stimuli's relevant feature (e.g., shape), by responding with the left or right hand. The Simon effect finds that response times (RTs) decrease when a stimulus' location is congruent with the response hand. RT distribution analyses have shown that the Simon effect fades over time, i.e., the differences in RTs between congruent and incongruent stimuli decrease as RT increases. The aim of the present study was to examine the neural substrates underlying latent cognitive processes involved in the resolution of spatial stimulus-

response conflict in visual and tactile domains using a model-based approach. The results are in line with the spontaneous decay account of the fading Simon effect and shed light on the modal specificity of processing conflict.
Nineteen participants undertook functional magnetic resonance imaging (fMRI) while performing a tactile and visual Simon task. In the visual task, participants were asked to discriminate between a rectangle and triangle, presented either on the left or right side of a centrally-presented fixation cross. In the tactile task, participants were instructed to discriminate between a pulse or continuous vibration presented on the left or right of the torso, delivered with a vibrotactile belt.
The fMRI data was subjected to a multivariate pattern analysis (MVPA) in regions-of-interest in the visual and somatosensory cortex. For unseen data, the MVPA predicted every trial as having a right or left stimulus presentation with a degree of similarity to seen right and left data classes. We found that trials in which MVPA classified the stimulus location to a higher degree also showed a larger Simon effect. This suggests that the sensory representation of the irrelevant stimulus feature is what drives, at least in part, the magnitude of the Simon effect. The irrelevant stimulus feature could not be decoded in tactile trials. In an ongoing study we aim to extract more distinct neural patterns in the visual and somatosensory sensory areas by using optimised stimulus presentations.
Our results show that the processing of irrelevant stimulus information in visual areas predicts the temporal dynamics of resolving response-conflict in the visual Simon task. This finding is in line with the spontaneous decay account, which stresses the influence of sensory processing on the difference between congruent and incongruent trials.

Disclosures: Y. Salzer: None. G. de Hollander: None. L. van Maanen: None. M. Salti: None. B.U. Forstmann: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.09/KKK6

Topic: H.02. Human Cognition and Behavior

Title: Perceptual confidence: a true reflection of objective human visual discrimination?

Authors: *L. ZIZLSPERGER[1], L. MEISSAUER[2], T. HAARMEIER[3];
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Abstract: Visual perception has been described as a process of probabilistic inference in which decision confidence contributes to successful choice formation and enactment[1]. Recently we demonstrated that while perceptual sensitivity and confidence in it increased with training, bias-free metacognitive sensitivity (the „precision“ of the metacognitive evaluation) did not[2]. For visual perception in healthy human subjects here we investigated how these three descriptions of perceptual performance vary under more difficult perceptual conditions. 26 emmetropic subjects performed two visual paradigms while presentation time was varied and / or visual acuity was diminished by the temporary inroduction of +2 dpt refraction lenses. In order to allow for comparisons between different stimulus modalities[3], we presented a random dot kinematogram (RDK) in the first and a Landolt C in the second task.
For the experimental myopia we found perceptual performance and the confidence in it to decrease significantly with the introduction of the refraction manipulation in the Landolt, but not in the RDK paradigm. The reduction of the presentation time significantly impaired objective and subjective performance for both visual stimuli. Metacognitive sensitivity evolved in parallel with the two other behavioral measures in both tasks.
In summary, we did not observe a dissociation of these three different performance measures - even for the combination of artificial myopia and short stimulus presentation - but in all experimental variations subjective and objective performance measures went in parallel. We were not surprised to see the changes in type-1 (perceptual) sensitivity differ between the two visual submodalities. But within each experimental condition, combining two presentation times with two different tasks, perceptual confidence and type-2 (metacognitive) sensitivity was observed to be a true reflection of the perceptual performance. In other words, experimental visual impairment by incorrect refraction not only changed objective behavioral performance for the worse, but also the metacognitive evaluation of one own’s perceptual efficiency. The findings suggest that type-1 and type-2 processes operate on the same input, possibly even sharing their neuronal implementation.
1.Pouget A et al. Confidence and certainty: distinct probabilistic quantities for different goals. Nat Neurosci. 2016; 2.Zizlsperger L et al. Metacognitive Confidence Increases with, but Does Not Determine, Visual Perceptual Learning. PLoS One. 2016; 3.Livingstone M and Hubel D. Segregation of form, color, movement, and depth: anatomy, physiology, and perception. Science. 1988.

Disclosures: L. Zizlsperger: None. L. Meissauer: None. T. Haarmeier: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.10/KKK7

Topic: H.02. Human Cognition and Behavior

Title: How does the brain infer the shape of an object based on hand pose and skin contacts?

Authors: *F. MEHRABAN POUR BEHBAHANI, G. SINGLA-BUXARRAIS, A. FAISAL; Imperial Col. London, London, United Kingdom

Abstract: A hallmark of human cognition is the ability to rapidly and accurately assign meaning to sensory stimuli. Even without visual feedback, humans can determine the shape of objects solely on the basis of haptic feedback. This feat is achieved despite large variability in sensory and motor estimation of hand pose and object location. The human hand can take advantage of rich dynamics in object interaction, but capturing the data and interpreting it with regards to well-defined physics models is challenging and has not been done in a systematic way. A principled way to understand the underlying neural computations is through first-principle Bayesian ideal observer models which have been very successful in explaining human visual, auditory and multi-sensory perception, yet have rarely been applied in the haptic domain. We propose a Bayesian perceptual model for recursive integration of noisy proprioceptive hand pose with noisy contact point information on the surface of the skin. The model simultaneously forms an optimal representation of a haptically explored object and estimates the true hand pose within that space. We present a proof-of-principle reconstruction of object shape from multi-finger tactile exploration with contact points reconstructed using a physics engine. The algorithm presented allows us to predict in real-time, from motion capture data in human experiments, an optimal Bayesian estimate of the object shape, given the same sensory information. Therefore, our work provides a framework for a principled study of human haptic exploration of complex objects in a similar principled manner that transformed vision research.

Disclosures: F. Mehraban Pour Behbahani: None. G. Singla-Buxarrais: None. A. Faisal: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.11/KKK8

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust

Title: The role of time in perceptual decision-making

Authors: *D. M. ZOLTOWSKI[1], Á. KOBLINGER[2], J. FISER[2], M. LENGYEL[1];
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Abstract: In perceptual decision-making, neural representations of environmental stimuli and uncertainty must be computed on short time scales. Here, we formulate two opposing probabilistic models in which stimulus time presentation is used for either evidence integration or probabilistic sampling. In evidence integration, time is used to iteratively collect evidence and update a posterior distribution over an environmental variable given the stimulus. Crucially, we assume that the posterior distribution is changing as evidence is integrated and it is represented exactly at each time point. Alternatively, under the sampling hypothesis time is used to approximately represent the posterior distribution by sequentially sampling from a static distribution. We devised a novel orientation-estimation task in which subjects reported their estimate of the orientation of a line segment as well as their uncertainty associated with their estimate, and we developed intuitive as well as formal theoretical predictions of how humans should behave in this task under the two models. On each trial of the task, we obtained a subjective measure of uncertainty along with a true measure of error. Both the evidence integration model and probabilistic sampling model predicted the error and uncertainty to decrease as a function of stimulus presentation time. The factor that distinguished the two models was the relationship between error and uncertainty. As a function of stimulus presentation time, under evidence integration we predicted the across-trial correlation between error and uncertainty to remain constant while under probabilistic sampling we predicted the across-trial correlation to increase for longer presentation times. The key insight is that under probabilistic sampling the representation of uncertainty becomes increasingly accurate through time as the number of samples increases. Finally, we collected data from human subjects completing our orientation-estimation task and obtained initial evidence supporting the probabilistic sampling model.

Disclosures: D.M. Zoltowski: None. Á. Koblinger: None. J. Fiser: None. M. Lengyel: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.12/KKK9

Topic: H.02. Human Cognition and Behavior

Support: CIHR grant MOP-115197

NSERC grant 327317-11

Title: Perceptual decoupling is not the same as mind-wandering: Using experience sampling to measure the dynamics of thought

Authors: *Q. RAFFAELLI[1], A. C. HERRERA-BENNETT[2], K. CHRISTOFF[2];
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Abstract: A prominent view of mind wandering defines it is a "shift in the contents of thoughts away from an ongoing task and/or from events in the external environment to self-generated thoughts and feelings" (Smallwood & Schooler, 2016, p488). This definition, however, fails to capture a crucial phenomenological characteristic of the wandering mind: its dynamic quality, including the tendency of the wandering mind to move in an unconstrained fashion (free movement) and to cover a broad conceptual scope (conceptual variability). Here we developed novel experience sampling items aiming to capture these so far neglected dynamic aspects of mind wandering. Participants answered several thoughts probes sent at random times within regular time intervals throughout the days during the course of several days. We were specifically interested in how freedom of thought's movement relates to the extent to which thoughts are perceptually coupled with the environment. Results suggest that being decoupled from (or unaware of) the external environment is not a significant predictor of the felt spontaneity of thoughts' movement. In addition, having one's thoughts related to the immediate surrounding was only marginally predictive of the spontaneity of thoughts' movement. Finally the predictive power of attending to something in the immediate surrounding disappeared when task relatedness was added as a predictive variable. Therefore, the degree of perceptual coupling with the environment appears relatively independent of the felt spontaneity of thought's movement, suggesting that freedom of movement captures something about the wandering mind that perceptual decoupling does not.

Disclosures: Q. Raffaelli: None. A.C. Herrera-Bennett: None. K. Christoff: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.13/KKK10

Topic: H.02. Human Cognition and Behavior

Support: HealthPAC (FP7-PEOPLE-2013-ITN)

Title: Age effects on visual perceptual decisions of ambiguous stimuli

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Abstract: The brain is constantly making choices while interpreting the environment. To understand how age affects visual decision-making, we investigated age-related changes in spontaneous percept switches and percept choices during intermittent presentations of ambiguous stimuli. Spontaneous switches can be triggered by different visual stimuli, such as monocular ambiguous visual stimuli or binocular rivalry images. An ambiguous visual stimulus has multiple and equally plausible interpretations, such as the bi-stable rotating sphere. In such a sphere two transparently moving dots are moving in opposite directions and due to structure-from-motion the stimulus is perceived as a 3-dimensional rotating sphere moving in one or the opposite direction. During binocular rivalry experiments, the left and the right eye receive different input simultaneously. During stimulus-presentation only one of the two presented images is perceived, and the other image is suppressed. Dominance durations (the time a percept remains dominant) are typically in the order of several seconds. In this study, 52 observers ranging from 17 to 72 years old, viewed bi-stable rotating spheres and binocular rivalry stimuli and were forced to make a choice between two percepts. Stimuli were presented continuously for 2 minutes or intermittently for 1 second, with a range of inter-stimulus intervals (0.125 - 2 seconds). The results show that dominance durations during continuous viewing are longer for older subjects for the binocular rivalry stimulus but not for the bi-stable rotating spheres. For the intermittent stimulus presentation, perceptual alternations decrease at an older age in binocular rivalry, while for the bi-stable rotating sphere there are only differences in perceptual alternations among different age groups at a short off-duration. Based on these results, we conclude that the effect of age is not a general phenomenon for ambiguous stimuli. Visual decisions are more stimulus dependent, rather than experience dependent.

Disclosures: E. Arani: None. R. van Ee: None. R. van Wezel: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.14/KKK11

Topic: H.02. Human Cognition and Behavior

Support: Neukom Institute CompX Grant

Title: Contributions of neural adaptation to value-based and perceptual choice

Authors: *O. HORNO[1], M. SPITMAAN[2], A. SOLTANI[2];
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Abstract: Despite the prevalent use of two-alternative choice paradigms to study different types of decision making, real-life decisions often involve selection among a set of options. The set of options, including the irrelevant options, can alter decision-making processes and result in preference reversal between the relevant options. This set of phenomena, referred to as context or decoy effects, has been extensively studied in value-based choice and has revealed important aspects of valuation and decision processes. Recently, we have proposed that trial-by-trial adjustments of neural representations to the set of presented options can account for some context effects (Soltani et al., 2012). Interestingly, a new study has shown that context effects can also arise in perceptual decision making (Trueblood et al., 2013). Considering the prevalence of neural adaptation in most brain areas, we hypothesized that neural adaptation could contribute to both value-based and perceptual context effects. To test this hypothesis, we conducted an experiment to measure context effects in two different tasks using a within-subject design. In one task, human subjects selected between monetary gambles with different reward probabilities and magnitudes in order to maximize payoff (value-based task). In another task, subjects chose the line with the longest shadow out of a set of slanted lines, and were rewarded based on the size of the shadow (perceptual task). In both tasks, however, subjects were shown three options at the beginning of each trial, and one was removed at the time of choice (phantom decoys). Finally, in order to test the effects of neural adaptation on longer timescales, we measured context effects after increasing the range of option values in another value-based task (high-range manipulation). Firstly, we found within-subject decoy effects for both value-based and perceptual decision-making tasks. However, in the perceptual task, the decoys were effective only when presented next to the more horizontal line. Secondly, we found a significant correlation between the overall decoy effects in the two tasks, indicating that similar trial-by-trial adaptation could underlie context effects in two different types of decision making. Finally, we did not find any difference between decoy effects during the main experiment and high-range manipulation, illustrating that adaptation on longer timescales may not contribute to context effects. Overall, our results extend previous findings on the role of neural adaptation in sensory processing to the realm of irrational choice behavior, and further indicate that adaptation contributes to different types of decision making.

Disclosures: O. Horno: None. M. Spitmaan: None. A. Soltani: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.15/KKK12

Topic: H.02. Human Cognition and Behavior

Support: Government of Ireland Postdoctoral Fellowship GOIPD/2015/481

Royal Society

ASUR (1014-C4-Ph1-071)

COST Action Open Multiscale Systems Medicine (OpenMultiMed) supported by COST (European Cooperation in Science and Technology)

Northern Ireland Functional Brain Mapping Facility (1303/101154803) funded by InvestNI and the University of Ulster

Title: Eye-hand interaction in perceptual decision making

Authors: A. ZGONNIKOV[1], *K. WONG-LIN[3], P. T. PIIROINEN[2], D. O'HORA[4];
[1]Sch. of Psychology, and Complex Systems Res. Ctr., [2]Sch. of Mathematics, Statistics & Applied Mathematics, and Complex Systems Res. Ctr., Natl. Univ. of Ireland, Galway, Galway, Ireland; [3]Ulster Univ., Derry-Londonderry, United Kingdom; [4]Sch. of Psychology, and Complex Systems Res. Ctr., Natl. Univ. Ireland, Galway, Galway, Ireland

Abstract: Tracking eye movements during decision making has provided numerous insights into possible mechanisms of attention and evidence accumulation preceding the actual choice. In a parallel stream of research, mouse/hand trajectories during choice execution are analysed under the assumption that cognition can “leak” into motor output, thereby leaving traces of decision maker’s thought process. At the same time, possible interactions between eye and hand dynamics during decision making remain largely unexplored. This study is the first one to report joint analysis of synchronized eye-tracking, pupilometry, and mouse-tracking data during decision making. We employ a well-known motion discrimination task (“random dots” paradigm) to investigate: (1) the basic patterns of eye-hand coordination during decision making; (2) how these patterns change with task difficulty (that is, motion coherence); and (3) individual differences in dynamics of such simple decision making, and possible relation of these differences to subjects’ personality (as assessed by BIS/BAS, PANAS, and Jackson-5 scales).

Disclosures: A. Zgonnikov: None. K. Wong-Lin: None. P.T. Piiroinen: None. D. O'Hora: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.16/KKK13

Topic: H.02. Human Cognition and Behavior

Support: ARO Contract W911NF-09-D-0001, subaward KK1323

Title: EEG-based classification of intended responses in a multi-modal crewstation simulation

Authors: *P. M. CONNOLLY, S. SIMONS, K. ZACHERY, D. KRNAVEK;
Teledyne Scientific and Imaging, LLC, Durham, NC

Abstract: Brain-Computer Interfaces (BCIs) show great promise in augmenting people's abilities. Non-invasive approaches such as EEG, have two fundamental control signals. The first is direct control which uses scalp potentials to decode the details of motor execution (position, velocity, angle, etc.) measured in primary motor cortical areas. The alternative control approach is goal selection. In a goal-directed BMI, goals are selected by detecting neural signals associated with the user's behavioral goals, while the machine enacts the details normally controlled by fine-grain motor commands. To develop our novel goal-directed BMI, we tested the ability to decode the intent of subjects performing identification tasks in a simulated crewstation of a military vehicle. Since crewstations (and many other applications) are inherently multi-modal, we constructed the task to have both auditory and visual stimuli. Therefore, we defined intent as the selection of a response having both an attentional context (visual or auditory) and a target goal (stimulus category). Within each attentional context, subjects had to select among three intended responses in response to the identified stimulus category. Importantly, the specific motor response (button press) was randomized on each trial, and a delay period was inserted between the stimulus presentation and the presentation of the randomized response mapping.Data were collected with a 32-channel active electrode EEG system and an eye tracker operating at 250Hz from n=15 subjects performing the identification tasks in the crewstation environment. In the visual context, subjects had to generate intended responses to stimuli that fell into the threat, friendly, or neutral category. In the auditory context, subjects heard range callouts and generated intended responses to identified ranges in the near, middle, or far categories. From these data we extracted spectral features, including phase coherence, for classification. Classification performance for determining the attentional context averaged 83.63% (chance = 50%) across subjects. Classification performance in selecting the target goal to auditory stimuli averaged 69.1% (chance = 33%), and 60.14% (chance = 33%) in the visual modality. These results show promise for the use of EEG in goal-directed BCIs for military applications. Funding for this research was provided by the Institute of Collaborative Biotechnologies, a University Affiliated Research Center (UARC) based at UCSB and sponsored by the Army Research Office.

Disclosures: P.M. Connolly: None. S. Simons: None. K. Zachery: None. D. Krnavek: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.17/KKK14

Topic: H.02. Human Cognition and Behavior

Title: Overimitation in a virtual environment

Authors: *M. LILJEHOLM, E. CASTILLO;
Neurosci., Univ. of California, Irvine, Irvine, CA

Abstract: Overimitation - the faithful copying of redundant, causally irrelevant, actions - has been demonstrated cross-culturally in human children as well as adults. To date, human overimitation has been studied using physical boxes, containers or contraptions, from which the observee, and subsequently the observer, extracts some object using a tool. A major limitation of this method is that it is difficult to combine with neuroscientific techniques, such as fMRI, thus impeding assessment of underlying neural computations. Another notable feature of previous demonstrations of human overimitation is that they involve observation of actual limb movements by a conspecific, raising the question of whether such observations are necessary for the phenomenon to occur. Here, we demonstrate overimitation using a computerized maze-learning task, in which participants were required to move an abstract avatar from a start location to a goal location in a 2D maze. Our methods and results pave the way for future assessment of the neural bases of overimitation.

Disclosures: M. Liljeholm: None. E. Castillo: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.18/KKK15

Topic: H.02. Human Cognition and Behavior

Title: Pre-movement stretch responses predict changes-of-mind.

Authors: *L. P. SELEN, J. MARESCH;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Tasks change continuously and are often ambiguous in everyday life. In order to act appropriately, our brain has to monitor the task goals and adapt our motor responses in real time. Signs of this real-time adaptation of responses have been shown in the form of changes-of-mind in reach paradigms where subjects evaluate additional information after having initiated their reach (Resulaj et al, 2009; Burk et al., 2014) and in the form of rapid motor response muscle activity that tracks the accumulated evidence in a random-dot-motion (RDM) discrimination task (Selen et al., 2009). Here we investigate whether stretch induced rapid motor responses prior to movement initiation only represent the accumulated evidence or also contain information about an upcoming change-of-mind. Human subjects held the handle of a robotic manipulandum (vBOT) and indicated the direction of a random-dot stimulus by a full arm movement to one of two targets, 30 cm away from the start position and separated by 40 degrees. Subjects were instructed to rapidly indicate the perceived direction once the RDM stimulus disappeared. At this moment the the robot displaced their hand (1cm in 60ms), extending the shoulder and eliciting rapid motor responses in the shoulder muscles (especially Pectoralis Major). We maximized the occurrence of change-of-mind by only presenting intermediate coherence levels of the dots (6.4%, 12.6%, 25,6%) and a limited range of viewing durations (range 0.2-0.6s, mean 0.40s). In order to control and shorten the reaction times, we used a beep paradigm to indicate the offset of the RDM. We recorded muscle activity from Pectoralis Major and quantified the magnitude of the rapid motor response in the first 100 ms after perturbation onset. For each change-of-mind trial we selected a normal trial with the same coherence level, but opposite direction, and the closest viewing duration. If the rapid motor responses reflect the likelihood of a change-of-mind, one would expect increased activity for right-to-left changes-of-mind compared to straight right choices. However, if the rapid motor responses just reflect accumulated evidence, the activity should be lower in change-of-mind trials because there is less evidence for the right target. In all 6 subjects rapid motor responses were lower in change-of-mind trials compared to their matched normal trial. These results confirm that the CNS continuously provides information from the ongoing decision process to the motor system, but that rapid motor responses do not, yet, reflect activity that is related to the upcoming change-of-mind.

Disclosures: L.P. Selen: None. J. Maresch: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.19/KKK16

Topic: H.02. Human Cognition and Behavior

Support: Grant-in-Aid for Challenging Exploratory Research 24240085 16K12995

Grant-in-Aid for JSPS fellows grant number 26-407

Title: An attack-and-defend competition as a redundant cognitive-motor system

Authors: *K. FUJII, Y. YOSHIHARA, Y. YAMAMOTO;
Nagoya Univ., Nagoya, Japan

Abstract: Humans interact by perceiving relevant information, deciding on and then executing suitable actions. Computational neuroscience has revealed the cognition and motor control of individual agents. However, competitive mutual anticipation and action in multiagent systems have no specific solution and exhibit a great diversity in their behavioral forms. To describe the interactive movements, researchers used a non-linear oscillator and revealed that the skirmish and deadlock states in sports emerged from the competitive interaction. However, these studies cannot explain the manner in which the cognition and motor systems function. Here we adopt a hybrid approach to solve the inverse (from measured data to model) and forward (model simulation) problems using previous measurement results and modeling an attack-and-defend competition. Three-layered redundant systems were constructed: two-agents, an individual agent, and cognitive-motor systems. We implemented two non-linear characteristics: one is the attacker's decision to move forward and attempt to pass the defender by observing oneself and the opponent. Second is the transferability, i.e., movement initiation delay, confirmed by our previous study. At the intra-agent level, in the determination phase of the outcome, the model replicated actual state transitions, wherein the defender's higher transferability led to a more successful defense and vice versa. Furthermore, the attacker's model of observing the defender's transferability revealed a higher probability of a successful attack than the non-observed model. At the inter-agent level, the measured and simulated behaviors were similar. In particular, the determination state of order in a successful attack was abruptly changed rather than gradually, from the skirmish state similar to oscillators. We revealed the overall processes in the two-agent competition and suggest the importance of always defending in high transferability and attacking when the defender's less transferability. In our further expanded model, we biomechanically defined transferability by implementing a redundant actuator system in this model. We anticipate developments in the scientific field of complex movement, which adapt to such uncontrolled environments.

Disclosures: K. Fujii: None. Y. Yoshihara: None. Y. Yamamoto: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.20/KKK17

Topic: H.02. Human Cognition and Behavior

Title: Motor affordance biases subjective value

Authors: *C. C. ROMERO[1], M. A. GOMEZ[1], M. WEBSTER[1], J. T. MCGUIRE[2], T. SCHONBERG[3], J. C. SNOW[1];
[1]Univ. of Nevada, Reno, Reno, NV; [2]Boston Univ., Boston, MA; [3]Tel Aviv Univ., Tel Aviv, Israel

Abstract: In everyday life food choices are most often made when consumers are confronted with real foods, yet our understanding of human decision-making is based almost exclusively on studies that have relied on computerized images. Although image interaction is common in the modern world, humans have presumably evolved to perceive, grasp, and consume real foods, not images. Here, we used a 'cue-approach training' procedure described by Schonberg et al., (Nature Neuroscience, 2014) to test whether or not the value of common snack foods can be modulated by passive exposure to real foods that have the potential for (but do not require) a physical motor approach response. We found that 2-AFC choices between 'high-value' (preferred) items were biased significantly in favor of snacks that were viewed as real objects, versus images. Importantly, display format did not influence decisions between low-value (non-preferred) food pairs, indicating that the biasing effect of the real foods was not attributable to factors other than preference. Our results suggest that subjective value is enhanced for items with a definite motor association or affordance.

Disclosures: C.C. Romero: None. M.A. Gomez: None. M. Webster: None. J.T. McGuire: None. T. Schonberg: None. J.C. Snow: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.21/KKK18

Topic: H.02. Human Cognition and Behavior

Support: ERC Starting Grant , ERC-2012-StG

Title: Optimized functional MRI in the subthalamic nucleus at 7 Tesla and 3 Tesla

Authors: *G. DE HOLLANDER[1], M. C. KEUKEN[1], W. VAN DER ZWAAG[2], R. TRAMPEL[3], B. U. FORSTMANN[1];
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [2]Spinoza Ctr. for Neuroimaging, Amsterdam, Netherlands; [3]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The basal ganglia (BG) form a group of nuclei in the midbrain that are important for cognitive processes, such as motor control and response inhibition. These nuclei also play a role in the pathogenesis of Parkinson's Disease (PD), where dopaminergic cell loss in the substantia nigra leads to severe (motor) symptoms. Deep brain stimulation (DBS) of the basal ganglia, specifically the subthalamic nucleus (STN), has been a successful therapy in alleviating motor symptoms of PD. However, the precise mechanisms underlying DBS and its side effects are not well-understood. An important hypothesis is that the subthalamic nucleus contains multiple subdivisions, related to different cortical networks, such as the 'limbic', 'cognitive', and 'motor' network, and that they are crucial to optimal DBS electrode positioning (Temel et al., 2005; but see Keuken et al., 2012).
In vivo functional magnetic resonance imaging (fMRI) of the basal ganglia, and specifically the STN, could help to elucidate its role in cognition, as well as those of its putative subdivisions. However, fMRI in the STN remains challenging, even with ultra-high field MRI (7 Tesla [T] and above), because of its small size, as well as its complicating magnetic properties, mainly due to its its high concentration of iron (de Hollander et al. 2015).
In this project we aimed to 1) optimize 7T fMRI sequences to be able to robustly show functional activation in the STN, as well as other basal ganglia nuclei, 2) compare fMRI data obtained at 7T to fMRI data obtained at 3T with identical resolution, 3) run a speeded decision-making task while collecting 7T fMRI data that was designed to tap into putative STN subdivisions. In the speeded decision-making paradigm, subjects were cued about the reward payoff structure of the coming trial, as well as a perceptual difficulty manipulation.
Our results show that care needs to be taken in optimizing fMRI sequences at 7T such that both robust BOLD contrast as well as an acceptable spatial and temporal resolution in the STN can be obtained. More concretely this means that "off-the-shelf" 7T fMRI sequences (usually optimized for cortex) are not suitable and that the impressive submillimeter functional MRI resolutions obtained in some parts of cortex, are currently not feasible in the STN. Our 3T fMRI data show that lower-field fMRI is not capable of delivering both acceptable BOLD contrast, as well as the spatial resolution that is needed to answer questions about putative subdivisions in the STN. Preliminary results from the factorial speeded decision-making task suggest that the STN is involved in adjusting decision strategies related to both reward payoff, as well as stimulus quality.

Disclosures: G. de Hollander: None. M.C. Keuken: None. W. van der Zwaag: None. R. Trampel: None. B.U. Forstmann: None.

Poster

267. Perceptual and Motor Decision Making

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 267.22/KKK19

Topic: H.02. Human Cognition and Behavior

Support: SHBT Fellowship 1539610

NIH/NIDCD R01-DC014924

Title: Dynamics of perceptual decision-making and choice confidence

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Abstract: Recent vestibular psychophysical studies have consistently shown that humans discriminate high frequency motions (with short duration) better than low frequency motions (with long duration) when performing binary direction-recognition forced-choice tasks. This behavior has been modeled with a high-pass filter (HPF) having a time constant (circa 0.5s) that is much shorter than all other behaviorally relevant time constants found in vestibular pathways [1,2]. Here, we hypothesize that an HPF with such a short time constant is part of the decision-making process. In other words, we propose that the brain may utilize an HPF to selectively weigh recent information that is more relevant for current state when making a perceptual decision. In order to investigate the dynamics of perceptual decision-making in a forced-choice paradigm, we quantitatively analyzed human choice behaviors and developed a computational model. Specifically, choice accuracy and confidence were concurrently measured as a function of observation duration in 12 normal human subjects while performing a direction-recognition forced-choice task using a tilted subjective visual vertical (SVV) display. Observation duration was varied between 105ms and 1600ms, during which a stationary Gabor patch with a 7° viewing angle was displayed. Both choice accuracy and confidence were consistent with response dynamics that included an HPF having average time constants of 134ms and 225ms, respectively. For our computational model, we incorporated an HPF into a preexisting decision-making model [3] of a sensory evidence accumulator. The proposed model predicted that both choice accuracy and confidence reach a plateau below 100% when given moderate sensory stimulus. This prediction matched the human behavioral data. The veracity of this model's predictions was also compared to predictions made by other published models of decision-making. The other three preexisting models were (a) a pure accumulator model [3], (b) a collapsing bound accumulator model [4], and (c) an urgency signal accumulator model [5]. The simulations showed that only our new accumulator model with an HPF matched choice accuracy,

discrimination thresholds, confidence histograms, and median confidence as functions of observation duration. These results show that (1) binary choice and confidence share a neural representation of the same noisy decision variable(s), and that (2) the dynamics of the shared processing can be captured by an HPF (or other equivalent leaky mechanism) combined with an accumulator.
[1] Lim & Merfeld 2012 [2] Merfeld et al 2016 [3] Ratcliff & McKoon 2008 [4] Milosavljevic et al 2010 [5] Churchland et al 2008

Disclosures: K. Lim: None. D.M. Merfeld: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.01/KKK20

Topic: H.02. Human Cognition and Behavior

Support: Bourse de relève académique pour jeunes chercheurs, Faculty of Biology and Medicine, University of Lausanne

Title: Structural and functional architecture of the brain network for social cognition

Authors: *A. A. SOKOLOV[1,2], M. ERB[3], F. E. POLLICK[4], R. S. J. FRACKOWIAK[5], K. J. FRISTON[2], M. A. PAVLOVA[3];
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Abstract: Intact perception of bodily non-verbal signals on intentions, dispositions and emotions of others is of fundamental value in our daily life. However, the architecture of the brain networks for social cognition remains largely unknown. We studied visual perception of emotional (angry and happy) and neutral body language in healthy participants, using a combination of functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI). Data processing was conducted with Statistical Parametric Mapping (SPM12) and FMRIB Software Library (FSL). The right superior temporal sulcus (STS) and caudate nucleus exhibit higher activation for happy body language, and the left inferior insula, perigenual anterior cingulate cortex (ACC) and posterior midcingulate cortex (MCC) for angry as compared to neutral body language. The cerebellar vermis (lobule IX) and right amygdala appear to signal an absence of emotional content in body movements. DTI tractography and effective connectivity analysis reveal the structural and functional architecture within this network for processing of

emotional body language. In summary, higher-order fronto-temporal regions, the cerebellum and basal ganglia are mainly involved in recognition of emotions conveyed by body motion, with a lateralization depending on emotional content. Better understanding of structure and function within the social cognition networks may contribute to further advance in research and management of neuropsychiatric disorders.

Disclosures: A.A. Sokolov: None. M. Erb: None. F.E. Pollick: None. R.S.J. Frackowiak: None. K.J. Friston: None. M.A. Pavlova: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.02/KKK21

Topic: H.02. Human Cognition and Behavior

Support: NSF CAREER BCS 1151805

Qualcomm Institute, Strategic Research Opportunities Program

Kavli Institute for Brain and Mind, UC San Diego, Innovative Research Award

UC San Diego Academic Senate Award

Title: Visual population codes for perceived actions in the human brain

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Abstract: Over the last decades neurophysiological, neuropsychological, neuroimaging and brain stimulation studies have identified a network of occipital, temporal, parietal, and frontal brain areas that support the visual processing of others' actions. The neural computations and representational properties in each area however need to be better specified. Here, we investigated the representational content of human brain areas involved in action processing using fMRI, representational similarity analyses (RSA), and computational modeling. Participants viewed video clips of 8 different actions performed by 3 different agents (both human and robot) during fMRI scanning. We derived two indices from the representational similarity matrices for each region of interest (ROI): Agent decoding index and action decoding index, reflecting the degree to which significant agent and action information can be

distinguished, respectively. We found significant Agent decoding in earlier visual areas and face and body sensitive visual areas (e.g., FFA, EBA), as well as the core nodes of the action observation network (posterior superior temporal sulcus (pSTS), inferior parietal and ventral premotor cortex). However, agent decoding index varied across ROIs. Notably, the right pSTS agent decoding index was significantly greater than those for the parietal and frontal ROIs ($p<0.05$). For actions, we found significant decoding in all visual ROIs as well as pSTS, parietal and ventral premotor regions. The overall action decoding index did not differ significantly across ROIs, but hierarchical clustering indicated the structure of action-related information representation differs between the levels of the cortical hierarchy. We next applied modeling to the experimental stimuli themselves (i.e., the action videos), characterising both the sensory information in the stimuli (using approaches from computer vision), and higher-level/semantic aspects (e.g., action type, target of the action, intention), and considering these model similarity matrices with those constructed from the brain responses. Based on these analyses, we were able to reveal differences in representational properties in the regions studied. We suggest pSTS pools information from earlier visual areas regarding the visual features of the actor/agent, and works with parietal and frontal regions that code higher-level aspects of actions. These results are consistent with existing computational models of action recognition, and can contribute to their extension with further analyses and modeling of representational properties of each region, as well as the network dynamics.

Disclosures: A.P. Saygin: None. B.A. Urgen: None. S. Pehlivan: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.03/KKK22

Topic: H.02. Human Cognition and Behavior

Support: Innovation Fund of Shanghai Designated for Undergraduate Students

Innovation Fund of USST Designated for Undergraduate Students

Title: Cortical processing of emotion: an electroencephalogram study

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Abstract: Emotion is a fundamental function of human brain, and has been widely studied by using traditional psychology paradigm like self-report rating or experimental method such as

electroencephalogram(EEG) and fMRI(functional Magnetic Resonance Imaging). Using affective movie to elicit subject's emotion state is a widely used method in traditional experiment taking advantage of ability of eliciting stable emotion for longer periods, while have not been broadly used in EEG experiment because of unreliable movie database or insufficient movie amount. The present study implemented 49 affective film clips from proved dependable emotion movie database(Emotional Movie Database, Carvalho et al. 2012) as stimulus and recorded EEG signal from 12 subjects(12 male, 20-21 years old) performing passive viewing task. These stimulus were estimated to induce five emotion states(horror, erotic, social negative, social positive and neutral) in the affective space. Various analysis methods included event-related potentials(ERP), Time-Frequency Analysis and low-resolution electromagnetic tomography (LORETA) were conducted to investigate the emotion process.
The ERP result confirms that previous findings, the early components such as P1,N1,P2 and slow components such as LPP,N450 ,can also be elicited by emotion movie stimulus. Also, amplitude of N1,LPP and N450 show significant differences among each emotion state. To further investigate how these differences were modified by cortex current potentials, we utilised LORETA to map the current density distribution during each component occurred. These results deduced that these motivated areas may relate with different emotion processing mechanism. Although ERP analysis revealed early-stage cortical information process of emotion, we also interested in which stable cortical network can be built during different affective state. We extracted band energy of each channel as frequency characteristic and conducted Analysis of Variance(ANOVA) to each characteristic between horror(negative) and erotic(positive) emotion. This statistic analysis shows there are significant differences in gamma band spatial pattern between two affective conditions. Furthermore, time-frequency analysis were operated to describe related gamma oscillation within and between different functional areas.

Disclosures: E.H. Zhang: None. O. Wang: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.04/KKK23

Topic: H.02. Human Cognition and Behavior

Title: Body motion presented upright and upside-down: Human ultra high field 9.4T fMRI

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Tübingen Med. Sch., Tuebingen, Germany; [4]High-field Magnetic Resonance, Max Planck Inst. for Biol. Cybernetics and Univ. of Tuebingen Med. Sch., Tuebingen, Germany

Abstract: Body motion is an indispensable source of information for social cognition and interaction. Yet display inversion severely impedes biological motion (BM) processing. The primary advantage of upside-down presentation is that an inverted display retains the same relational structure and absolute motion as an upright one, thereby keeping the same amount of sensory information available. This is why display inversion often serves as a control for proper BM processing in patients with neurodevelopmental, neurological and psychiatric disorders. It is still unclear how brain networks underpinning body motion processing are affected by display inversion. To address this issue, we used ultra-high field fMRI at 9.4T providing for highest spatial resolution and sensitivity in humans. Typically developing adults performed a two-alternative-forced-choice (2AFC) task, indicating whether an upright point-light walker or control displays (the same movies inverted 180 deg) were presented. An upright walker elicited most pronounced clusters of fMRI activity in the bilateral superior occipital cortices and the right middle temporal cortex, whereas the inverted display results in bilateral activity of lower occipital cortices, primarily, the lingual cortices and the left fusiform gyrus. Activation in these areas exhibited specific temporal dynamics: a decrease in activation in the second 5 s of stimulus duration, with a recurrent increase afterwards that presumably reflects back propagating influence. Most importantly, we uncovered pivots of activity in the distributed network driven by cognitive processing of the similar visual input: Perceivers who did not recognize upside-down displays as a walker exhibit several clusters of activity in the right hemisphere including the lingual, postcentral cortices, and the pars operculum. The outcome provides novel insights on the brain networks underlying BM processing and its functional neuroanatomy.

Disclosures: M. Pavlova: None. M. Erb: None. G. Hagberg: None. K. Scheffler: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.05/KKK24

Topic: H.02. Human Cognition and Behavior

Title: Event-related synchronization-desynchronization features of social altruistic and selfish decision

Authors: *I. P. KUZNIETSOV, O. RAKOVETS, N. IEVPAK, T. KACHYNSKA, O. ABRAMCHUK;
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Abstract: Human brain is higly specialized on conducting social cognitive functions, which results in specifics of brain cortex structural and biochemical organization. This, in turn, should lead to differences in brain electrical activity in persons, who have different types of social-oriented activity. While different types of social activity, either altruistic or selfish, based on different types and estimation mechanisms of social reward, we expected to find more expressed differences in activity of executive systems under conditions of making a social interactions specific decision. Since the activity of executive systems can be in general described by interplay of dopamine and norepinephrine mediator systems, which activity is characterised by specific reactions in alpha and beta EEG bands, we supposed, that persons with more expressed prosocial behavior should have more reactive EEG responses in the alpha-2 and beta-bands. Our study was conducted on 45 volunteers, which were classified as altruistic-oriented (24 persons) or selfish-oriented (21 person), based on the results of Leary test and relations of altruistic and selfish decisions they made during the experiment. To obtain altruistic-oriented and selfish-oriented decisions we used two experimental probes: "Stag hunt" game (choosing a stag was considered as an altruistic decision, choosing a rabbit was considered as a selfish decision) and an original game, which mimicked the decision making during a basketball game (a pass is considered as an altruistic decision, a throw as a selfish decision). ERD/ERS reaction was analyzed in P3 and P4 electrode sites. It was shown, that the major difference in EEG reaction between altruistic-oriented and selfish-oriented persons is observed in 15-18 Hz frequency range between 1000 and 2000 ms after a stimulus presentation independently on the stimulus type. The origin of this difference are the sychnronization processes in persons of selfish-oriented type and desynchronization processes in persons of altruistic-oriented type. We consider this effect to be the evidence of a more thorough estimation of stimuli importance by altruistic-oriented persons.

Disclosures: I.P. Kuznietsov: None. O. Rakovets: None. N. Ievpak: None. T. Kachynska: None. O. Abramchuk: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.06/KKK25

Topic: H.02. Human Cognition and Behavior

Support: ESRC PhD student scholarship

Title: Brain structure and social belonging: expectations of social pleasure and pain are reflected in regional brain volumes

Authors: *B. CRAWFORD[1,2], N. MUHLERT[2,3], G. MACDONALD[3], A. D. LAWRENCE[1]; [1]Sch. of Psychology, Cardiff Univ., Cardiff, United Kingdom; [2]Sch. of Psychological Sci., Univ. of Manchester, Manchester, United Kingdom; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: Human beings have a fundamental need to form and maintain social connections (a "need to belong"). Satisfaction of this leads to social pleasure, while failure to satisfy these needs results in social pain. Previous research has focused on the neural correlates of reactions to social rejection and acceptance. However, much of the pleasure and pain of social life occurs in *anticipation* of things that have yet to come. To measure individual differences in such anticipatory processes, we have developed a novel self-report measure, the levels of dispositional expectancies for social threat and reward scale (LODESTARS). The LODESTARS is a 10-item inventory examining the extent to which participants expect to experience social reward and social punishment during an imminent vividly imagined social encounter with previously unknown individuals. Data from 848 participants demonstrate that the LODESTARS has a clear two-factor structure and excellent psychometric properties. The brain-structural correlates of dispositional social threat and reward expectancies were examined using voxel-based morphometry (VBM). Regional grey matter volume (GMvol) of 100 healthy participants (mean age 24 years; 26 males) was assessed. High-contrast T1-weighted anatomical images were acquired using a 3-Tesla MRI scanner and analysed using SPM12. To correct for multiple comparisons across the whole brain, non-stationary cluster extent correction was implemented. Age, gender and total brain volumes were accounted for. Higher social threat expectancies were associated with greater GMvol in brain regions involved in social attention and perception, including the right superior temporal sulcus (pSTS). This may reflect attentional bias and hypervigilance directed towards potential social threat signals in the environment. Supporting this interpretation, pSTS GMvol correlated *positively* with amygdala GMvol in our sample (Pearson's $r = .23$; $p = .021$). Higher expectancies of social reward and lower expectancies of social threat were associated with greater GMvol in brain regions implicated in emotion regulation, particularly right ventromedial PFC (vmPFC). Previous findings suggest that this region may function as a hub that modulates negative affective responses (perceived fear and aversiveness) across a broad range of paradigms. Corroborating this, vmPFC GMvol was *negatively* correlated with amygdala GMvol in our sample (Pearson's $r = -.27$; $p = .008$). Our findings may have implications for understanding the consequences of social connection on brain structure, as well as brain structural dispositions to mood disorder risk, including social anxiety and social anhedonia.

Disclosures: B. Crawford: None. N. Muhlert: None. G. MacDonald: None. A.D. Lawrence: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.07/KKK26

Topic: H.02. Human Cognition and Behavior

Support: Healthy Brain Grant 1-A-2015.

Title: Brain rhythms during aesthetic experience

Authors: *E. JIMENEZ[1], L. E. ROLDÁN MORALES[2], F. GARCÍA-PALMA[3], V. CALDERÓN-ORTÍZ[4], M. ARIAS-GARCÍA[5,4];
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Abstract: Neuroaesthetics is a new field of research that combines multiple disciplines to elucidate the neurobiological foundations of the cognitive and affective processes involved in aesthetic and artistic experiences. One important method that can be applied for gaining an understanding of the underpinning of aesthetics in the brain is that of electrophysiology. Electroencephalography (EEG), allows the recording and identification of brain rhythms in a mental processing. We presented a Mexican short film named “MUSA” while simultaneously evaluated the EEG activity and the ocular movements, in order to explore the possible differences in cognitive processing between people with and without aesthetical education (dance, theater, painting, sculpture, architecture and movie making). Our results showed significant differences between the groups in Alpha (8-12 Hz) and Beta (13-30 Hz) components between the two groups studied, suggesting that the previous educational experience, could shape the brain rhythms implicated in an aesthetic and artistic experiences.

Disclosures: E. Jimenez: None. L.E. Roldán Morales: None. F. García-Palma: None. V. Calderón-Ortíz: None. M. Arias-García: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.08/KKK27

Topic: H.02. Human Cognition and Behavior

Title: Effects of repetitive transcranial magnetic stimulation (rTMS) on human movement responses to the Rorschach test and its electrophysiological correlates

Authors: *G. Z. SOGHOYAN[1], A. ANDO[2], Y. QUN[3], L. GIROMINI[2], A. ZENNARO[2], M. BOHM[1], D. MARYANOVSKY[1], J. A. PINEDA[1];
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Abstract: According to the Rorschach literature, human movement responses (M) demonstrate higher cognitive functioning because of the implied ability to empathize with a human being [1]. Using EEG mu suppression as a proxy biomarker for mirror neuron activation, Giromini, and colleagues [2] suggested that strong internal representation of the "feeling of movement" may be sufficient to trigger MNS activity even when minimal external cues are present. Specifically, by conducting an EEG study with the Rorschach stimuli, these authors have shown that attributing M responses yields greater EEG mu suppression. These findings were replicated by a second study [3] and then further confirmed by additional analyses on the same data [4]. Furthermore, Ando' and colleagues [5], by using Transcranial Magnetic Stimulation (TMS) showed that temporary disruption of left inferior frontal gyrus (LIFG; presumably implicated in mirroring activity), but not Vertex, yielded a statistically significant reduction in the attribution of M responses to the Rorschach. The goal of the current study was to use rTMS to determine the role that prefrontal cortex plays in M production and characterize its electrophysiological correlates. All participants were exposed to the Rorschach stimuli during a baseline (without rTMS but with EEG data recording) and during the experimental condition (with rTMS and with EEG). For the control group, 1 Hz rTMS at 90% of rMT was applied for fifteen minutes over the Vertex (this site was chosen as control site since it is often used to test for non specific rTMS effects); for the experimental group, 1 Hz rTMS at 90% of rMT was applied for fifteen minutes over the LIFG. Mu suppression over sensorimotor cortex (including scalp locations C3, Cz and C4) was calculated: the first 500ms were used as reference point, and the ongoing mu power computed relative to that baseline. We expected that mu suppression would not occur when the stimulation was applied over LIFG. By testing a mixed, 2 (between-subject: vertex vs. LIFG) by 2 (within-subject: baseline vs. rTMS) ANOVA, we observed that disrupting LIFG, but not vertex, decreased the number of M responses produced by the participants, with a highly significant interaction effect, $F(1, 26) = 24.60$, $p = .000$, *Partial* $\eta^2 = .486$. Within the LIGF group, we noted a relative reduction of Mu suppression from baseline (EEG data recording without rTMS

condition) to experimental condition. These findings further suggest an intimate link between action simulation and action perception, provide support for the use of EEG to measure MNS activity, and support the role of IFG in mirroring.

Disclosures: G.Z. Soghoyan: None. A. Ando: None. Y. Qun: None. L. Giromini: None. A. Zennaro: None. M. Bohm: None. D. Maryanovsky: None. J.A. Pineda: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.09/KKK28

Topic: H.02. Human Cognition and Behavior

Title: Attachment styles and their relation to respiratory sinus arrhythmia and mu suppression

Authors: *N. C. CASTRO[1], M. WIDDOWSON[1], M. BOHM[1], D. MARYANOVSKY[1], A. MINICHINO[1,2], J. PINEDA[1];
[1]Cognitive Sci., Univ. of California San Diego, La Jolla, CA; [2]Sapieniza Univ. of Rome, Roma, Italy

Abstract: Little is known about the neurobiological underpinnings of attachment orientations and their impact on social and affective behaviors. Theories of adult attachment emphasize two dimensions of attachment insecurity: anxious attachment, associated with exaggerated appraisal of threat in social contexts and hyper-activation of coping strategies, and avoidant attachment, characterized by deactivating coping strategies in emotionally relevant situations. We hypothesized: 1) anxious attachment to be associated with amplified autonomic and mirror neuron system activity; and 2) avoidant attachment to be associated with reduced autonomic and mirror neuron system activity, both in response to video clips of others' painful facial expressions.
Forty-eight participants (secure, anxious, and avoidant attachment style groups) underwent EEG and respiratory sinus arrhythmia (RSA) recording while watching videos of painful, neutral, and blurred facial expressions. To assess attachment styles, participants completed the Experiences in Close Relationships Revised questionnaire (ECR-R). Scalp electrode EEG recordings of mu rhythm (8–13 Hz) over the sensorimotor cortex during the session were used to calculate mu wave suppression, a biological marker of empathic resonance. A comprehensive battery of social cognitive, empathic, and personality measures was also administered to each participant. Preliminary correlation analyses on RSA data (22 subjects) and EEG data (16 subjects) were consistent with our hypotheses: 1) the anxious attachment subscale of the ECR-R was significantly associated with both RSA-indices ($r = -.47$, $p = 0.039$) and increased mu suppression

(r= -.54, p= <0.05), such that subjects with higher scores on this ECR-R subscale had lower RSA indices and greater mu suppression; and 2) the avoidant attachment subscale of the ECR-R was significantly associated with RSA indices (r= .60; p= 0.003), such that subjects with higher scores on this ECR-R subscale had higher RSA indices.
These results suggest that the tendency of individuals with anxious attachment to exaggerate appraisal of threat could be explained by an increased activation of the autonomic (lower RSA indices) and mirror neuron system. On the other hand, the deactivation of coping strategies typically showed by individuals with an avoidant attachment could be associated with a lower activation of the autonomic system (higher RSA indices). Our results provide insights on psychopathological conditions where attachment dysregulation is likely to play an important (causal) role.

Disclosures: N.C. Castro: None. M. Widdowson: None. M. Bohm: None. D. Maryanovsky: None. A. Minichino: None. J. Pineda: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.10/KKK29

Topic: H.02. Human Cognition and Behavior

Title: Understanding the moral brain: a meta-analysis of regions involved in sociomoral processing using multilevel kernel density analysis and activation likelihood estimation

Authors: *S. J. FEDE[1,2], C. L. HARENSKI[2], K. A. KIEHL[1,2];
[1]Psychology, Univ. of New Mexico, Albuquerque, NM; [2]Mind Res. Network and Lovelace Biomed. and Envrn. Reserch Inst., Albuquerque, NM

Abstract: The neurobiology of moral cognition is a relatively young field. However, the literature has expanded considerably with more than 100 related publications on the topic. Although previous meta-analysis was conducted (Bzdok et al., 2012), the rapidly expanding body of literature merits follow-up to consider recent findings. Additionally, understanding differences in the meta-analytic procedures involved in Multilevel Kernel Density Analysis (MKDA) and Activation Likelihood Estimation (ALE) can inform not only the field of socioemotional decision making but cognitive neuroscience meta-analysis in general. Here, ALE and MKDA were used to conduct neuroimaging meta-analyses of the moral cognitive neuroscience literature. This meta-analysis included 1542 participants across 36 experiments. MKDA, but not ALE, replicated previous main effect findings of the neural correlates of moral cognition: the left amygdala, medial prefrontal cortex (mPFC), bilateral temporoparietal junction

(TPJ), and posterior cingulate (PCC). On the other hand ALE, but not MKDA, replicated findings in the temporal gyrus. These results provide support for the network of brain regions proposed in theories of moral emotion and cognition, but also indicate that the meta-analytical techniques of ALE and MKDA may not individually provide a complete picture of neuroimaging literature and should be interpreted cautiously on their own.

Disclosures: S.J. Fede: None. C.L. Harenski: None. K.A. Kiehl: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.11/KKK30

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant 16H02839 and 15H01671

Title: Effect of cheering on functional connectivity between the player's and the observer's brain activity during a competitive game

Authors: *T. KOIDE, S. SHIMADA;
Meiji Univ., Kawasaki-Shi, Japan

Abstract: Mirror neuron system (MNS) is the brain region that is activated not only when performing an action by themselves, but also when observing the same action performed by others. In this study, we utilized functional near-infrared spectroscopy (fNIRS) hyperscanning technique to examine the functional connectivity between the player's and the observer's brain activity during a competitive game. Thirty-two pairs of male subjects participated in the experiment (aged 21.3 ± 1.6 years, mean ± SD). One of the subjects (the player) performed the rock-paper-scissors (RPS) game against the experimenter, and another (the observer) observed the player performing playing RPS game. We assigned half of the pairs to the cheering group in which the observer was instructed to cheer for the player during the RPS game and the other half to the control group in which the observer was instructed to judge whether the player performed RPS game with or without cheating. The experiment was repeated until more than 10 trials were obtained for each condition of outcome (WIN, LOSS and DRAW). The hemodynamic responses were assessed by using 48-ch fNIRS (OMM-3000, Shimadzu, Japan). The channels were divided to 24-ch each for the left hemisphere of each subjects (ch_p1 - ch_p24 for the player, ch_o1 - ch_o24 for the observer) which were placed on the left sensorimotor area (near C3 of international 10-20 system), a main component of the MNS. First, we employed general linear model (GLM) analysis on fNIRS data and found that the observer's MNS was located over the

premotor, the primary motor and the parietal cortices. We then applied the psychophysiological interaction (PPI) analysis and found that the functional connectivity between the player's premotor cortex and the observer's MNS was stronger in the cheering group than in the control group (ch_p2-ch_o7: $t(30) = 2.49$, $p < 0.05$, corrected; ch_p2-ch_o12: $t(30) = 2.19$, $p < 0.1$, corrected). Furthermore, functional connectivity between the player's premotor cortex (-20 -14 76) and the observer's parietal cortex (-32 -62 65) was correlated with the observer's feeling of the sense of unity with the player ($r = 0.590$, $p < 0.05$). These results suggest that cheering enhances the assimilation of the observer's internal motor state with that of the player during observation of a competitive game.

Disclosures: T. Koide: None. S. Shimada: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.12/KKK31

Topic: H.02. Human Cognition and Behavior

Support: Simons Seed Grant, SCSB, MIT

Feodor Lynen Postdoctoral Fellowship of the Alexander von Humboldt Foundation to DK

Title: Task and stimulus representation in face responsive cortex in autism spectrum disorder

Authors: *D. KLIEMANN, S. ANZELLOTTI, D. AYYASH, J. GABRIELI, R. SAXE; MIT, Cambridge, MA

Abstract: Understanding the neural basis of the social difficulties in Autism Spectrum Disorder (ASD) is essential to develop effective interventions, test their efficacy and predict individual treatment outcome. Cognitive neuroscience offers a key level of analysis towards this endeavor by bridging the gap between molecular and cellular mechanisms to the behavioral phenotype. One hallmark symptom in Autism Spectrum Disorder (ASD) is disproportionate social impairments, including difficulty processing information from emotional faces. Despite an enormous body of research on this matter, the neural basis for the affected behavior remains unclear with weak and conflicting results. Here, we present a new approach focusing on naturalistic dynamic social contexts with the goal of finding robust, replicable neural correlates of difficulties in social information processing in ASD that generalize across individuals and stimuli. We investigated whether information in patterns of neural responses to emotional faces

represent i) the valence of emotional facial expressions, ii) task of the subject and iii) the flexibility of neural patterns to task demands. In a pilot study, we measured blood-oxygen-level-dependent signal (BOLD) responses while neurotypical subjects watched short naturalistic movie clips of dynamic positive and negative facial expressions. For each movie clip, subjects were instructed to judge either the person's age or the valence of their emotional expression. Split-half multivoxel pattern analyses (MVPA) suggest that the task of the subject (attending to age vs emotion) could be robustly decoded from patterns in face-responsive regions (bilateral anterior and posterior superior temporal sulcus (STS), fusiform face area (FFA) and medial prefrontal cortex (MPFC)), independent of the stimulus. The property of the stimulus (valence of the facial emotion) was represented in STS and MPFC (replicating prior reports), however weaker than the task representation. Next, we compared patterns of neural responses in ASD (n = 17) versus neurotypical participants (n = 20) in a follow up study, and found evidence suggesting aberrant task representation in MPFC in ASD. Additionally, a region in right STS showed reduced flexibility in neural activity to task demands: while neurotypical participants showed increased valence decoding when judging emotion versus age of facial expressions, this modulation was absent in ASD. Our results suggest that atypical cortical representation of social information in ASD might reflect reduced neural flexibility of response patterns to optimally integrate task demands and perceptual input.

Disclosures: D. Kliemann: None. S. Anzellotti: None. D. Ayyash: None. J. Gabrieli: None. R. Saxe: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.13/KKK32

Topic: H.02. Human Cognition and Behavior

Support: National Science Foundation BCS-1455866

Title: Neural control strategies underlying human-human physical joint interaction for object manipulation

Authors: *K. MOJTAHEDI, J. FINE, M. SANTELLO;
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Abstract: Research into the behavioral and neural foundations of social coordination have typically employed tasks, such as finger tapping, that focuses on visual or auditory coupling between actors. However, a large component of non-verbal social coordination involves physical

interaction. Furthermore, most studies have yielded mixed results when examining the distribution of roles, i.e., leader and follower, as there are no clear task manipulations that consistently result in individuals taking on particular roles. The current study aimed to further investigate how people self-organize into roles during physical interactions, and how these roles are reflected in each actor's neural dynamics. We examined how the EEG dynamics of leaders (or followers) differed when they are assigned that role or when it emerges naturally. The experiment involved two participants lifting and balancing a U-shaped object, using one hand each. Dyads of participants were either assigned to a group with either no a-priori role assignment (Human-Human; H-H group), or with one person being assigned a role (Leader-Follower; L-F group). Cortical activity was assessed using electroencephalography (EEG) recorded from both subjects. EEG activity was examined using time-frequency (wavelet) analyses to determine complementary patterns of brain activity in the H-H and L-F groups. Behavioral results revealed subjects naturally took on leader-follower roles in the H-H group. EEG results in frontal alpha band activity (11-14 Hz) showed a significant discrimination between leader and follower in both the H-H and L-F groups. These differences in frontal activity are likely driven by a tendency for leaders to exert more active control in action planning and corrective processes during coordination. We also found group differences in the low gamma band (30-35 Hz). Gamma band modulation was more pronounced over central areas for H-H dyads, whereas it was larger over frontal areas for L-F dyads. This result implies that being assigned a role versus taking it on naturally can have differential consequences in terms of what cortical areas are recruited. Overall, our results indicate that certain brain areas are commonly recruited in leaders and followers, whereas different areas are recruited pending how this role distribution was determined, i.e., assigned or emergent. *Key words: social coordination; physical interaction; leader and follower; object manipulation; sensorimotor control.*

Disclosures: K. Mojtahedi: None. J. Fine: None. M. Santello: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.14/KKK33

Topic: H.02. Human Cognition and Behavior

Support: EU Marie Curie Global Fellowship

NINDS R3721135

Nielsen Corporation

Title: The role of the orbitofrontal cortex in regulation of interpersonal space: Evidence from lesions and intracranial recordings

Authors: *A. PERRY[1], J. STISO[1], C. DEWAR[1], J. LIN[2], R. T. KNIGHT[1];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]Univ. of California, Irvine, Irvine, CA

Abstract: Interpersonal distance is central to communication and complex social behaviors but the neural correlates of interpersonal distance preferences are not defined. Previous studies suggest that damage to the orbitofrontal cortex (OFC) is associated with impaired interpersonal behavior. To examine whether the OFC is critical for maintaining appropriate interpersonal distance (IPD), we tested 8 patients with OFC damage in a real-world behavioral interpersonal distance task and compared them to healthy controls and to patients with lesions restricted to the lateral prefrontal cortex. Only patients with OFC damage showed abnormal interpersonal distance preferences. The comfortable distances these patients chose with strangers were closer than controls ($p < 0.005$) and resembled distances normally used only with close others. We then utilized the spatial and temporal advantages of intracranial cortical recordings to test when neuronal populations in the OFC are activated during an interpersonal distance computerized task. We tested 6 patients with intractable epilepsy, who had been implanted with electrode grids or depth electrodes over or in OFC for pre-operative monitoring using a computerized IPD task. Some patients had depth electrodes in additional regions of interest, such as the amygdala and insula. Increases in spectral power in the high frequency band range (HFB: 70-150 Hz) were used to index neuronal activation. Our results show complex patterns of HFB activation in the OFC, as well as in other limbic regions during the interpersonal distance task. Together, these studies provide evidence for a key role of the orbitofrontal cortex in regulating interpersonal behavior.
Support: This work was supported by the EU Marie Curie Global Fellowship (to AP); NINDS R3721135 and the Nielsen Corporation (to RTK)

Disclosures: A. Perry: None. J. Stiso: None. C. Dewar: None. J. Lin: None. R.T. Knight: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.15/KKK34

Topic: H.02. Human Cognition and Behavior

Title: Facial affect recognition in schizotypy using the n170 and n250 of the event-related potential

Authors: *J. MORALES, S. SARKISSIANS, J. ABARA, M. SERGI;
Psychology, California State Univ. Northridge, Northridge, CA

Abstract: Deficits in social cognition are consistently found in persons with schizophrenia. Neurocognitive studies of persons with schizophrenia and schizotypy suggest a wide range of deficits, including attention, working memory, emotion recognition, and response inhibition. Facial emotion recognition is a key aspect of social cognition. Schizophrenia is associated with impaired facial processing as observed in the N170 and N250. The N170 and N250 are two event-related potentials (ERPs) that have been studied in relation to face processing in schizophrenia. The N170 component relates to facial features encoding. Whereas, the N250 component relates to affect decoding.

There has been little research on facial affect recognition in psychosis-prone or persons with schizotypy. Schizotypy is conceptualized as a subclinical manifestation of the same biological factors that give rise to schizophrenia and other schizophrenia spectrum disorders. Examining emotion recognition in schizotypy eliminates many confounds associated with schizophrenia research such as medication effects, social isolation, chronic hospitalization, and generalized cognitive deficits. This grants the examination of whether emotion recognition deficits reflect susceptibility to schizophrenia.

Electroencephalography studies of schizophrenia and schizotypy have yielded varied results on the N170 and N250. The aim of this study was to examine affect decoding and encoding in controls (low) and psychometric schizotypes (high) using the N170 and N250 components. For the present study, participants were screened utilizing the Schizotypal Personality Questionnaire-Brief and given an emotion recognition continuous performance task (CPT) utilizing Ekman's emotional facial expressions. ERPs were recorded while participants performed a computer-administered visual Happy-Happy CPT. The ERP waveform for fear emotion was evaluated, in which the N170 and N250 were identified.

This study hypothesized differences between controls and schizotypes on the amplitude of the N170 and of the N250. Repeated measures analysis of the amplitude for the N170 and for the N250 yielded no significant difference between controls and schizotypes, $F(1,12) = .79$, $p = .391$. Controls and schizotypes do not differ in early stages of facial affect recognition. This finding suggests that schizotypes produced similar neural activation patterns for facial features encoding and affect decoding as those produced in controls.

Disclosures: J. Morales: None. S. Sarkissians: None. J. Abara: None. M. Sergi: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.16/KKK35

Topic: H.02. Human Cognition and Behavior

Support: NRF-2011-0027921

Title: Cortical activity during others' preference estimation

Authors: ***J. PARK**[1], S.-P. KIM[1], J.-W. SOHN[2];
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Abstract: Humans often attempt to predict what others prefer in social interactions. As one of the theoretical bases for how humans can predict the preference of others, perspective taking highlights the human ability of inhibiting one's own preference while estimating emotions or thoughts of others from their point of view. Previous neuroimaging studies have revealed that the network of dorsal medial prefrontal cortex (dmPFC) and right temporoparietal junction (rTPJ) is related to the ability of predicting others' preference. However, it remains unknown whether cortical brain oscillations also play a role in prediction of others' preference. In particular, brain oscillations that are modulated differently when people report their own preference and predict the preference of others would suggest a neural basis for the perspective taking process. To investigate the role of the brain oscillations in perspective taking process, we carried out human experiments in which information of other's preference is minimally available while measuring subject's electroencephalography (EEG). Twenty participants (all female, average age: 21.86) conducted the task of predicting the preference of others while only a facial picture of other's is given. In each trial of the task, participants were shown a picture of others or self for three seconds, followed by the presentation of a movie poster at which participants estimated preference as liking or disliking. A time-frequency analysis was used to analyze temporal changes of the power of brain oscillations. Participants could predict others' preference for movies with accuracy of 56.89 ± 3.16 % and ten out of twenty participants exhibited prediction accuracy higher than a chance level (95% interval, permutation test). There was a significant difference in the power of the right parietal alpha (9~14Hz) oscillation when participants reported self preference and predicted others' preference ($p < 0.05$). The power of the left temporal beta (18~25Hz) oscillation (0~0.5 s after the onset of poster presentation) for all the trials was significantly but marginally correlated with individual prediction accuracy of participants ($r^2 = 0.2903$). In contrast, beta power ratios between the trials of predicting other's preference and reporting self preference showed a much higher correlation with individual accuracy ($r^2 = 0.7857$), indicating that left temporal beta oscillations may reflect the ability of taking other's perspective while suppressing self preference. Our results suggest that right parietal alpha and left temporal beta oscillations may be correlated with one's perspective taking ability to predict what others prefer.

Disclosures: J. Park: None. S. Kim: None. J. Sohn: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.17/KKK36

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH049334

NIH Grant T32 MH018870

American Psychiatric Foundation

Leon Levy Foundation

NIH Bench to Bedside Award

Title: Disruptions in connectivity to frontal theory of mind networks underlies deficits in naturalistic social cognition in schizophrenia

Authors: *G. H. PATEL[1], S. ARKIN[1], N. STRAUSS[1], H. M. DE BAUN[1], C. C. KLIM[1], R. BERMAN[2], D. LEOPOLD[2], D. C. JAVITT[1];
[1]Columbia Univ., New York, NY; [2]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Introduction: Deficits in social cognition are a major cause of psychosocial disability in schizophrenia patients (SzP). Major components of social cognition include the detection and use of facial affect information to understand the intentions of others. Underlying these operations are visual processing areas, face processing areas, attention networks, and theory of mind networks. SzP have been shown to be impaired in potentially all three networks, especially in dynamic situations. This study investigates the neural and cognitive bases of poor social cognition by tracking eye-movements during a naturalistic social cognition task along with resting-state functional connectivity in SzP vs. healthy controls (HC).
Methods: 18 SzP and 18 HC both performed The Awareness of Social Inference Test (TASIT) with eye-tracking, which involves answering questions about short video clips of social situations, and also underwent both resting-state and task functional magnetic resonance imaging (fMRI). For each subject, the distance of eye-position from the mean position of the HC on each video frame was averaged across frames (HC eye-position was compared to mean of all other HC). Task fMRI data were used to localize the visual, face-processing, attention network, and theory of mind areas, which were then used to calculate interareal connectivity at rest. Connectivity was then correlated with performance on TASIT.
Results: SzP performed worse in answering questions about TASIT videos involving sarcasm vs. lies. Sarcasm performance also correlated with the percentage of time spent within 2 standard deviations of the mean eye-position of HC (r=.35). In HC, performance on TASIT sarcasm

videos was significantly correlated with the connectivity within and between visual and attention areas, whereas in SzP performance significantly correlated with connectivity of visual, face-processing, and attention areas with frontal theory of mind areas.
Conclusions: This study demonstrates the utility of using naturalistic stimuli to study social cognition deficits in Sz and related psychiatric disorders. The difference in eye-tracking patterns may reflect impaired orientation to socially relevant features of the environment and/or impaired interpretation of these features. The functional connectivity-performance correlations indicate that connectivity to theory of mind areas underlies these deficits.

Disclosures: G.H. Patel: None. S. Arkin: None. N. Strauss: None. H.M. De Baun: None. C.C. Klim: None. R. Berman: None. D. Leopold: None. D.C. Javitt: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.18/KKK37

Topic: H.02. Human Cognition and Behavior

Support: NIMH IRP

NIMH Bench-to-Bedside Award

Title: Naturalistic viewing and neuronal signatures in a ketamine model of schizophrenia

Authors: *R. A. BERMAN[1], G. H. PATEL[2,3], D. C. JAVITT[2,4], D. A. LEOPOLD[1];
[1]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Columbia Univ., New York City, NY; [3]New York State Psychiatric Hosp., New York City, NY; [4]Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: To navigate the social world successfully, primates rely on dynamic visual cues that convey critical information about actions, intentions, and emotions. Studies of schizophrenic patients suggest that impairments in dynamic perception may contribute to the cognitive and social deficits that are central to the disease (Butler et al. 2009, Martinez et al 2012). In the present study, we investigated the neural mechanisms of dynamic visual perception in the macaque, using a free-viewing paradigm and naturalistic videos to measure gaze behavior and accompanying neuronal activity in a ketamine model of schizophrenia. Monkeys freely viewed a series of five-minute nature videos, which were either "social movies" featuring macaque monkeys engaged in social interactions, or "non-social movies" featuring natural scenes with no animals present (Russ et al. 2015). Monkeys viewed the same set of social and non-social movies

multiple times, both under control conditions and after systemic administration of subanesthetic doses of the NMDA antagonist ketamine (0.25-1.0mg/kg). We measured eye movement behavior and additionally recorded the activity of neurons in the anterior fundus face patch (AF) using specialized chronic microwire bundles that permit longitudinal recording from single cells. As recently reported by our laboratory (McMahon et al. 2015), neurons in AF are reliably driven during free-viewing of the movie stimuli, with each cell exhibiting a unique, signature response across repeated viewings of given movie. This property allowed us to monitor neuronal responses to the dynamic movies across many days and across conditions (control and ketamine). Behavioral analysis of eye movement data showed an increase in fixational drift after ketamine injection, but monkeys nonetheless continued to direct gaze to the content of the movies, suggesting that ketamine did not strongly affect the overall pattern of movie viewing. By contrast, the responses of single AF neurons were often altered by ketamine administration, with an effect that depended on the type of movie stimulus (social or non-social). Specifically, we found that ketamine caused a decrease in neuronal responses during the social movies, whereas responses to non-social movie stimuli were slightly increased under ketamine conditions. These results indicate that the encoding of biological stimuli may be particularly affected by ketamine administration, and suggest that dynamic paradigms will be an important tool for delineating the neural bases of perceptual deficits in schizophrenia.

Disclosures: R.A. Berman: None. G.H. Patel: None. D.C. Javitt: None. D.A. Leopold: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.19/KKK38

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH049334

NIH Grant T32 MH018870

American Psychiatric Foundation

Leon Levy Foundation

Title: Engagement of visual, face,, attention, and theory of mind areas in schizophrenia during naturalistic viewing

Authors: *S. ARKIN[1], C. C. KLIM[2], R. BERMAN[3], D. LEOPOLD[3], D. C. JAVITT[2], G. H. PATEL[2];

[1]Psychiatriy, [2]Psychiatry, Columbia Univ., New York, NY; [3]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Introduction: Deficits in social cognition are a major cause of psychosocial disability in schizophrenia patients (SzP). A major component of social cognition is social communication, which requires the engagement of multiple networks, including those underlying visual processing, face processing, control of attention, and theory of mind operations. Separate studies of SzP have demonstrated potential deficits in all four networks, but it remains unclear whether a deficit in one network propagates to the other networks or the functioning of all four networks is deficient. To study this, we recorded brain activity evoked by the watching of a cinematic movie in SzP vs. healthy controls (HC) to determine which networks were deficient.
Methods: 11 SzP and 6 HC underwent functional magnetic resonance imaging (fMRI) while watching 15 minutes of a cinematic movie ("The Good, the Bad, and the Ugly"), as well as resting state and task fMRI. Task fMRI data were used to localize the visual, face-processing, attention network, and theory of mind areas. The timecourse for each voxel of BOLD activity evoked in each subject by the movie was correlated with the average timecourse of the HC (inter-subject synchronization; each HC correlated with average all other HC). Resting state data was used to calculate interareal correlation of activity at rest, which was then compared to the resting state data from 500 subjects collected in the Human Connectome Project.
Results: Visual, ventral-stream face-processing, and attention areas were strongly synchronized in both groups. Contrasting the SzP and HC maps revealed that HC demonstrated significantly more synchronization of middle and posterior superior temporal sulcus (STS) and both dorsomedial and lateral frontal areas, overlapping with face-processing networks and frontal theory of mind areas. SzP had more synchronization of posterior cingulate, angular gyrus, and parts of ventromedial frontal cortex, corresponding to the default mode network. Resting state functional connectivity analyses found that middle and posterior STS areas are functionally connected with each other and with the frontal areas.
Conclusions: This study demonstrates the utility of naturalistic viewing paradigms to simultaneously evaluate the functioning of multiple networks in Sz and other neuropsychiatric disorders. We find that visual processing and attention areas are equally engaged in SzP and HC in viewing of the movie, while face-processing and theory of mind network areas fail to be engaged in SzP. Pairing these findings with resting state functional connectivity reveals potential connectivity deficits that may underlie this failure.

Disclosures: S. Arkin: None. C.C. Klim: None. R. Berman: None. D. Leopold: None. D.C. Javitt: None. G.H. Patel: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.20/KKK39

Topic: H.02. Human Cognition and Behavior

Support: Bernstein Award to J.G. (BMBF 01GQ1006))

Title: Characterizing mentalizing processes during cooperative decisions using EEG-hyperscanning

Authors: *T. RUSCH[1], M. SPEZIO[3], J. P. GLÄSCHER[2];
[1]Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamurg, Germany; [3]Psychology, Scripps Col., Claremont, CA

Abstract: Theory of Mind and mentalizing capacities are classically examined with the so called "False Belief Task". We transferred this task's basic concept into the realm of social decision making to investigate how people predict other agents' behavior during value guided choices. Two individuals engage in a simple choice task, in which probabilistic outcomes that also depend on the partner's choice have to be learned. Cooperative choices are highly rewarded. After periods of successful cooperation one player's (Learner's) outcome distribution is reversed, but only the other player (Teacher) is informed about the reversal. The Learner thus has a false belief about the state of the world. To maximize the reward, the Teacher must track how the Learner's false belief evolves over time and make choices to "communicate" the contingency switch to the Learner. The Learner on the other hand needs to detect and recognize the Teacher's incentive to react accordingly. On each trial both players make explicit predictions of their partner's choices before making their own. Throughout the course of the task both players need to act as Teacher and Learner an equal number of times. Furthermore, EEG is continuously recorded from both players synchronously while they engage in the interactive task. Continuous choice behavior and low RTs during periods of successful cooperation suggest, that the players rely on in the learning period established beliefs about the state of the world and the partner's belief. However, after a reversal the Teacher initially predicts unchanged behavior for the Learner in line with the Learners' false belief. Nevertheless, he/she switches his/her own choice. On the one hand, the Teacher's choice switch assures a maximal possible outcome for both players given the Learner's false belief, on the other hand, it informs the Learner about the contingency switch. The Learner detects the Teacher-signal and adapt his/her predictions about the Teacher's choices accordingly. After accumulating enough evidence the Learner reacts to the new reward distribution and adjust his/her choice. The Teacher accurately predicts the learning curve and matches his/her own choices. Increased mentalizing processes required when acting as

Teacher or Learner in comparison to periods of stable cooperation are reflected in decreased theta, alpha as well as beta power. The interactive nature of the task, the necessity to track and incorporate the partners beliefs into the own decision making process and continuous EEG Hyperscanning allow the examination of mentalizing processes on a behavioral and neuronal level in an ecologically valid setting.

Disclosures: T. Rusch: None. M. Spezio: None. J.P. Gläscher: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.21/KKK40

Topic: H.02. Human Cognition and Behavior

Title: Food-related symptoms in autism are related to aberrant gustatory cortex intrinsic functional connectivity

Authors: *M. A. COLLINS[1], S. C. MILLEVILLE[1], S. E. WOHLTJEN[1], J. E. INGEHOLM[1], G. L. WALLACE[2], W. K. SIMMONS[3], S. J. GOTTS[1], A. MARTIN[1];
[1]NIMH, NIH, Bethesda, MD; [2]Dept. of Speech and Hearing Sci., George Washington Univ., Washington DC, DC; [3]Laureate Inst. for Brain Res., Tulsa, OK

Abstract: Abnormal sensory processing is a well-established characteristic of autism spectrum disorder (ASD). Aberrant food selectivity and gustatory sensations are clinically relevant features of ASD, yet the neural bases of these phenomena are largely unknown. In a previous report on neural responses to gustatory stimulation and pictures of appetizing food in typically developing (TD) subjects we identified three critical areas in insula cortex; the left and right mid-dorsal insula and the left anterior insula (Simmons et al., 2013). In the current study we used these regions as seeds in an analysis of resting state fMRI scans to look for differences in neurocircuitry between ASD subjects without intellectual disability (N = 50, mean age: 19.2 ± 8.5, IQ: 112±14.7) and TD subjects (N = 45, mean age: 18.2 ± 3.4, IQ: 116 ± 10.7) matched on age, sex, IQ, and head motion. All ASD subjects met standard clinical and research diagnostic criteria and all subjects completed the Adolescent/Adult Sensory Profile, which contains a number of questions addressing food preferences. Each insula seed region was associated with widespread reductions in correlation for the ASD, relative to the TD subjects in regions typically associated with social processing including the right STS, right post-central gyrus, pre-central and superior frontal gyri bilaterally, and right cingulate cortex ($p<0.05$, FDR corrected). Abnormally increased connectivity between the thalamus and all three seed regions was also observed ($p<0.05$, FDR corrected). Preliminary analyses indicate that similar reductions in seed-

based connectivity are significantly related to increased food-related symptoms ($p<0.01$) in ASD. These findings suggest that reduced connectivity between the insula and other brain regions may underpin food-related symptomatology in ASD.

Disclosures: M.A. Collins: None. S.C. Milleville: None. S.E. Wohltjen: None. J.E. Ingeholm: None. G.L. Wallace: None. W.K. Simmons: None. S.J. Gotts: None. A. Martin: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.22/KKK41

Topic: H.02. Human Cognition and Behavior

Title: Discriminating between sarcasm and lying using neural correlates from the amygdala and cingulate cortex

Authors: *Y. PATHAK[1], E. H. SMITH[1], S. SINHA[2], G. M. MCKHANN, II[1], S. A. SHETH[1]; [1]Neurolog. Surgery, Columbia Univ., New York, NY; [2]Neurosurg., Rutgers Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Emotional recognition is an important aspect of social cognition and allows us to cultivate interpersonal relationships and navigate social contexts. Complex semantic constructs communicated through language such as sarcasm are uniquely human. Deficits in identifying these stimuli are associated with neurological and psychiatric disorders, including autism and psychosis. The socio-emotional network, delineated through lesion studies and neuroimaging, includes the limbic system, facial processing system and mirror-neuron network. Major nodes of these systems include amygdala, anterior cingulate cortex (ACC), prefrontal cortices and hippocampus. The role of the amygdala in identifying faces and emotional valence is well established. The ACC has known involvement in cognitive control and conflict. In this study, we propose that sarcasm represents a level of semantic conflict since the verbal and non-verbal communication is incongruent. Therefore, we hypothesize that neural data from the amygdala and the ACC can be used to classify between sarcastic cues and lying.
We analyzed local field potentials (LFPs) recorded from depth electrodes implanted to monitor epilepsy patients. Specifically, we recorded from the amygdala and the ACC while 4 subjects were engaged in The Awareness of Social Interference Test (TASIT), a task designed to examine social perception and emotional recognition. Power spectral density (PSD) was computed based on subject-perceived condition using the multi-taper method. We also implemented a linear discriminant analysis (LDA) to classify between the perception of lying and sarcasm. Data from all four subjects were combined to increase the power of our analysis. We trained on 74% (68

trials) of the dataset and used z-scored spectrograms from the last 10 seconds of each trial as our classification feature. The remaining 26% (24 trials) of the data was used as the test set.
For both trial types, we observed an increase in theta power (4-7 Hz) at the amygdalar contacts and an increase in theta and alpha power (8-12 Hz) at the ACC contacts compared to baseline. The maximum prediction accuracy of identifying sarcasm versus lying from the LDA classification was 67% for the amygdala and 63% for the ACC.
The results confirm that the amygdala and the ACC play a significant role in discerning complex semantic constructs in human language. Classification features tested in this study could serve as potential inputs for closed-loop systems designed to treat conditions such as autism. Further work will examine other emotional conditions, as well as field-field and spike-field coherence within and between amygdala and ACC.

Disclosures: Y. Pathak: None. E.H. Smith: None. S. Sinha: None. G.M. McKhann: None. S.A. Sheth: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.23/KKK42

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI 26350987

MEXT KAKENHI 16H01486

JSPS KAKENHI 15H01846

Title: Common neural substrates of initiator and responder in spatial and feature-based joint attention: a hyperscanning fMRI study

Authors: A. YOSHIOKA[1], T. KOIKE[2], E. NAKAGAWA[2], M. SUMIYA[2], S. OKAZAKI[2], N. SADATO[2], *H. C. TANABE[1];
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Abstract: Joint attention (JA) is the ability to coordinate and share attention between two persons regarding objects. In a dyad, one person initiates JA (IJA) while the other respond to a JA bid (RJA). It seems important issue to underpin the core neural substrate of initiative and responding to JA bid. In the previous studies, however, the gaze-related brain activity was contaminated because the task has to use spatial attention. To overcome this problem, we

developed a feature-based JA task. Here, we explore the core neural substrates of initiative and responding to JA using spatial-based and feature-based JA task during a real interactive hyperscanning functional magnetic resonance imaging (fMRI). Twenty-two pairs (44 volunteers) were participated in the fMRI study. In the feature-based JA task, stimuli were constructed with 4 DIMENSIONS such as number, shape, color, and pattern. Each stimulus has one of 4 CHARACTERS in each dimension. The initiator freely chose one of 4 DIMENSIONS of the stimulus and informed it by utterance (IJA) during 2 sec silent period. Immediately after the initiator's direction, the responder attended the same dimension of the object and identified a CHARACTER of this dimension (RJA). In the next scan period, the responder answered the character of this designated dimension during the 2 sec silent period. The initiator judged whether the answer is correct or not immediately and feedback verbally in the same period. The results showed that common IJA regions in both feature-based and spatial JA were anterior cingulate cortex (ACC) expanding to caudal medial prefrontal cortex (MPFC), striatum, right cerebellar hemisphere Crus II, whereas common RJA regions were bilateral superior temporal sulcus/gyrus, rostro-medial MPFC, posterior MPFC, and bilateral anterior cerebellum IX. As for the response and feedback period, the anterior to middle cingulate cortex, bilateral putamen, amygdala, superior temporal region, supramarginal gyrus, premotor cortex, and right inferior frontal gyrus were commonly activated in all four conditions. To take our previous results into account, ACC and superior temporal region seems to be the important region in IJA and RJA, respectively.

Disclosures: A. Yoshioka: None. T. Koike: None. E. Nakagawa: None. M. Sumiya: None. S. Okazaki: None. N. Sadato: None. H.C. Tanabe: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.24/KKK43

Topic: H.02. Human Cognition and Behavior

Title: Cognition of biological body movement as a risk factor for social bullying in unaffected siblings of children with autism spectrum disorders

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Albuquerque, NM; [5]MGH/MIT AA Martinos Ctr. Biomed. Imaging, Massachusetts Gen. Hosp., Boston, MA

Abstract: Introduction: Children with autism spectrum disorders (ASD) and their unaffected siblings (US) are frequent targets of social bullying, which leads to severe cognitive, emotional and social consequences. Understanding the risk factors is essential for developing preventative measures. Discriminating different biological body movements (BBM) requires fast and flexible processing and interpretation of visual cues. As previously reported, children with ASD show difficulties in tasks with BBM. Since US show an autism trait endophenotype we expect that they will also display BBM deficits. We investigate whether their deficits relate to simple visual-spatial perception, interpretation of social intentions expressed in BBM, or selection/execution of behavioral responses. **Methods**: *Participants*: 8 US, 8 matched TD children age 7-14; *Tasks/Measurements*: Blue Man Test (BMT, Ciesielski, 2007), visual-spatial N-Back stop-response task (accuracy, RTs, ERPs); Social BMT for Narrative Interpretation of social cues and intentions expressed by BBM of BMT; analysis of narratives with a latent Dirichlet allocation [LDA]); Neuropsychological Test Battery; Social Experience Questionnaires with children and parents. **Results**: As compared to TD the US displayed: (i) Prolonged RTs in visual identification of complex figures and BBM stimuli, but not in simple visual-spatial perception; (ii) Low self-awareness of social bullying in contrast to high parental reports; (iii) Lower quality and repetitious words in social interaction narratives (LDA); (iv) Abnormally reduced amplitude of ERPs P300, a physiological marker of decision making/memory update, despite high accuracy of performance on the visual-spatial BMT. **Conclusions**: The abnormally prolonged visual information processing of complex visual information in US group may be at the foundation of difficulty in interpretation of fast social cues from peers leading to poor awareness that a bullying act has occurred and to less flexibility of words to describe it. The ERP P300 component may elucidate the neural basis of the prolonged complex visual processing and suggest novel preventative measures.

Disclosures: V.E. Newton: None. I. Solis: None. C. Bouchard: None. C. King: None. G.E. Avina: None. J. McClain: None. K.R. Ciesielski: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.25/KKK44

Topic: H.02. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship

CNI Innovation Grant

Templeton Foundation Varieties of Understanding Grant

Title: Neural integration of costs and outcomes in evaluations of prosocial actions

Authors: *N. VELEZ, H. GWEON;
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Abstract: The ability to evaluate other people's actions is critical for deciding whom to trust, befriend, or ask for help. Such evaluations rely on the benefits accrued from others' actions, but this is not the only relevant factor; for example, if a stranger holds a door open for you, you might think that they are being helpful, even if they were mistaken and don't provide any real benefit. Thus, evaluations of others' actions are driven not only by the external outcomes (value) of the action, but also by the intentions behind it, which can often be gleaned from the cost incurred by the actor.

The present work investigates neural signals involved in evaluation of prosocial actions within two networks of brain regions. First, we explored brain regions that are recruited when reasoning about other people's mental states, including medial prefrontal cortex (MPFC) and the temporoparietal junction (TPJ). Second, we examined neural populations involved in the anticipation and evaluation of rewards—including social rewards—such as the striatum and subregions in the MPFC. We asked whether distinct neural populations encode the *cost* and *outcome* of others' actions, and what neural populations might be involved in integrating cost and outcome to form social evaluations.

In an fMRI study, we presented 18 participants with 48 stories in which one character gave a gift to another character. In each story, we orthogonally varied the *cost* of the gift (i.e., the difficulty of obtaining the gift), and the *outcome* (i.e., whether the recipient liked it). After each story, participants were asked how much *praise* the giver deserved.

We found separable neural signals that reflected the *cost* and *outcome* of the giver's actions: activity in a subregion of the striatum reflected cost, while patterns of activation within dorsal MPFC distinguished between positive and negative outcomes. Critically, neural activity in ventral MPFC showed a positive correlation with participants' ratings of praiseworthiness. Our results suggest that brain regions that have been traditionally implicated in mentalizing and reward processing contribute to forming evaluations of other people's actions. Further work will systematically examine how neural representations of outcomes and costs are integrated in vMPFC.

Disclosures: N. Velez: None. H. Gweon: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.26/KKK45

Topic: H.02. Human Cognition and Behavior

Support: This work was supported by JSPS KAKENHI grant numbers 16H02839 and 15H01671

Title: Socially anxious tendency affects discrimination of emotions in other's gaze

Authors: *Y. TSUJI, S. SHIMADA;
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Abstract: Socially anxious tendency is considered as a potential for social anxiety disorder (SAD), which is characterized by excessive fear to a social situation associated with being evaluated or embarrassed by others. Especially, gaze of others is known to frequently induce social anxiety. Here we employed an other's gaze emotion discrimination task to examine the effect of socially anxious tendencies on social cognition. We generated emotionally ambiguous gazes by employing a morphing technique (neutral, disgust 10-100% in 10% steps, and happy 10-100% gazes). Subjects were required to judge whether the stimulus was positive or negative. Seventeen male adult subjects (aged 21.2 ± 1.35, mean ± SD) participated in the study. Two subjects were excluded from analysis because they did not correctly perform the experiment. The participant's level of social anxiety was examined by means of the Japanese version of the Social Phobia Inventory (SPIN-J). The SPIN-J is a 17-item questionnaire, each rated on a 5-point Likert-type scale. The total score of SPIN-J ranges from 0 to 68, and the clinical cut-off point is at 30. The SPIN-J is designed to measure three symptom dimensions: fear, avoidance, and physiological arousal. The subjects were assigned to the high (HSA: n = 9) or low socially anxious tendency groups (LSA: n = 6) on the basis of clinical cut-off point. To examine the differences in judgment between the LSA and HSA groups, logistic curves were fitted to the subject's response function in the emotion discrimination task. We estimated the point of subjective equality (PSE) from the logistic curve where positive and negative judgment probabilities are equal (50%). The mean PSE in the HSA group (disgust = 14 ± 8.3%, mean ± SE) was significantly smaller than that in the LSA group (disgust = 30 ± 11%) (Wilcoxon's Rank Sum Test: $Z = 1.77$, $p < 0.05$). We also found a negative correlation between the score of the avoidance, which is a subscale of SPIN-J, and PSE (correlation coefficient $\rho = -0.64$, $p < 0.01$). These results suggest that highly socially anxious subjects have a tendency to recognize ambiguous emotional gazes as negative.

Disclosures: Y. Tsuji: None. S. Shimada: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.27/KKK46

Topic: H.02. Human Cognition and Behavior

Title: An fNIRS study of cooperativeness during synchronized tapping task

Authors: *A. MURAKAMI, H. YOKOUCHI, S. HIWA, T. HIROYASU;
Doshisha Univ., Kyotanabe-shi, Japan

Abstract: [Introduction] Communication is an important process for our human beings. Especially, the ability to behave in adapting to others' feelings and willingness is referred to as cooperativeness. Higher cooperativeness allows us to communicate with others better and understand each other easier. Cooperativeness varies between different people, and the brain activities when we have an interaction with others have not been investigated well. If the relation between cooperativeness and the brain activities would be revealed, it may help diagnosis and treatment of communication disorders. Our goal of this study is to examine human brain activity during cooperative work. In this paper, we investigated the brain activity during human-machine cooperative work as a preliminary study for human-human cooperative work. We discussed how human processed the information from others and determined their behaviors. [Method] Cooperativeness is often associated with synchronization with others. Therefore, a synchronized tapping task between a subject and a machine has been applied to investigate a mechanism of human predictive behavior for synchronization. In this task, the subject attempted to synchronize a button press with an auditory stimulus. The stimulus intervals were periodically changed. Sixteen healthy male subjects participated in this experiment, and their cerebral blood flow changes were measured using functional near-infrared spectroscopy (Hitachi ETG-7100). Furthermore, social skills of the subjects were evaluated using a questionnaire called KiSS-18. [Result & Discussion] We analyzed their task performances using synchronization error (SE) calculated by a time difference between auditory stimulus and tap interval. The average of time-course SE of all subjects indicated that they could adapt to the change of rhythm within a few times of tapping. From the data of blood flow changes, regions which were activated among over 80 percent of subject were the DLPFC in frontal region and the angular gyrus in left temporal region. The subjects were divided into high-performance (3 subjects) and low-performance (3 subjects) groups based on KiSS-18 scores, and the activated regions differed between the groups. When the rhythm of a stimulus is changed, people attempt to adjust timing to the changed rhythm. It is reported that the angular gyrus has the function of recognition and the DLPFC have the function of behavior control. Our results suggest that the subjects recognized the change of rhythm and controlled behavior of tapping to adjust timing to the changed rhythm. [Conclusion] It was suggested that cooperativeness could be associated with DLPFC and angular gyrus.

Disclosures: A. Murakami: None. H. Yokouchi: None. S. Hiwa: None. T. Hiroyasu: None.

Poster

268. Neural Processes of Social Cognition

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 268.28/KKK47

Topic: F.02. Behavioral Neuroendocrinology

Title: Visual erotic stimulation: different cerebral responses in homosexual and heterosexual men

Authors: *C. GÓMEZ[1], C. AMEZCUA-GUTIÉRREZ[1], M. HERNÁNDEZ-GONZÁLEZ[2], M. A. GUEVARA[2];
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Abstract: Visual stimuli with sexual content have been widely-used to induce a state of sexual arousal that is associated with psycho-physiological reactions and the activation of different brain areas, including the prefrontal and parietal cortex, which are involved in detecting and processing stimuli. In humans, a whole range of individual, social and cultural factors -for example, sexual preference- affect the perception of, and responses to, sexual or erotic stimulation. Given this background, the aim of this work was to determine the functioning of these cortical areas in homosexual and heterosexual men during observation of visual stimuli with sexual content. The study included 20 healthy men, 10 homosexual and 10 heterosexual, aged 18-30 years. Their electroencephalographic activity (EEG) was recorded for 3 minutes at rest and then while observing a video with erotic content (5 min) or a neutral video (5 min). Absolute power (AP) was analyzed in the frontopolar (Fp1, Fp2), frontal (F3, F4) and parietal (P3, P4) cortices for the following EEG bands: delta (1-3.5 Hz), theta (4-7.75 Hz), alpha1 (8-10.5 Hz), alpha2 (11-13.5 Hz), beta1 (14-19.5 Hz), beta2 (20-30 Hz), and gamma (31-50 Hz). A sexual arousal scale was applied to measure the responses of all subjects after each video. Although all subjects, heterosexual and homosexual, reported only mild subjective sexual arousal, it was sufficient to induce changes in cerebral functionality. During both videos, the heterosexual group presented a lower theta AP in parietal areas and, like the homosexual men, a decrease of alpha in almost all derivations. While watching both videos, the homosexual men presented a higher theta AP at F2, whereas viewing the erotic video generated higher AP in beta2 and gamma at F3. These data show that during a state of moderate sexual arousal, the functionality of frontopolar, frontal and parietal areas varied depending on sexual preference. The results of this study can contribute to a better understanding of the cortical functionality that

underlies differential processing and assigning of incentive value to stimuli in homosexual and heterosexual men during sexual arousal.

Disclosures: C. Gómez: None. C. Amezcua-Gutiérrez: None. M. Hernández-González: None. M.A. Guevara: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.01/KKK48

Topic: H.03. Schizophrenia

Title: Preclinical characterization of SUVN-1507035 with multimodal activity alleviating psychosis and depression in preclinical models

Authors: ***N. MUDDANA**, R. SUBRAMANIAN, S. EDULA, S. PETLU, S. YATHAVAKILLA, R. ABRAHAM, P. JAYARAJAN, R. PALACHARLA, J. THENTU, R. DYAVARASHETTY, H. KALYANI, A. MOHAMMED, S. RAVELLA, R. NIROGI; Suven Life Sci., Hyderabad, India

Abstract: Psychosis such as schizophrenia is a debilitating disease. Most patients undergoing treatment for psychosis are often not socially productive. The currently marketed atypical as well as typical antipsychotics drugs (APD) have a number of draw backs with respect to efficacy and safety profile. APD are not successful in addressing the various domains of schizophrenia. SUVN-1507035 is one of our multimodal molecules having affinity for 5-HT/dopamine receptors. SUVN-1507035 was assessed in the conditioned avoidance response for efficacy. SUVN-1507035 was also assessed in the open field using two different classes of psychomimetics, MK-801 (NMDA antagonist) and amphetamine (Dopaminergic agonist). The antidepressant like activity of SUVN-1507035 was assessed using the differential rReinforcement at low rate 72s (DRL-72s). SUVN-1507035 was tested for its effect on dopamine synthesis rate in reserpinized rats. The safety profile of SUVN-1507035 was investigated using rotarod and the catalepsy assay. SUVN-1507035 was found to have the characteristic response of antipsychotics in the conditioned avoidance response. It reversed hyperlocomotor activity induced by both MK-801 and amphetamine. It also showed antidepressant like activity in the DRL-72s. SUVN-1507035 decreased the dopamine synthesis rate indicating agonistic activity at dopamine receptor in vivo. The efficacy doses were devoid of motor impairment as observed from the rotarod and catalepsy assays, i.e., a separation between the doses which produced efficacy and side effects. SUVN-1507035 is a promising new molecule for the treatment of schizophrenia and associated comorbid disorders.

Disclosures: **N. Muddana:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Subramanian:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Edula:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Petlu:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Yathavakilla:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Abraham:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **P. Jayarajan:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Palacharla:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **J. Thentu:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Dyavarashetty:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **H. Kalyani:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **A. Mohammed:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Ravella:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Nirogi:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.02/KKK49

Topic: H.03. Schizophrenia

Support: NIH/NIMH R01 MH059803

NIH/NIMH R01 MH094320

Title: Pharmacologic augmentation of cognitive training (PACT): Amphetamine enhances gains in auditory discrimination training in adult schizophrenia patients

Authors: ***N. R. SWERDLOW**[1], M. TARASENKO[1], S. G. BHAKTA[1], J. A. TALLEDO[1], A. I. ALVAREZ[1], E. L. HUGHES[1], B. K. RANA[1], S. VINOGRADOV[2], G. A. LIGHT[1]; [1]UCSD Sch. of Medicine, Dept of Psychiatry, LA Jolla, CA; [2]UC San Francisco, Dept of Psychiatry, San Francisco, CA

Abstract: Background: Targeted cognitive training (TCT) of auditory processing drives improvements in higher-order cognition in schizophrenia (SZ) patients. Gains in TCT

performance can be detected after one session of training. We assessed a pharmacological augmentation of cognitive training (PACT) strategy by testing if the psychostimulant, amphetamine, augments TCT gains in auditory processing speed (APS) in SZ patients and healthy subjects (HS).
Methods: Carefully characterized HS and SZ patients were tested in a screening session (test 1: no pill), followed by a double-blind balanced crossover design (tests 2-3), comparing placebo (PBO) vs. 10 mg of AMPH (1 week between tests). On each test day, 1-h of Posit Science "Sound Sweeps" training was bracketed by 2-4 min pre- and post-training assessments of APS. Training consisted of a speeded auditory time-order judgment task of two successive frequency modulation (FM) sweeps. Screen day testing also included measures of acoustic startle prepulse inhibition (PPI), and event-related potentials (ERPs).
Results: Baseline performance (APS, trials completed) was impaired in patients vs. HS. Auditory system "learning" (APS post- vs. pre-training) was enhanced by AMPH, and this effect tended to be more robust in patients than in HS. Greater sensitivity to AMPH-enhanced learning was associated with higher screening levels of PPI and shorter P3a latency, but not with ERP measures of mismatch negativity or P3a amplitude, or with a list of clinical, demographic or physiological variables, including the rs4680 polymorphism of catechol O-methyl transferase. AMPH-enhanced APS learning in patients was not "state-dependent".
Discussion: AMPH enhances auditory discrimination learning in SZ patients, most likely via an enhancement of intact brain mechanisms in the service of the attentional demands of training. Sensitivity to these effects of AMPH was associated with higher screening levels of PPI and faster P3a latency, potentially reflecting more intact forebrain circuitry; these measures might serve as biomarkers to identify sensitive clinical populations. We do not know whether gains in APS observed in patients after 1-h of TCT predict clinical benefits after a full course of 30-50 h of auditory system exercises. If AMPH can enhance or accelerate the therapeutic effects of TCT, this "PACT" approach could represent a transformative treatment paradigm for SZ.

Disclosures: **N.R. Swerdlow:** None. **M. Tarasenko:** None. **S.G. Bhakta:** None. **J.A. Talledo:** None. **A.I. Alvarez:** None. **E.L. Hughes:** None. **B.K. Rana:** None. **S. Vinogradov:** 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; serves as a site PI on an SBIR grant to Positscience. **G.A. Light:** F. Consulting Fees (e.g., advisory boards); Astellas, Forum Pharmaceuticals, Boehringer Ingelheim, Neuroverse.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.03/KKK50

Topic: H.03. Schizophrenia

Support: A reserch funding agency was renascience

Title: High-dose pyridoxamine add-on treatment for schizophrenia with enhaced carbonyl stress

Authors: *M. MIYASHITA[1], M. ARAI[1], K. TORIUMI[1], T. ICHIKAWA[2], Y. HORIUCHI[1], A. KOBORI[1], K. TAKAHASHI[3], T. TOKUNAGA[3], K. ISHIMOTO[3], H. YUZAWA[4], S. USAMI[5], T. YOSHIKAWA[6], Y. OKAZAKI[3], S. WASHIZUKA[7], N. AMANO[7], S. TAKIZAWA[4], T. MIYATA[8], M. ITOKAWA[1];
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Abstract: Objective: The aim of this clinical trial was to obtain a proof of concept whether high dose pyridoxamine, one of the three forms of vitamin B6, would be a novel treatment for schizophrenia with enhanced carbonyl stress. Method: 10 Japanese schizophrenic patients with high plasma pentosidine that is representative biomarker of enhanced carbonyl stress were recruited in a 24 week, open trial. High dose pyridoxamine ranging from 1200mg to 2400 mg per a day was administered on the present anti-psychotic regimen. Positive and Negative Syndrome Scale (PANSS) and Brief Psychiatric Rating Scale (BPRS) were conducted as efficacy assessments. We also performed Drug Induced Extra-Pyramidal Symptoms Scale (DIEPSS) and Columbia Suicide Severity Rating Scale (C-SSRS) as safety assessment, respectively. Result: As one patient dropped out of the trial due to physical exacerbation, 9 patients completed the trial. Mean decrease rate of plasma pentosidine levels were 26.8% and mean rate of improvement regarding PANSS and BPRS were 8.1% and 10.8%, respectively. Two patients were markedly improved in their psychological symptoms. One patient who harbor frameshift mutation in Glyoxalase 1 (GLO1), which means genetically vulnerable to carbonyl stress, also showed considerable improvement of psychosis accompanied with moderate decrease of plasma pentosidine levels. Additionally, some patients demonstrated improvement of rapport and emotional expression. More than 20% reduction in the assessment scale of drug induced Parkinsonism were found in four patients. Although there are no severe suicide related idea or

behavior, Wernicke's encephalopathy-like adverse drug reactions occurred in two patients followed with completely recovery by thiamine supplementation. Conclusion: High dose pyridoxamine add-on treatment was, at least in part, effective for subpopulation of schizophrenic patients with enhanced carbonyl stress. However it also caused Wernicke's encephalopathy-like adverse drug reactions. Further placebo controlled, randomized trial with careful monitoring will be required to validate the efficacy of pyridoxamine for these patients.

Disclosures: **M. Miyashita:** 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; renascience. **M. Arai:** None. **K. Toriumi:** None. **T. Ichikawa:** None. **Y. Horiuchi:** None. **A. Kobori:** None. **K. Takahashi:** None. **T. Tokunaga:** None. **K. Ishimoto:** None. **H. Yuzawa:** None. **S. Usami:** None. **T. Yoshikawa:** None. **Y. Okazaki:** None. **S. Washizuka:** None. **N. Amano:** None. **S. Takizawa:** None. **T. Miyata:** None. **M. Itokawa:** None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.04/KKK51

Topic: H.03. Schizophrenia

Support: NIH Grant R01MH085666

Title: Juvenile treatment with mGluR2 3 agonist LY379268 ameliorates learning deficits via enhancing NMDAR GluN2B function in a developmental model of schizophrenia

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Abstract: Cognitive deficits are early symptoms of schizophrenia (SZ) with glutamatergic dysfunction during early neurodevelopment contributing to the pathological process. Group II mGluR2/3 agonist has been proposed to be a therapeutic target, but a recent clinical trial failed. However, early intervention targeting mGluR2/3 remains to be an attractive strategy. Indeed, a recent study has shown that mGluR2/3 agonist treatment confers benefits for the early course of SZ but not for the patients with long-standing illness. We hypothesize that early intervention with the mGluR2/3 agonist LY379268 (LY37) will ameliorate cognitive deficits of SZ by targeting NMDA receptors. We tested this hypothesis by using a neurodevelopmental model of SZ based on prenatal administration of methylazoxymethanol (MAM) at embryonic day 17. We found that learning deficits in juvenile MAM rats can be rescued by repeated LY37 treatment in

the early stage. The improved behavioral performance was associated with an increased protein expression of GluN2B and an increased phosphorylated glycogen synthase kinase-3β ser9 level in the PFC. Furthermore, we found that bath application of LY37 induced significant increases in amplitude and decay time of NMDAR-mediated miniature and evoked EPSCs in PFC neurons, indicating an improved function of GluN2B-containing NMDARs. To determine the potential side effect of LY37 treatment during the juvenile period, we examined neuronal excitability and synaptic transmission of developing prefrontal neurons from rats treated with LY37 versus saline (SAL). One hour after the last injection of the 1-week LY37 treatment during P21-P28, decreased excitability of pyramidal neurons but increased amplitude of spontaneous EPSCs were observed. However, there were no apparent differences in neuronal excitability and synaptic transmission between LY37 treatment and SAL control groups after 1, 5 and 10 weeks following drug cessation, indicating that juvenile LY37 exposure and withdrawal did not alter the electrophysiological properties of prefrontal neurons. These results suggest that early treatment with mGluR2/3 agonist improved the learning deficits in a developmental model of SZ by enhancing GluN2B-mediated NMDA receptor function without clear side effects in the PFC neurons. Thus, early intervention has therapeutic value for SZ treatment.

Disclosures: B. Xing: None. M. Wang: None. M.A. Snyder: None. W. Gao: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.05/KKK52

Topic: H.03. Schizophrenia

Support: Grant NN107234 of OTKA

Grant 294313-SERRACO of ERC

KTIA_13_NAP-A-III/1

Title: The role of P2X7 receptors in a rodent PCP-induced schizophrenia model

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Abstract: In this study the expression and function of central P2X7 receptors (P2rx7) were examined in a phencyclidine (PCP)-induced schizophrenia model in mice. In P2rx7+/+ mice,

PCP (2 and 5 mg/kg i.p.) induced hyperlocomotion, stereotype behavior, ataxia and decreased social interactions. In mice, genetically deficient in P2X7 receptors (P2rx7-/-), the social interactions were increased, whereas the PCP induced hyperlocomotion and stereotype behavior were alleviated. The selective P2X7 receptor antagonist JNJ47965567 (30 mg/kg i.p.) partly replicated the effect of gene deficiency on PCP-induced behavioral changes and counteracted PCP-induced social withdrawal. mRNA encoding P2X7 receptors were upregulated in response to PCP in the prefrontal cortex and the hippocampus, respectively. P2rx7 activation released [^{3}H]glutamate from acute prefrontal cortex slices, which was enhanced by preceding in vivo PCP treatment. The amplitude of NMDA evoked currents recorded from layer V pyramidal neurons of cortical slices were slightly decreased by both genetic deletion of P2rx7 and by JNJ47965567 (100 nM). PCP increased mRNA expression encoding NR2A, NR2B and neuregulin 1 and decreased mRNA expression of NR1 and, GABA α1 subunits in the prefrontal cortex of young adult (56 days) P2rx7+/+ mice, but not in juvenile (18 days) or P2rx7-/- animals. In summary, we report here for the first time the alleviation of PCP induced behavioral changes by the inhibition of P2X7 receptors. Functional alterations of glutamatergic transmission in the prefrontal cortex and subsequent changes in the expression of glutamate receptor subunits and other pre- and postsynaptic proteins might underlie these changes.

Disclosures: B. Sperlagh: None. B. Koványi: None. C. Csölle: None. S. Calovi: None. E. Kató: None. L. Köles: None. A. Bhattacharya: None. J. Haller: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.06/KKK53

Topic: H.03. Schizophrenia

Support: Sydney Baer Foundation

Title: Neurophysiological correlates of cognitive impairment in schizophrenia inpatients

Authors: *W. ZHANG[1], A. SHILUK[1], S. RACKELMANN[1], S. T. PIANKA[2], J. SPROCK[1], A. W. BISMARK[1], C. KAUFFMAN[3], M. L. THOMAS[1], M. TARASENKO[1], G. A. LIGHT[1]; [1]Psychiatry, UCSD, San Diego, CA; [2]UCLA, Los Angeles, CA; [3]Alpine Special Treatment Center, Inc, San Diego, CA

Abstract: Deficits in early auditory processing contribute to higher-level cognitive functioning in schizophrenia. The current researchers have previously shown that MMN and P3a are reliable EEG measures of auditory processing, and that they are related to cognition and functioning in

clinically stable schizophrenia outpatients. This relationship has never been assessed in highly symptomatic patients with protracted illness requiring public guardianship and longer-term inpatient care. Moreover, the feasibility of administering these EEG measures to chronically ill patients with severe attentional and motivational deficits has been not yet been demonstrated. The current study aims to determine whether measures of early auditory processing are associated with cognitive functioning in an inpatient schizophrenia sample.
Twenty-five schizophrenia patients under public conservatorship were recruited from a transitional care facility and tested following a minimum of one month of acute stabilization with the approval of the Public Conservator's offices in San Diego and Los Angeles County. All participants' conservators were provided with informed consent and each participant assented to being part of the study. MMN and P3a ERPs were measured with a passive oddball paradigm utilizing a 1000Hz, 50msec standard tone and five duration- and/or frequency-modulated deviant tones. Global cognitive functioning was assessed with The MATRICS Consensus Cognitive Battery (MCCB). Total testing time was approximately two-and-a-half hours.
All patients completed the testing battery and provided usable data. Larger frontocentral amplitudes of MMN and P3a were significantly associated with MCCB Working Memory (MMN: $r=0.45$, $p=.02$; P3a: $r=0.62$, $p=0.001$). Moreover, shorter P3a latencies were significantly associated (all $r's>0.40$, $p's < 0.05$) with better performance in Neuropsychological Assessment Battery Mazes (NAB Mazes) and Hopkins Verbal Learning Test-Revised (HVLT-R), MCCB tests that respectively assess problem solving and verbal learning.
The present study demonstrates that EEG measures of early auditory processing - MMN and P3a - are robust correlates of cognitive functioning accounting for substantial variance in schizophrenia inpatients undergoing longer-term hospitalization. These measures require little attention or motivation and are therefore ideal for incorporation into a clinical testing battery, even within intensive inpatient treatment settings. Future longitudinal follow up will evaluate MMN and P3a for use as biomarkers for predicting and monitoring treatment response in this inpatient cohort.

Disclosures: W. Zhang: None. A. Shiluk: None. S. Rackelmann: None. S.T. Pianka: None. J. Sprock: None. A.W. Bismark: None. C. Kauffman: None. M.L. Thomas: None. M. Tarasenko: None. G.A. Light: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.07/KKK54

Topic: H.03. Schizophrenia

Support: JSPS Grant 25253074

JSPS Grant 15K14973

JSPS Grant 26870878

Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers S2603

Title: Effect of deficiency of vitamin B6 on mouse behavior and monoaminergic system

Authors: *K. TORIUMI[1,2], M. MIYASHITA[1], A. KOBORI[1], Y. HORIUCHI[1], I. NOHARA[1], N. OBATA[1], M. ITOKAWA[1], G. KONOPKA[2], M. ARAI[1];
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Abstract: Schizophrenia is a heterogeneous psychiatric disorder characterized by positive and negative symptoms and cognitive impairment. Recently, we have reported that about 20% of schizophrenia patients show accumulation of pentosidine, one of advanced glycogen end products (AGEs), and lower level of vitamin B6 (VB6), which works as a scavenger against AGEs, in the peripheral blood. Furthermore, our clinical study found that the VB6 level is inversely proportional to the score of PANSS, suggesting that the loss of VB6 might contribute to development of the symptom in schizophrenia. In order to uncover the relationship between VB6 level and schizophrenia, we developed VB6-deficient mice by feeding C57BL/6J male mice with a VB6-lacking diet containing low VB6 at 5μg/100g pellets from 8 to 12 weeks of age, while control mice were fed with a normal diet in which VB6 is contained at 1.4mg/100 g pellets. After the feeding for 4 weeks, the plasma VB6 level in VB6-deficient mice decreased to about 3% of that in control mice. Moreover, the body weight of VB6-deficient mice did not increase during the feeding of VB6-lacking diet, leading to significant difference in the body weight between VB6-deficient and control mice. Next, to evaluate the effect of the low VB6 on mouse behavior, we performed behavioral tests using VB6-deficient mice. In the social interaction test, VB6-deficient mice showed less interaction compared with the control mice, corresponding to an increased negative symptom-like behavior. These behavioral data suggest that the VB6 deficiency might be associated with the negative symptoms. Finally, to investigate whether the VB6-deficiency affected the function of monoaminergic neuronal systems, the tissue contents of monoamines and their metabolites in various regions of the brains were measured. A marked increase in 3-Methoxy-4-hydroxyphenylglycol (MHPG) was shown in all brain regions compared to that in the control, which is consistent with many clinical reports that increased MHPG level was shown in schizophrenia patients. Furthermore, because of the increased MHPG, the ratio of MHPG to noradrenaline significantly increased in VB6-deficient mice, suggesting that the activities of noradrenergic neuronal systems were increased in VB6-deficient mice. These results suggest that VB6-deficiency might be involved in schizophrenia symptoms via the enhancement of noradrenergic system.

Disclosures: K. Toriumi: None. M. Miyashita: None. A. Kobori: None. Y. Horiuchi: None. I. Nohara: None. N. Obata: None. M. Itokawa: None. G. Konopka: None. M. Arai: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.08/KKK55

Topic: H.03. Schizophrenia

Support: NIH Intramural Grant

Title: Tolcapone improvement of cortical information processing in patients with schizophrenia is modulated by COMT val158met genotype

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Abstract: Dopamine (DA) dysregulation is thought to underlay deficits in working memory and prefrontal cortical (PFC) physiology seen in patients with schizophrenia. Converging evidence indicates that DA focuses and stabilizes the prefrontal cortical network by modulating NMDA, non-NMDA and GABAergic currents. In this context, catechol-O-methyl transferase (COMT) may play a unique role in regulating dopamine flux in the prefrontal cortex. COMT has a common functional polymorphism (Val[158]Met) that affects prefrontal function and working memory capacity (Weinberger et al, 2001) and has been associated with cognitive deficits in schizophrenia (Egan et al, 2001). Early studies showed that tolcapone, a COMT inhibitor that penetrates the blood brain barrier, improves working memory and PFC efficiency as measured by BOLD fMRI in normal volunteers. We also previously found that tolcapone's effect on neuropsychological tasks was modulated by COMT val-met genotype, such that Val/Val homozygotes benefited from the drug treatment more than Met/Met homozygotes (Apud et al., 2007). In the current study, we sought to determine whether tolcapone (100 mg three times a day on the first day and 200 mg three times a day for the next 6 days) or placebo would have an effect on prefrontal recruitment of neuronal resources in patients with schizophrenia. We randomized subjects following a within-subjects, counter-balanced study design and enrolled them to participate in a double-blinded, cross-over, placebo-controlled trial. Subjects underwent BOLD fMRI on the seventh day of each arm while performing the N-Back working memory task with three levels of difficulty (1Back, 2Back, 3Back). Comparing the three COMT genotypes, the thirty-three subjects (six women) are not different in age, gender, IQ, race and day of tocapone administration (day 1 vs. day 2). We performed a 3-way ANCOVA analysis with Drug, Load, and Genotype as factors in SPM12. Pulled across all genotype groups and load conditions, the drug effect was not significant. However, post-hoc ROI analysis showed that greater working

memory load yielded greater activation in bilateral dorsolateral prefrontal cortex ($p<0.001$). Post-hoc analyses showed that, compared to the placebo condition, only Val/Val homozygotes on tolcapone showed less activation ($p<0.045$). Our results are consistent with previous findings in healthy volunteers of Val/Val homozygotes benefiting from tolcapone's COMT inhibitory effect by improving information processing. These findings suggest that the effect of tolcapone on prefrontal efficiency is modulated by COMT Val-Met genotype.

Disclosures: **J.A. Apud:** A. Employment/Salary (full or part-time): OCD-NIMH-NIH. 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; NIMH funded. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); No. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); No. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); No. F. Consulting Fees (e.g., advisory boards); No. **Y. Tong:** A. Employment/Salary (full or part-time): CTNB-NIMH-NIH. 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; No. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); No. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); No. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); No. F. Consulting Fees (e.g., advisory boards); No. **T. Vargas:** A. Employment/Salary (full or part-time): NIMH employee. **J.H. Callicott:** A. Employment/Salary (full or part-time): NIMH employee. **B. Kolachana:** A. Employment/Salary (full or part-time): NIMH employee. **D.R. Weinberger:** A. Employment/Salary (full or part-time): Lieber Institute, Johns Hopkins University. **V.S. Mattay:** A. Employment/Salary (full or part-time): Lieber Institute for Brain Development. **K.F. Berman:** A. Employment/Salary (full or part-time): NIMH employee.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.09/KKK56

Topic: H.03. Schizophrenia

Title: Cortisol levels in schizophrenic patients treated with typical and atypical antipsychotic drugs

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Abstract: The objective of the study was to analyze salivary cortisol levels in a sample of patients with paranoid schizophrenia, treated with typical and atypical antipsychotic drugs, compared with the data obtained in a control group of healthy participants. Participated two groups of 15 paranoid schizophrenics patients (diagnosed under the ICD-10 criteria). One was treated with typical and other atypical antipsychotic drugs (average 11.6 years of evolution of disease); 15 healthy subjects as a control group. The age range was 24 to 38 years. To measure cortisol levels were collected over 3 sessions saliva samples and analyzed with the method enzyme immunoassay (EIA). The results showed higher salivary cortisol levels in patients treated with atypical antipsychotics compared to patients treated with typical antipsychotic drugs, in the control group lower cortisol levels found. Statistically significant differences between groups ($F_{[2,42]}$=4.81; p=0.02) were found. The analysis of multiple comparisons of Tukey-Kramer showed that the group of patients treated with atypical antipsychotics had higher salivary cortisol levels than the control group ($p < 0.05$). No statistically significant differences between groups of schizophrenic patients treated with typical and atypical antipsychotics, neither among patients treated with typical antipsychotic drugs and control groups were found. For their condition, schizophrenic patients show higher vulnerability to stressful events. The increase in stress levels are associated with enhanced secretion of cortisol, which can activate apoptotic processes in the central nervous system and negatively impact on their cognitive performance. Future research would be directed at determining the effect of these two types of antipsychotics drugs on cortisol secretion and its possible influence on the cognitive impairment shown by patients with schizophrenia. Taking into account variables such as the chronicity of psychiatric symptoms, adherence treatment, age of onset of symptoms and duration of illness.

Disclosures: C. Vega Michel: None. M. Rojas: None. S. Meneses-Ortega: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.10/KKK57

Topic: H.03. Schizophrenia

Support: 31-116689

310030_135736/1

51AU40_125759

Title: N-acetyl-cysteine and environmental enrichment reversed the long-lasting effect of oxidative stress on PVI circuitry: relevance for schizophrenia

Authors: *D. DWIR, J.-H. CABUNGCAL, P. STEULLET, M. SCHNIDER, M. CUÉNOD, K. DO;
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Abstract: A hallmark of the pathophysiology of schizophrenia is a dysfunction of parvalbumin-expressing fast-spiking interneurons (PVI), which are essential for neuronal synchrony during sensory and cognitive processing. Oxidative stress and inflammation, as observed in schizophrenia, affects the highly metabolically active PVI. Some schizophrenia patients have decreased brain glutathione (GSH) levels due to genetic and functional origin. GSH dysregulation, by increasing vulnerability to oxidative stress and inflammation during early development leads to impaired cortical circuitry, specifically the PVI and the perineuronal nets (PNN) that surround them. We tested whether a combined treatment of N-acetyl-cysteine (NAC) and enriched environment (EE), during adolescent, prevents the deleterious effect of oxidative insult on PVI and PNN. We used a transgenic mouse model with GSH deficit (GCLM KO) that shows SZ related phenotype, increased oxidative stress and microglia activation. Here, we confirmed previous findings that an additional oxidative stress, using a dopamine reuptake inhibitor (GBR), in early postnatal days (P10-20) led to long-lasting effects in adult GCLM KO: increase in oxidative stress, activation of microglia, increase in MMP9-IR, and PVI and PNN impairment These effects were completely reversed by the combination of NAC treatment (given between P21-35) and EE (during P35-56). Interestingly, MMP9-IR was also reversed by NAC treatment alone. The fast rhythmic oscillations reflecting neuronal synchronization of PVI was decreased in the GBR-treated GCLM KO, and recovered by NAC/EE. Thus, an early oxidative insult induces long-lasting effects on PVI and PNN which can be reversed by a combined NAC and EE, even after the challenge. In analogy, individuals carrying genetic risks to redox dysregulation potentially vulnerable to early-life insults could benefit from a combined pharmacological and psycho-social therapy.

Disclosures: D. Dwir: None. J. Cabungcal: None. P. Steullet: None. M. Schnider: None. M. Cuénod: None. K. Do: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.11/KKK58

Topic: G.07. Other Psychiatric Disorders

Support: nSF grant IOS1557755

nih grant R03DA038734

boettcher young investigator award

narsad young investigator award

Title: Targeting an upstream neuromodulator of dopamine release rectifies a propsychotic response in a DREADD induced hyperdopaminergic state.

Authors: *T. COOMER, E. E. RENN, R. DAS, E. B. OLESON;
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Abstract: Conditioned avoidance is a classical animal model of the antipsychotic response in which a warning signal prompts an animal to press a lever to avoid electrical foot shock. All known antipsychotics disrupt the ability of an animal to show a conditioned avoidance response, an effect our recent data suggest is due to their ability to block dopaminergic encoding of the warning signal. A sensitized dopamine response to cues predicting drug availability is also theorized to cause craving and motivate drug seeking. As such, a hyperdopaminergic neuropathology that facilitates conditioned avoidance may also increase motivation for drugs of abuse through enhanced attribution of incentive salience to drug predictive cues. Due to recent technical advances, we can now directly test this theory by assessing the causal role of heightened dopamine release in behaviors relevant to the co-morbid expression of schizophrenia and addiction. Here, we use designer-receptor exclusively activated by designer drug (DREADD) technology to assess the effects of producing a transient hyperdopaminergic state in conditioned operant avoidance and in a behavioral-economics based cocaine self-administration task. Our data reveal that a DREADD-induced transient hyperdopaminergic state is sufficient to produce a pro-psychotic response and increase motivation for cocaine. We then attempt to counteract that response pharmacologically. Historically, both typical and atypical antipsychotics target the dopamine D2 receptor, but a number of issues exist with these pharmacotherapies that result in poor compliance. We propose an alternative method of treatment that targets upstream modulators of dopaminergic neurons in the mesocorticolimbic pathway that will potentially ameliorate both the schizophrenic symptoms, and drug-seeking behaviors. To achieve this, our group artificially induces a hyper dopaminergic state in transgenic rats by utilizing Gq-coupled DREADD virus technology. We then systemically administer an orexin OX1 receptor antagonist, which was previously demonstrated to modulate dopamine neural activity. Our data demonstrate that an orexin antagonist dose-dependently decreases conditioned avoidance, exclusively when tested in a DREADD-induced hyperdopaminergic state. The implication of this latter observation suggests upstream modulators of dopamine release may offer pharmacotherapeutic utility for schizophrenia.

Disclosures: T. Coomer: None. E.E. Renn: None. R. Das: None. E.B. Oleson: None.

Poster

269. Schizophrenia: Experimental Therapeutics

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 269.12/KKK59

Topic: H.03. Schizophrenia

Support: Funded in part with Federal funds (UL1TR000101) from the National Center for Advancing Translational Sciences (NCATS), NIH.

VA Capitol Health Care Network (VISN 5) Mental Illness Research, Education, and Clinical Center Pilot Study Program.

Title: Impact of exercise training on prefrontal gray matter volume in schizophrenia

Authors: J. W. CHO[1], T. TESLOVICH[2], X. YOU[3], L. C. KORSHAK[1], J. P. POWELL[1], C. J. VAIDYA[4], P. KOKKINOS[1], *B. L. SCHWARTZ[1];
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Abstract: Schizophrenia is associated with structural and functional abnormalities in the prefrontal cortex (PFC). Specifically, gray matter (GM) volume reduction has been reported in the dorsolateral PFC, a region linked to performance on cognitive tasks designed to measure executive functioning and working memory. The benefits of exercise and physical fitness, including increases in GM and cognitive performance, have been observed in healthy adults. Brain-derived neurotrophic factor (BDNF) is proposed to play a role in mediating such exercise-induced neuroplasticity. Here, we investigated the effects of exercise on BDNF levels and the middle frontal gyrus (MFG), a region that includes the dorsolateral PFC, using magnetic resonance imaging, in patients with schizophrenia. Participants were 10 veterans, ages 30 to 67 years (M = 54.3 years), who met DSM 5 criteria for schizophrenia or schizoaffective disorder. Participants were enrolled in a 12-week exercise program (3 times a week) tailored to each participant's level of fitness at baseline. Each 1 hour session comprised 30 minutes of aerobic activity and 30 minutes of strength training. Structural images were acquired on a 3.0 T Siemens Trio at baseline and endpoint. Cortical parcellation and volumetric measures were generated via FreeSurfer's automated processing stream. Serum BDNF levels were measured at baseline and endpoint. Nine of the 10 participants successfully completed the exercise intervention. Aerobic fitness, as measured by metabolic equivalent level, increased significantly from baseline (9.3± 2.4) to endpoint (10.9± 1.9) ($t(7) = -3.06$, $p < 0.05$). While average serum BDNF levels did not significantly increase, baseline levels were predictive of change in BDNF following the intervention. Specifically, lower initial levels were associated with greater change ($r(8)= -.64$, $p < 0.05$). Structurally, a significant increase in volume was seen in the right rostral MFG following the intervention (baseline=0.91±.09, endpoint=0.93±.09), $t(8) = -2.36$, $p < .05$),

independent of age. Significant increases in both physical fitness and PFC volume were seen following a 12-week exercise intervention for schizophrenia patients. As greater GM volume in the PFC has been linked to increased cognitive performance, the inclusion of physical fitness training in the treatment of schizophrenia may be an efficient and cost-effective tool to improve aspects of cognition, such as executive functioning and working memory. These preliminary data provide support for a future controlled study to explore and elucidate the mechanisms of exercise-induced improvements in cognition and brain function in schizophrenia.

Disclosures: J.W. Cho: None. T. Teslovich: None. X. You: None. L.C. Korshak: None. J.P. Powell: None. C.J. Vaidya: None. P. Kokkinos: None. B.L. Schwartz: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.01/KKK60

Topic: I.04. Physiological Methods

Support: UCSD Center for Brain Activity Mapping (CBAM)

NIH U01NS094232

NIH NS057198

NIH EB00790

NIH S10RR029050

Title: Mutlicolor two-photon excitation for increasing fluorescence excitation depth

Authors: M.-H. YANG[1], *C. G. FERRI[2], P. A. SAISAN[2], M. ABASHIN[2], P. TIAN[2,4], A. DEVOR[3,5], Y. FAINMAN[1];
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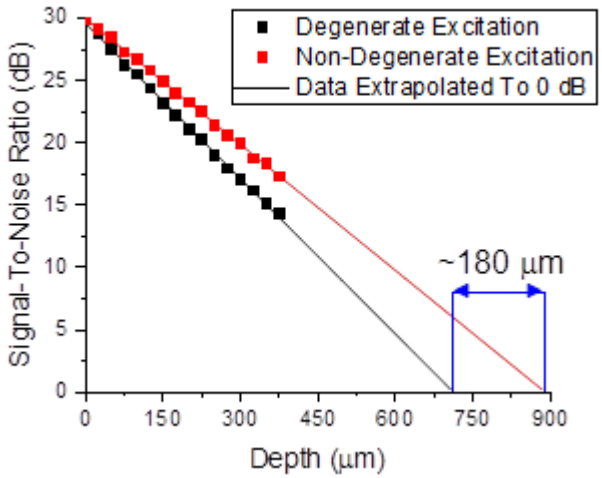
Abstract: The advent of 2-photon microscopy has enabled *in vivo* imaging of cerebrocortical structure and function with micron resolution. Yet, penetrating deep into the cortex remains a challenge due to the scattering and absorption of light by cerebral tissue. The combined unwanted effect of scattering and absorption can be reduced by employing longer illumination wavelengths in the range of 1300-1700 nm. This range, however, is not suitable for conventional,

a.k.a. “degenerate” 2-photon excitation (D-2PE) of visible probes (e.g., with red or green emission). Here, we address this problem by multicolor 2PE, a.k.a. non-degenerate 2PE (ND-2PE). The principle of multicolor 2-photon imaging has been demonstrated *in vivo* for simultaneous excitation of multiple chromophores [1]. We are adapting this technology for deep imaging.

We excite the fluorophores via the simultaneous absorption of two photons of different energy (i.e., wavelength) using two different pulsed lasers sources. The lasers are spatially aligned and temporally synchronized. One laser is tuned to the near infrared (NIR, 700-900 nm), the second laser is tuned to the infrared (IR, 1300-1700nm) to match the energy needed for excitation of the fluorophore. The IR laser is used to compensate for NIR power loss due to tissue scattering. Furthermore, IR wavelengths are scattered less by tissue than NIR, allowing for deeper penetration into the cortex.

We excited fluorescein (FITC) submerged within an intralipid suspension of varying concentration. Excitation was provided by a pulsed Ti:Sapphire laser tuned to 825nm and an optical parametric oscillator tuned to 1315nm. Under these conditions, we can compare the D-2PE and ND-2PE fluorescence. We find that ND-2PE fluorescence decreases over a longer distance within the intralipid as compared to D-2PE fluorescence for all concentrations. These results are summarized in the attached figure. Thus, we have demonstrated a proof-of-principle for increased fluorescence excitation depth using non-degenerate excitation.

[1] Mahou, P., et al. (2012). *Nature Methods*, *9*(8), 815-818.



Disclosures: M. Yang: None. C.G. Ferri: None. P.A. Saisan: None. M. Abashin: None. P. Tian: None. A. Devor: None. Y. Fainman: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.02/KKK61

Topic: I.04. Physiological Methods

Support: HHMI

Title: Mesoscale mapping of cortical neuron activity

Authors: ***N. J. SOFRONIEW**, D. FLICKINGER, J. FREEMAN, K. SVOBODA;
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Abstract: Cellular imaging is widely used to link neural activity in populations of neurons with behavior. Neural activity related to particular animal behaviors is distributed across multiple brain areas, often separated by millimeters. Most microscopes with subcellular resolution have small fields-of-view and therefore cannot image neurons in multiple brain areas at the same time. Large field-of-view microscopes do not resolve single cells, especially in the axial dimension. To overcome this limitation we designed and built a 2-photon random access mesoscope (2p-RAM) that provides rapid access for fast raster scanning anywhere within a large tissue volume. The 2p-RAM has subcellular resolution (lateral, 0.66 μm, axial 4.09 μm at the center) across a large imaging volume (diameter 5 mm x 1 mm cylinder). A fast three-dimensional scanning system allows efficient sampling of neural activity in arbitrary regions of interest across the entire imaging volume. We used the 2p-RAM to map neural activity in transgenic mice expressing protein calcium sensors as they navigated in tactile virtual reality. We first performed low frame rate (1.9 Hz) imaging of a large (4.2 mm x 4.2 mm) area overlapping somatosensory and parietal cortex, containing more than 2500 active neurons. We then used high frame rate (9.6 Hz) imaging to characterize four smaller (600 um x 600 um) patches in more detail. We describe mesoscale cortical organization of neuronal tuning to tactile input and locomotion.

Disclosures: N.J. Sofroniew: None. D. Flickinger: None. J. Freeman: None. K. Svoboda: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.03/KKK62

Topic: I.04. Physiological Methods

Support: NIH R01 MH083686 (DWT)

NIH U01 NS090541 (DWT)

Simons Foundation SCGB (DWT, JWP)

NIH T32 MH065214 (ASC)

McKnight Foundation (JWP)

NSF CAREER Award IIS-1150186 (JWP)

Title: Two-photon imaging of neurons using stereoscopy (TwINS)

Authors: *A. SONG[1], A. S. CHARLES[2], S. Y. THIBERGE[3], J. L. GAUTHIER[2], S. KOAY[2], J. W. PILLOW[2], D. W. TANK[2,3,4];
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Abstract: Two photon laser scanning microscopy of calcium dynamics is a widely used imaging method for large scale recording of neural activity *in vivo*. Here we demonstrate a volumetric calcium imaging method—Two-photon Imaging of Neurons using Stereoscopy (TwINS) —that increases the number of recorded neurons without the sacrifice of frame rate in sequential multi-plane volume imaging. TwINS exploits sparsity of neural activity by creating a single 2D projection image of the volume and relying on demixing algorithms to extract signals from individual neurons. A projection along a single axis, however, both loses depth information and creates significant spatial overlap of signals from axially-aligned neurons, compromising demixing. Thus, to both preserve depth information and produce a spatially distinct image profile for each neuron, TwINS raster-scans a V-shaped point spread function (PSF; 30-50μm axial FWHM) to illuminate neurons across a volume. When an active neuron in the volume is scanned, each arm of the V shaped PSF makes a neuron-shaped contribution to the 2D projection, but the contributions from each arm are spatially shifted by the distance between the two arms at that depth in the volume. Thus neurons that might overlap on the image with a single excitation beam will have spatially distinct depth-encoding "image pairs" on the projection, facilitating demixing. The TwINS microscope consists of a beam-shaping module and a conventional two-photon microscope. In the beam shaping module, two parallel beams,

displaced lateral to each other, are created with a birefringent block. The two beams (either low NA Gaussian beams, or Bessel beams created with an axicon) are directed into a custom two-photon microscope. Volume imaging at 30Hz is performed using conventional resonant scanners and a PMT. For analysis, neural activity is first separated from background activity using a denoising algorithm that estimates pixel activity above a background level and uses a Laplacian Scale Mixture prior to incorporate sparsity and spatial cohesion. The denoised data is then fed into a somatic pair finding algorithm using orthogonal matching pursuit, which exploits the fact that neurons appear as image pairs. This process returns demixed activity traces for individual neurons and their inferred 3D position. We demonstrate the use of TwINS to image activity both in layer 2/3 neurons in primary visual cortex under a visual stimulus in an awake mouse and from populations of CA1 neurons in mouse hippocampus during behavior. Our results demonstrate that TwINS is a relatively simple to implement approach for volumetric two-photon imaging while maintaining a high volume frame-rate.

Disclosures: A. Song: None. A.S. Charles: None. S.Y. Thiberge: None. J.L. Gauthier: None. S. Koay: None. J.W. Pillow: None. D.W. Tank: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.04/KKK63

Topic: I.04. Physiological Methods

Support: NETT Grant P36496

Title: Characterisation of spontaneous hippocampal circuit dynamics by two-photon minimal inertia scanning

Authors: *R. SCHUCK[1], P. QUICKE[2], A. FOUST[2], L. A. ANNECCHINO[2], C. COPELAND[2], S. GARASTO[2], S. R. SCHULTZ[2];
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Abstract: Understanding how the dynamics of activity in neural circuits relates to information processing is a key problem in understanding perception, memory and brain disorders. To characterise the dynamics of large and dense neural populations, we employ two photon (2P) calcium imaging. Standard raster scanning of a 2P spot scales extremely poorly with population size, limiting acquisition rates and/or signal to noise ratio. Improvements to cellular sampling rate can be achieved by scanning directly from soma to soma. While such algorithms markedly increasing sampling rates over raster scanning, their trajectories require the beam to execute

sharp turns, necessitating slowdowns to overcome galvanometric scanner (GS) inertia. Our recently developed Adaptive Spiral Scanning (SSA) algorithm (Schuck et al. 2014, IEEE EMBS) quickly samples all cells in the field of view while avoiding inertia-imposed slowdowns. Here, we apply this algorithm to achieve fast cellular-resolution sampling of granule cell activity in the dentate gyrus of the hippocampus. 400µm horizontal hippocampal slices were taken from wild type mice (P14-P17) in 1-4°C ventilated (95%O2, 5% CO2) ACSF. Slices were recovered at 37°C for 30min before being placed in 2.5mL of rACSF at 37°C and "painted" with 10µL of a solution (50µg Cal520 dissolved in 2µL of Pluronic and 48µL of DMSO), then incubated and washed for 30 minutes. We compared the scanning efficiency of the SSA with a “shortest-path” algorithm (Travelling Salesman Algorithm, TSA), characterizing performance as a function of the number, density, and geometry of sampled targets. Calcium transient acquisition rates of around 200Hz to 300Hz for 25 targets were achievable with SSA. Both TSA and SSA substantially outperformed raster scanning for simultaneous acquisition of signals from many cells, with high signal to noise ratio and temporal fidelity. We analysed the dynamics of spontaneous activity in the dentate gyrus using two approaches: (i) computation of an information distance metric between all signals, followed by dimensionality reduction using multi-dimensional scaling. This allowed us to visualise the similarity of the information conveyed by each cell. (ii) embedding of the entire spatial pattern dynamics into a space constructed using the cosine distance, followed by multidimensional scaling. This approach allows us to characterise how patterns of activity traverse response space during spontaneous activity, and to study the dynamics following electrical perturbation. We hypothesize that these dynamics may be disturbed during neurodegenerative disorders which affect learning and memory.

Disclosures: R. Schuck: None. P. Quicke: None. A. Foust: None. L.A. Annecchino: None. C. Copeland: None. S. Garasto: None. S.R. Schultz: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.05/KKK64

Topic: I.04. Physiological Methods

Title: Miniaturized two-photon microscopy enabled single-spine resolution brain imaging in freely-behaving mice

Authors: *W. ZONG, R. WU, M. LI, H. JIA, Y. HU, Y. LI, H. RONG, Y. ZHANG, A. WANG, Z. ZHOU, L. CHEN, H. CHENG;
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Abstract: An ultimate goal of neuroscience is to decipher the principles underlying neuronal information processing in freely-behaving animals at the subcellular, cellular, circuit, and even higher levels. Recently, a flurry of effort has been dedicated to the development of light-weight and compact optical imaging systems that can be carried by awake and mobile animals. A major unmet challenge, however, is to image individual synaptic activity (of sub-micron sizes, operate at the millisecond scale, and are often buried deep within the brain) in freely-behaving animals. Owing to its intrinsic optical sectioning and deep tissue penetration, multi-photon microscopy has been the technique of choice for the *in vivo* three-dimensional (3D) imaging of dendrite spine structures and activities. However, to date, none of current miniaturized 2PMs has been able to achieve sub-micron imaging of synaptic activity in a freely-moving animal. Here we report the design, optimization, and construction of a miniaturized imaging device that can be head-mounted to a freely-behaving mouse and enables fast-scanning, high-resolution, two-photon fluorescent or second-harmonic generation (SHG) imaging. We have named it the "video-rate high-resolution dual-detection-mode miniaturized two-photon microscope" (VIHID-m2PM). The headpiece consists of a fast two-axis microelectromechanical-systems (MEMS) raster scanner, a miniature compound objective lens with an NA of 0.8, and a Z-scanning micromotor of 4-mm travel. Emission photons are detected either by a micro-photomultiplier tube (μPMT) directly attached to the headpiece (3.25 g) or gallium arsenide phosphide (GaAsP) photodetectors remotely connected to the headpiece (2.1 g) *via* a custom-made super-supple fiber bundle (SSFB). Overall, the VIHID-m2PM provides 0.65-μm lateral and 3.75-μm axial resolution at a 40-Hz (256 × 512 pixels) frame rate or 10 kHz linescan rate. Moreover, we demonstrate its superb performance for resolving Ca^{2+} signals in cell bodies, dendritic shafts, and single spines in the primary visual cortex of mice as they freely explore and interact with the surrounding environment.

Disclosures: W. Zong: None. R. Wu: None. M. Li: None. H. Jia: None. Y. Hu: None. Y. Li: None. H. Rong: None. Y. Zhang: None. A. Wang: None. Z. Zhou: None. L. Chen: None. H. Cheng: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.06/KKK65

Topic: I.04. Physiological Methods

Support: NSF IDBR DBI-1353757

NIH DC00566

NIH HD041697

Title: An implantable two-photon fiber-coupled microscope with electrically-tunable focus for applications to awake behaving mouse brain imaging

Authors: ***B. OZBAY**[1], G. L. FUTIA[1], M. MA[2], J. T. LOSACCO[2], D. RESTREPO[2], E. A. GIBSON[1];
[1]Bioengineering, [2]Cell & Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: We demonstrate a miniature fiber-coupled microscope (FCM) with a variable focus lens for two-photon brain tissue imaging of GFP and tdTomato fluorescent markers. The FCM is coupled to a laser-scanning microscope with pulsed excitation light centered at 950 nm. The pulse duration is 130 fs out of the fiber, as characterized using frequency resolved optical gating. The fluorescence emission passes through a bandpass filter (450 - 700 nm) and is detected on a non-descanned photon counting PMT. The FCM is coupled through a coherent fiber-bundle for lateral scanning with ~2 μm resolution. Axial-scanning is achieved using a small electrically-tunable lens distal to the fiber-bundle and allows for a z-scan range of ~500 μm. We achieved a total image volume of 250 μm diameter x 500 μm in depth.
Our FCM demonstrates a mechanically simple and lightweight 3D imaging system for rodent brain imaging. The FCM plastic enclosure is manufactured using 3D printing with a resolution of 0.04 mm and uses commercial optical components, which allows for greater accessibility and customization. The assembled device is lightweight (< 4 g) and is designed for mounting onto the skull of a mouse for brain imaging during behavior. We also show that the FCM can be chronically implanted on a mouse skull for recurrent imaging.

Disclosures: B. Ozbay: None. G.L. Futia: None. M. Ma: None. J.T. Losacco: None. D. Restrepo: None. E.A. Gibson: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.07/KKK66

Topic: I.04. Physiological Methods

Support: U01 NS094286-01

T32 NS048004

T32 NS058280

David Geffen School of Medicine Dean's Fund

Title: Miniscope.org: an open-source miniature wide-field imaging platform and online collaborative resource

Authors: *D. AHARONI[1,2,3,4,5], T. SHUMAN[1,4,5], D. J. CAI[2,3,4,5], T.-Y. LU[2,6], S. FLORES[1,4,5], M. LA-VU[2,3,4,5], B. S. KHAKH[2,6], A. J. SILVA[2,3,4,5], P. GOLSHANI[1,4,5];
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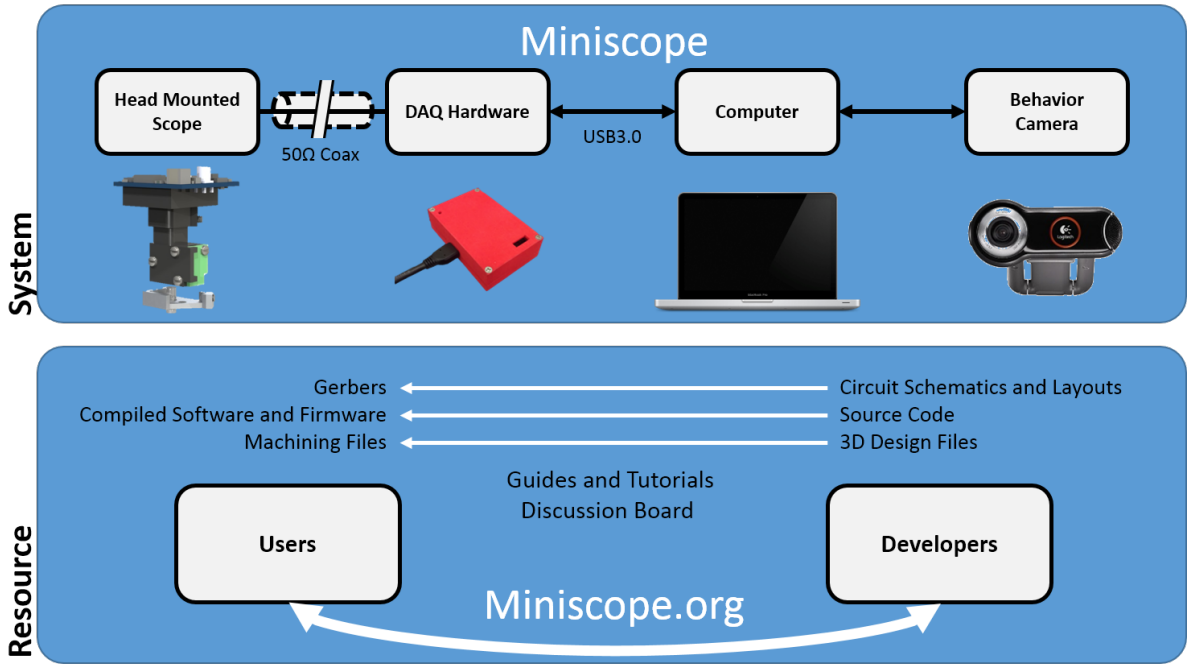
Abstract: Over recent years, increased access to affordable, high quality, low volume manufacturing and online collaborative platforms has led to a transformative open-source movement in the neuroscience community. A goal of this movement is to accelerate neuroscience research through the open sharing and collaborative advancement of research tools. Here we present the current status of our open-source Miniscope project which aims to advance miniature wide-field fluorescence microscopy through the open sharing of design files, parts lists, and tutorials on a collaborative, online resource, miniscope.org.
The Miniscope system consists of all necessary hardware, software, and firmware to image large-scale neural activity in awake, freely behaving animals. The head mounted scope (3 grams) connects to a custom designed Data Acquisition (DAQ) box through a single, commutator compatible, coaxial cable (down to 0.3mm in diameter, over 3m in length) carrying power, imaging data, and control signals. The DAQ box communicates to a computer over USB3.0 and can be controlled with our own DAQ software or through any third party DAQ software capable of connecting to a webcam. The electronics support the vast majority of commercial CMOS imaging sensors, giving access to the full range of resolutions/framerates available among commercial imaging sensors while requiring minimal circuit redesign. The optical elements in the scope are modular, allowing for the use of different filter sets, excitation sources, and imaging lenses.
To share this system we have built miniscope.org, an online resource constructed on a Wiki backbone. Miniscope.org contains part procurement and system assembly tutorials/videos for labs interested in using the system as well as detailed schematics and source code for labs to customize and innovate the Miniscope. The site also supports collaborative tools such as discussion boards, member pages, and the ability for members to create their own project pages around new developments.



Disclosures: D. Aharoni: None. T. Shuman: None. D.J. Cai: None. T. Lu: None. S. Flores: None. M. La-Vu: None. B.S. Khakh: None. A.J. Silva: None. P. Golshani: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.08/DP09 (Dynamic Poster)

Topic: I.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Video-rate volumetric functional imaging of the brain with synaptic resolution

Authors: ***R. LU**[1], W. SUN[1], Y. LIANG[1], A. KERLIN[1], J. BIERFELD[2], J. SEELIG[1,3], D. WILSON[4], M. TANIMOTO[1], B. SCHOLL[4], B. MOHAR[1], M. KOYAMA[1], D. FITZPATRICK[4], V. JAYARAMAN[1], M. ORGER[2], N. JI[1];
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Abstract: Neurons and neural networks often extend hundreds to thousands of micrometers in three dimensions. To capture all the calcium transients associated with their activity, we need volume imaging methods with sub-second temporal resolution. Such speed is challenging for conventional two-photon laser scanning microscopy (2PLSM) to achieve, because of its dependence on serial focal scanning in 3D and the limited brightness of calcium indicators. Here we present an optical module that can be easily integrated into standard 2PLSMs to generate an elongated focus approximating a Bessel beam. Scanning the Bessel focus in 2D turns frame rate into volume rate and enables video-rate volumetric imaging. Using Bessel beams optimized to maintain synaptic-level lateral resolution *in vivo*, we demonstrated the power of this approach in contributing discoveries for neurobiology by studying the activity of volumes of neurons and synapses in intact brains of fruit flies, zebrafish larvae, mice, and ferrets.

Disclosures: R. Lu: None. W. Sun: None. Y. Liang: None. A. Kerlin: None. J. Bierfeld: None. J. Seelig: None. D. Wilson: None. M. Tanimoto: None. B. Scholl: None. B. Mohar: None. M. Koyama: None. D. Fitzpatrick: None. V. Jayaraman: None. M. Orger: None. N. Ji: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.09/KKK67

Topic: I.04. Physiological Methods

Support: NIH Grant R01 OD019037

Title: Optimized volumetric live imaging with light field microscopy and selective volume illumination.

Authors: *T. V. TRUONG[1], A. ANDREEV[2], S. MADAAN[2], D. HOLLAND[1], S. E. FRASER[1]; [1]Translational Imaging Ctr., [2]BME, USC, Los Angeles, CA

Abstract: Recent efforts to carry out cellular functional imaging across large areas of the brain could greatly benefit from a volumetric live imaging modality, where a single snapshot could capture spatial information over a tissue-scale axially-extended volume, while maintaining single- or few-cell resolution. Such capability has been recently demonstrated with Light Field Microscopy (LFM), where the light field coming from the sample space is recorded in a single 2D image, capturing information from the 3D volume extended above and below the native focal plane, albeit at reduced spatial resolution due to the diffraction limit. Computational reconstruction is used to generate the 3D images of the sample from the 2D light field images.

Up until now, LFM has been done with widefield illumination, essentially lighting up the entirety of the sample, even though only a part of the sample is meaningfully captured in the reconstruction. Thus, widefield illumination introduces extraneous background and noise, degrading the contrast and accuracy of the final reconstructed images. Here, we implement Selective Volume Illumination (SVI) to LFM, illuminating only the volume of interest, thus significantly reducing the background and providing higher contrast and accuracy for the light field image reconstruction. We demonstrate SVI-LFM with both 1-photon and 2-photon excitation, the latter providing higher penetration depth in scattering tissue. Additionally, our implementation allows recording a high resolution, optically-sectioned 3D image of the same sample, in addition to the light-field-reconstructed volumetric, but low-resolution, image. Having both the high-resolution/low-speed, and low-resolution/high-speed image data of the same sample facilitates optimized observation of dynamical processes, and provides the potential for using the high-resolution image data to help speed up and constrain the reconstruction of the light field data.

Disclosures: T.V. Truong: None. A. Andreev: None. S. Madaan: None. D. Holland: None. S.E. Fraser: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.10/KKK68

Topic: I.04. Physiological Methods

Title: Optical monitoring of behavior-related calcium dynamics in 12 brain regions using a novel multi-fiber array

Authors: *Y. SYCH, F. HELMCHEN;
Lab. of Neural Circuit Dynamics, Brain Res. Institute, Univ. of Zurich, Zuerich, Switzerland

Abstract: Methods for a brain-wide monitoring of neural activity in subcortical areas are scarce. Here, we present an implantable multi-fibre array and an optical system for a brain-wide monitoring of calcium signalling. The lightweight (0.3 g) multi-fibre implant consists of a ferrule, fixation pins and 12 fibres (100 micron core diameter) fitted into a guiding structure. Implanted fibres are bevelled to 45 degrees, facilitating smooth penetration into deep brain areas. Thereby, the far-field illumination profile of each fibre is modified such that fluorescence is mainly excited in the tissue volume normal to the bevelled surface. The multi-fibre implant is connected via a linear 12-fiber bundle to an imaging system. The fibre-optic signal was cross-validated with two-photon scanning microscopy and electrophysiological recording of multi-unit

spike activity. We applied the novel multi-fibre array to simultaneously measure population calcium signals (GCaMP6 s/m virally delivered) in 12 brain areas during multiple behavioural sessions. Mice were trained in a whisker-based texture discrimination task. Preliminary data of the mesoscale brain network activity indicate that multiple cortical regions (S1BF, M1 L5/6) and subcortical brain areas *e.g.* striatum (CPu), thalamus (VPM, VPL, Po) and hippocampus (CA1, CA2, CA3) are recruited during sensorimotor processing in the task. Moreover, we observed temporal reorganization of a network activity during learning from naive to expert level mice. The multi-fibre array thus is a versatile tool to chronically study large-scale dynamics of the network of interacting brain regions.

Disclosures: Y. Sych: None. F. Helmchen: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.11/KKK69

Topic: I.04. Physiological Methods

Support: BRAIN Initiative Grant 5U01NS094296-01

Department of Defense, MURI Grant W911NF-12-1-0594

Kavli Foundation

NSF Graduate Research Fellowship Program, #2014189230

Simons Collaboration on the Global Brain #350520

Title: Swept confocally aligned planar excitation (SCAPE) microscopy for the identification of neural circuits in adult *Drosophila*

Authors: *W. LI[1], N. MISHRA[2], E. S. SCHAFFER[2], V. VOLETI[1], E. M. C. HILLMAN[1,3]; [1]Biomed. Engin., [2]Neurosci., [3]Radiology, Columbia Univ., New York, NY

Abstract: The identification of functional neuronal circuits in the brain is an important step towards developing predictive models of behavior. Although our neurogenetic understanding of the fly allows us to identify and label different neuronal subsets, there is still a huge gap between structural information provided by, for example, a connectome map and inferred functionality. To this end, one of the largest challenges is an inability to observe whole-scale brain activity within a normal, behaving animal.

Here, we use Swept Confocally Aligned Planar Excitation (SCAPE) microscopy to image whole-brain activity of adult behaving Drosophila at speeds of 10 brain volumes per second. SCAPE is a form of light-sheet imaging which uses a single objective lens, and a novel sheet scanning-descanning approach that enables translationless volumetric imaging at very high speeds. This imaging geometry is ideally suited to head-fixed imaging the fly brain, without requiring body restraint or immersion of the fly that would otherwise interfere with behavior. During *in vivo* imaging, the fly can be subjected to an odor stimulus controlled via an olfactometer while performing tracked walking behavior on an air-suspended ball.
To test the efficacy of SCAPE whole brain imaging as a tool for the functional identification of neural circuits, we express GCaMP6f pan-neuronally and ask whether known olfactory circuits can be identified from brain-wide calcium signals. Our latest results will be presented.

Disclosures: W. Li: None. N. Mishra: None. E.S. Schaffer: None. V. Voleti: None. E.M.C. Hillman: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.12/KKK70

Topic: I.04. Physiological Methods

Support: Simons Foundation Grant

NIH Grant EY024067

Title: Design of a modular microscope for flexible, high-resolution imaging of neural activity in the non-human primate

Authors: *J. CHOI[1], V. GONCHAROV[2], J. KLEINBART[1], A. ORSBORN[1], B. PESARAN[1]; [1]New York Univ., New York, NY; [2]Janelia Res. Campus, Ashburn, VA

Abstract: Recent advances in genetically encoded neural activity indicators, in vivo two-photon imaging, and optical stimulation have enabled cellular-resolution recording and stimulation of cortical activity with high signal quality and population specificity. These technologies need to be deployed in awake primate models to elucidate pathologies and phenomena most relevant to the human brain. Unfortunately, no existing microscope can be used for work in non-human primates without significant customization due to the size and shape of the primate brain. The large size of the primate cortex presents numerous technical challenges for studying large-scale distributed functions, which potentially involve many brain regions in concert. Areas of interest

span centimeters, imposing stringent demands for optical access across curved, expansive surfaces and suppression of large movement artifacts from cardiac, respiratory, and behavioral sources. These problems underscore the need for flexible and repeatable positioning of the objective lens relative to the cortical surface. At least five degrees of freedom are required to achieve parallel alignment of the focal plane and cortex. While some non-primate studies have achieved this by rotating the subject's body along two axes and moving the microscope in position alone, this method does not extend well to the large angles required for primate cortex. Therefore, a rotating optical assembly is required. Axial brain movement produces artifacts that are difficult to correct, and methods are needed for applying and maintaining pressure to a transparent artificial dura. To achieve and maintain optical access to multiple brain areas, an annular skull implant is designed that provides hygienic isolation and adequate space for placing multiple low-profile imaging chambers. Each chamber is designed to fit the curvature of the skull and provides optical access through an artificial dura. The low profile accommodates large, high numerical-aperture objectives with small working distances and would allow multiple non-overlapping imaging fields to be serially scanned over the course of an experiment. Over long-term implantation, a common failure mode of optical chambers is growth of a translucent neomembrane---a problem that must be addressed to allow recording over the months or years typical of primate experiments. Here we present a novel microscope and surgically-implanted chamber system designed to provide solutions to these issues. We propose that novel instrumentation will pave the way for comprehensive neuronal recording from primate cortex to study large-scale distributed networks.

Disclosures: J. Choi: None. V. Goncharov: None. J. Kleinbart: None. A. Orsborn: None. B. Pesaran: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.13/LLL1

Topic: I.04. Physiological Methods

Title: Optical adaptor for subcortical two-photon microscopy

Authors: *G. JUHASZ[1], L. JUDAK[2], G. SZALAY[2], G. KATONA[1], P. MAAK[3], M. VERESS[3], B. ROZSA[2];
[1]Fac. of Information Technol. and Bionics, Pazmany Peter Catholic Univ., Budapest, Hungary; [2]Inst. of Exptl. Med., Budapest, Hungary; [3]Dept. of Atomic Physics, Budapest Univ. of Technol. and Econ., Budapest, Hungary

Abstract: Light-based neuroimaging techniques are suitable to functionally image neural tissue elements from the area range down to the synaptic elements, thereby being an indispensable tool to understand brain function. A common drawback of optical imaging is the depth limit due to light scattering in live tissues. The usual working depth of these systems do not exceed a few hundred micrometers, hardly the width of the rodent cortex. There are engineering approaches to solve this problem, since deeper neural tissue elements also play crucial role in shaping cognition and behavior. So far these solutions all suffer from one or several constraints of being highly invasive, not precise, or of limited scope. Here we describe a possible solution along with *in vivo* test measurements to use with 2 photon laser scanning imaging of genetically encoded calcium indicators allowing to image deep tissue functions in considerable volumes.

Disclosures: G. Juhasz: None. L. Judak: None. G. Szalay: None. G. Katona: None. P. Maak: None. M. Veress: None. B. Rozsa: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.14/LLL2

Topic: I.04. Physiological Methods

Support: Grant-in-Aid for JSPS Fellows Number 15J10687

The VLSI Design and Education Center (VDEC), University of Tokyo, in collaboration with Cadence Design Systems, Inc.

Title: Highly sensitive implantable imaging device for flavoprotein fluorescence imaging

Authors: *Y. SUNAGA, M. HARUTA, T. YAMAGUCHI, H. TAKEHARA, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA;
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Abstract: To observe neural activities in the brain of an animal, fluorescence imaging technology is very useful. Especially, Green Fluorescent Protein (GFP) or autofluorescence of mitochondrial flavoprotein is very useful because its intensity is changed by neural activities without any treatment to an animal [1]. In usual, fluorescence reactions of a tethered animal is observed by a fluorescent microscope. However, it is difficult to observe the brain of an animal under freely moving condition with a fluorescent microscope. In order to observe fluorescence reaction associated with animal behavior, we have developed a miniaturized fluorescence imaging device based on a CMOS image sensor. Its dimensions are $1 \times 2.7 \times 0.15$ mm^3, and its

weight is less than 0.05 g. Furthermore, the device and its LEDs can be driven using only six wires. By implanting the imaging device into an animal brain such as a mouse or a rat, fluorescence reactions can be observed even under freely-moving conditions with minimal invasiveness. The imaging device was set on the brain surface and was fixed with the skull by a dental cement. To avoid interfering daily behavior of an animal, wires are connected only while imaging experiments are performed. In our previous works, we have developed an implantable imaging device for green fluorescence imaging at brain surface [2], and achieved to observe flavoprotein fluorescence reaction associated with visual stimulation [3]-[4]. However, the previous device needed many trials to reduce influence of noise by averaging the obtained data. To solve this problem, we have tried to develop a new implantable imaging devices for highly sensitive imaging. In this work, we introduced a new image sensor to improve flavoprotein fluorescence imaging quality. In flavoprotein fluorescence imaging, light detection sensitivity of the image sensor is more important than its spatial resolution, because flavoprotein fluorescence changes very weakly in wide range. To improve detection sensitivity, we designed a new image sensor that has 4 times larger pixels than that of the previous sensor. This new sensor enables us to observe weaker fluorescence with high signal-to-noise ratio compared with previous devices. We are now working to confirm the performance of the improved imaging device in *in vivo* experiments. [1] K. Shibuki *et al*. J. Physiol.. 549(3), 919927, 2003. [2] Y. Sunaga *et al.*, IEEE BioCAS 2014, Lausanne, Swiss. [3] Y. Sunaga *et al.*, M&BE 2015, Tokyo, Japan. [4] Y. Sunaga *et al.*, Jpn. J. Appl. Phys., 55, 3S2, 2016.

Disclosures: Y. Sunaga: None. M. Haruta: None. T. Yamaguchi: None. H. Takehara: None. T. Noda: None. K. Sasagawa: None. T. Tokuda: None. J. Ohta: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.15/LLL3

Topic: I.04. Physiological Methods

Support: LSRF

HHMI

Title: Deep brain fluorescence imaging using an ultra-thin optical fiber

Authors: *S. OHAYON[1], A. M. C. AGUIRRE[2], R. PIESTUN[2], J. DICARLO[1];
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Abstract: A major challenge in studying the brain is to image neural activity in deep structures that lie beyond the depth range of two-photon microscopes (500-1000um). Existing methods for imaging deep structures either use bulky objectives or GRIN lens (gradient refractive index) that are rigid, short, suffer from various optical aberrations, have large diameters (500-100um) and cause considerable damage upon insertion into tissue. We present a minimally invasive technique to image in-vivo fluorescence using standard multi-mode fibers (50-100um in diameter) that can be lowered to target structures at any arbitrary depth. In contrast to fiber-photometry, where all spatial information is lost and the recovered signal is averaged across the fiber core, our technique maintains full spatial information and can reconstruct the full 3D volume beneath the fiber. The system has reasonable optical properties (in plane FWHM 1um, optical sectioning: ~6um) sufficient to image cell bodies (neurons). To demonstrate the capabilities of this technique and to validate it we present results from a series of experiments. We show that fluorescence beads (15um) can be imaged in-vivo in mice. We also show that calcium dynamics can be recorded in-vitro (full frame acquisition speeds range between 0.5-15Hz, depending on spatial resolution). The technique has the potential to be used as an all-optical tool for both monitoring and perturbing neural activity. On-going efforts are now taking place to achieve in-vivo functional imaging. We hope this would pave the path for calcium imaging in deep brain structures in genetically intractable animals (e.g non-human primates).

Disclosures: S. Ohayon: None. A.M.C. Aguirre: None. R. Piestun: None. J. DiCarlo: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.16/LLL4

Topic: I.04. Physiological Methods

Support: EC Grant 604102 (Human Brain Project)

EC Grant 284464 (LASERLAB-EUROPE)

Italian Ministry for Education, University and Research, Flagship Project NanoMAX

Private Foundation "Ente Cassa di Risparmio di Firenze"

Title: High-fidelity functional and structural whole-brain imaging with Bessel-beam light-sheet microscopy

Authors: *C. MUELLENBROICH, L. SILVESTRI, L. TURRINI, A. P. DI GIOVANNA, T. ALTERINI, A. GHEISARI, P. RICCI, L. SACCONI, F. VANZI, F. S. PAVONE;
LENS, Univ. of Florence, Sesto Fiorentino, Italy

Abstract: Light-sheet microscopy (LSM) has proven a useful and versatile tool in neuroscience and is particularly well suited to image the entire brain with high frame rates at single cell resolution. On the one hand, LSM is often employed in combination with tissue clearing methods like CLARITY which allows for the reconstruction of the neuronal or vascular anatomy over cm-sized samples like the entire mouse brain. On the other hand, LSM has been paired with intrinsically transparent samples for the real-time recording of neuronal activity with single cell resolution across the entire brain, using calcium indicators like GCaMP6. Despite its intrinsic advantages in terms of high imaging speed and reduced photobleaching, LSM is very sensitive to residual opaque objects present in the sample, which cause dark horizontal stripes in the collected images. In the best case, these artefacts obscure the features of interest in structural imaging; in the worst case, dynamic shadowing introduced by red blood cells significantly alters the fluorescence signal variations related to neuronal activity. We show how the use of Bessel beams in LSM can dramatically reduce such artefacts even in conventional one-sided illumination schemes, thanks to their non-diffractive and "self-healing" properties. On the functional side, Bessel-beam LSM allows recording neuronal activity traces without any disturbing flickering caused by the movement of red blood cells. Furthermore, using this approach it is possible to extend high-resolution calcium imaging in less transparent samples or regions, like older or slightly pigmented Zebrafish larvae. On the structural side, our proposed method is capable of obtaining anatomical information across the entire volume of whole mouse brains - without the selection of 'nice regions' - allowing tracing blood vessels and neuronal projections also in poorly cleared specimens.

Disclosures: C. Muellenbroich: None. L. Silvestri: None. L. Turrini: None. A.P. Di Giovanna: None. T. Alterini: None. A. Gheisari: None. P. Ricci: None. L. Sacconi: None. F. Vanzi: None. F.S. Pavone: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.17/LLL5

Topic: I.04. Physiological Methods

Support: BRAIN Initiative 5U01NS094296-01

NSF CAREER 0954796

Kavli Foundation

Title: Towards two-photon SCAPE microscopy for extended depth, fast volumetric imaging of brain function.

Authors: *H. YU[1], P. T. GALWADUGE[1], W. LI[1], V. VOLETI[1], K. PATEL[1], E. M. C. HILLMAN[1,2,3];
[1]Biomed. Engin., [2]Radiology, [3]Mortimer B. Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY

Abstract: Reduced scattering at near-infrared wavelengths enables two-photon laser scanning microscopy (2PLSM) to image deeper into scattering biological tissue. However, conventional point-scanning implementations of 2PLSM are limited by slow volumetric imaging rates, preventing the capture of fast, 3D dynamics. Swept confocally-aligned planar illumination (SCAPE) microscopy is a recently developed new technique capable of very high speed 3D microscopy in living targets including the awake behaving mouse brain. SCAPE achieves high speed 3D imaging by using light-sheet illumination instead of point scanning. SCAPE's unique single-objective geometry and scan-descan approach enables rapid movement of the illuminated plane through the sample without needing physical translation of the objective or sample. However, current implementations of SCAPE utilize a 488 nm laser source for single-photon excitation, limiting the imaging depth of SCAPE to less than ~ 300 μm in in-vivo mouse brain.Implementing SCAPE with two-photon excitation would provide several benefits including deeper imaging and improved background rejection and contrast. However, it is challenging to combine two-photon excitation with light-sheet illumination owing to the need for much more laser power, particularly in the context of acquiring data at very high speeds. Here, we will describe our latest progress towards two-photon implementations of SCAPE.

Disclosures: H. Yu: None. P.T. Galwaduge: None. W. Li: None. V. Voleti: None. K. Patel: None. E.M.C. Hillman: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.18/LLL6

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS078223

NIH Grant K25NS083754

Title: Non-invasive functional neuroimaging in mice using structured illumination diffuse optical tomography

Authors: *M. REISMAN, Z. MARKOW, A. Q. BAUER, J. P. CULVER;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: The study of correlated spontaneous activity in functionally-related brain regions using functional connectivity MRI (fcMRI) has allowed comprehensive mapping of distributed brain networks in humans. Although studies utilizing fMRI in mice have recently seen an increased application towards mapping resting state networks, the difficulty of obtaining high resolution images with sufficient signal to noise in mice using fMRI has led to optical intrinsic signal (OIS) techniques providing most of the observations of fc in the mouse brain. While effective, OIS requires scalp retraction and is limited to superficial cortical tissues. Diffuse Optical Tomography (DOT) provides non-invasive imaging, but current DOT systems are either too sparsely sampling to match the cortical resolution of OIS or are too slow for capturing spontaneous hemodynamics in the mouse brain. Here we develop a DOT system that combines the spatial sampling of camera-based systems with the rapid-imaging of structured illumination (SI) to non-invasively map activity in the mouse cortex. The non-invasive mouse SI-DOT system is comprised of a sCMOS camera and a digital micromirror device (DMD) that allow for rapid illumination and detection with high spatial resolution (Fig 1A). Custom data quality assessment metrics allow for measurement optimization and improved accuracy of an input light model (Fig 1B). The sensitivity between each measurement and brain voxel is used to reconstruct hemodynamic activity. Evoked responses in the somatosensory cortex of the mouse upon electrical stimulation of the forepaw are observed non-invasively, through the intact scalp (Fig 1C). Establishing analogous functional imaging in both mouse and man is one of the most promising strategies for providing clinical translation. SI-DOT addresses many of the problems limiting other techniques from full 3D mouse brain imaging. Evoked responses are seen non-invasively, and the system's complete flexibility of wavelengths, illumination patterns, and detector binning provide a powerful framework for 3D mapping of mouse brain networks.

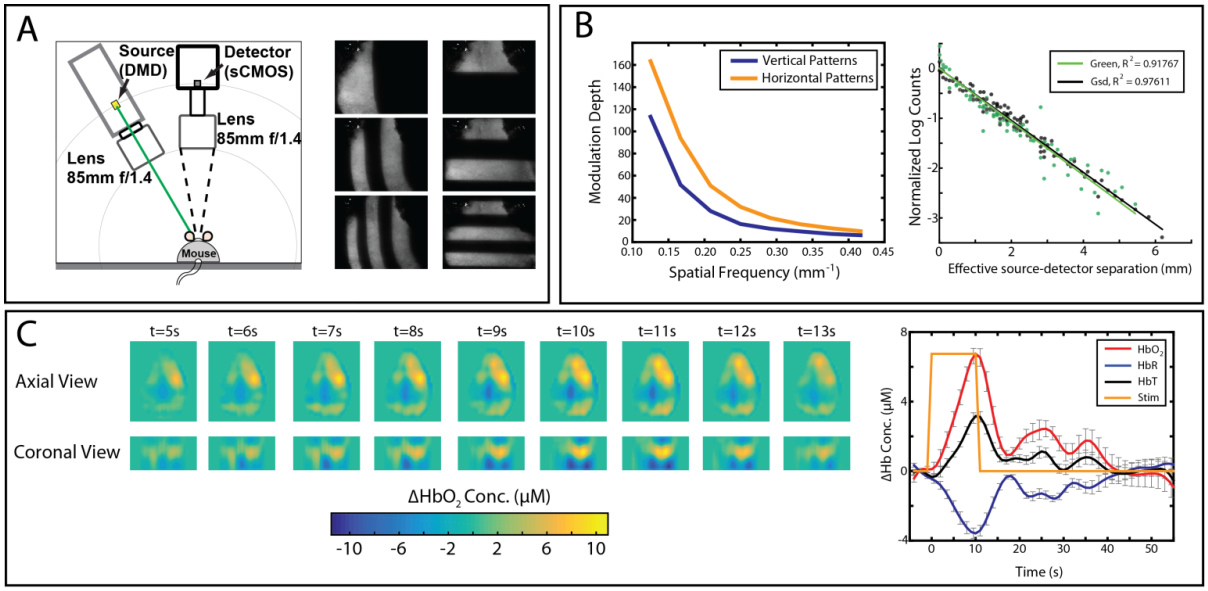


Fig 1. (A) System drawing showing spatial orientation of DMD source projection and Andor Zyla sCMOS detector. Six sample patterns show the illumination on the head of a mouse with the scalp intact and the sCMOS FOV. (B) Modulation depth shows the dynamic range of each illumination pattern as a function of spatial frequency, and allows optimization of illumination patterns by removing those with too low of a modulation depth. A comparison of the light falloff between the raw data and the modeled data show good agreement, confirming the accuracy of the light model and sensitivity matrix. (C) Reconstructions non-invasively show the evoked response in the somatosensory cortex of a mouse following an electrical stimulus of the left forepaw. The coronal slice through the maximum activation in the axial view provides previously unattainable depth information, up to 2mm beneath the surface of the scalp. A time trace of the signal in voxels >50% of the max response shows the expected increase in oxy- and total hemoglobin and decrease in deoxy-hemoglobin.

Disclosures: M. Reisman: None. Z. Markow: None. A.Q. Bauer: None. J.P. Culver: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.19/LLL7

Topic: I.04. Physiological Methods

Support: JSPS Research Fellowship for Young Scientist

the program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from MEXT and AMED

Scientific Research (No. 15H02350) from MEXT

Title: Super-field two-photon microscopy for simultaneous imaging of multiple cortical areas at cellular resolution

Authors: *S.-I. TERADA[1,2], M. OHKURA[3], J. NAKAI[3], M. MATSUZAKI[1];
[1]Dept. of Cell. and Mol. Physiol., The Univ. of Tokyo, Tokyo, Japan; [2]Grad. Sch. of Biostudies, Kyoto Univ., Kyoto, Japan; [3]Grad. Sch. of Sci. and Engin., Saitama Univ., Saitama, Japan

Abstract: Understanding the dynamics of cortico-cortical communications is essential because information processing in the brain is not only performed in intra-areal circuits but also through inter-areal interactions. Recent advances in two-photon microscopy have allowed us to simultaneously image multiple brain areas at cellular resolution. However, it is still difficult to image a continuous large field (>2 mm) and two distant (>3 mm) brain areas at cellular resolution using a single two-photon microscope. Here, we introduce super-field two-photon microscopy through a single objective, which allows the imaging of two distant (up to 6 mm apart) cortical areas and a large continuous area (up to 3 mm) at cellular resolution. The method depends on placing a novel optical device under a high-NA objective with a long working distance in a standard two-photon microscope. The device is composed of a pair of mirrors and a holder to rotate the mirrors perpendicular to the optical axis. By controlling the rotation timing and angle using custom-made software, the field of view can be rapidly switched without moving either the objective or the sample. By rotating the mirror pair back and forth between two angles, we conducted sequential two-photon calcium imaging of neuronal activities in two distant areas up to 6 mm apart and at a depth of up to 800 μm from the cortical surface. Furthermore, by stitching the fields of view, we succeeded in imaging a 3 mm × 1 mm continuous area. To prove that this optical system is effective for the study of the dynamics of cortico-cortical communications, we applied it to concurrent calcium imaging of layer 2/3 and layer 5 neurons in rostral and caudal motor cortical areas while the mice performed a lever-pull task (Hira et al., J. Neurosci 33, 2013). We will discuss how the neural activities in these fields are coordinated during motor execution. Importantly, the optical device together with the controller can be easily installed on a standard two-photon microscope. Its adaptation by neuroscientists should open the door to the study of information processing in brain networks at cellular resolution.

Disclosures: S. Terada: None. M. Ohkura: None. J. Nakai: None. M. Matsuzaki: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.20/LLL8

Topic: I.04. Physiological Methods

Support: NINDPS DP2NS087725-01

NEI R01EY023756-01

New York Stem Cell Foundation

David and Lucille Packard Fellowship

Arnold and Mabel Beckman Foundation

Title: 3D functional brain activity recording in zebrafish with compressive light field microscopy

Authors: *D. P. MOSSING[1], N. PEGARD[2], L. WALLER[3], H. ADESNIK[4];
[1]Grad. Program in Biophysics, [3]Electrical Engin. and Computer Sci., [4]Mol. and Cell Biol., [2]Univ. of California, Berkeley, Berkeley, CA

Abstract: Understanding the neural code requires minimally invasive instruments that are capable of monitoring brain activity in large volumes with cellular resolution and millisecond precision. Although genetically encoded dyes can report neural activity in the behaving animal, strong optical scattering in brain tissue generally limits performance. Most functional imaging devices attempt to overcome scattering using point scanning, non-linear two-photon excitation, or adaptive optics. These are complex and expensive, and suffer from either poor temporal or low spatial resolution. Here, we present a new microscopy technique for 3D functional imaging in live brain tissue aiming to maximize the number of neurons that can be reliably observed, while minimally perturbing animal behavior. The device is a simple light field fluorescence microscope allowing full volume acquisition in a single shot. Our computational methods first rely on the spatial and temporal sparsity of fluorescence signals to identify and precisely localize neurons and/or dendrites. For each neuron, we compute a unique pattern called the light-field signature that accounts for the effects of optical scattering and aberrations. The technique then yields precise localization of active neurons as well as a quantitative measurement of activity in all identified neurons within the 3D field of view. A single shot frame enables acquisition with single neuron spatial resolution and at high speeds, without ever reconstructing a volume image. This strategy is computationally efficient and compatible with strong optical scattering conditions. Experimental results are shown on live 5-day old zebrafish.

Disclosures: D.P. Mossing: None. N. Pegard: None. L. Waller: None. H. Adesnik: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.21/LLL9

Topic: I.04. Physiological Methods

Support: New York State Division of Science, Technology and Innovation

Title: 48-channel hyperspectral multiphoton microscopy in live mouse cortex

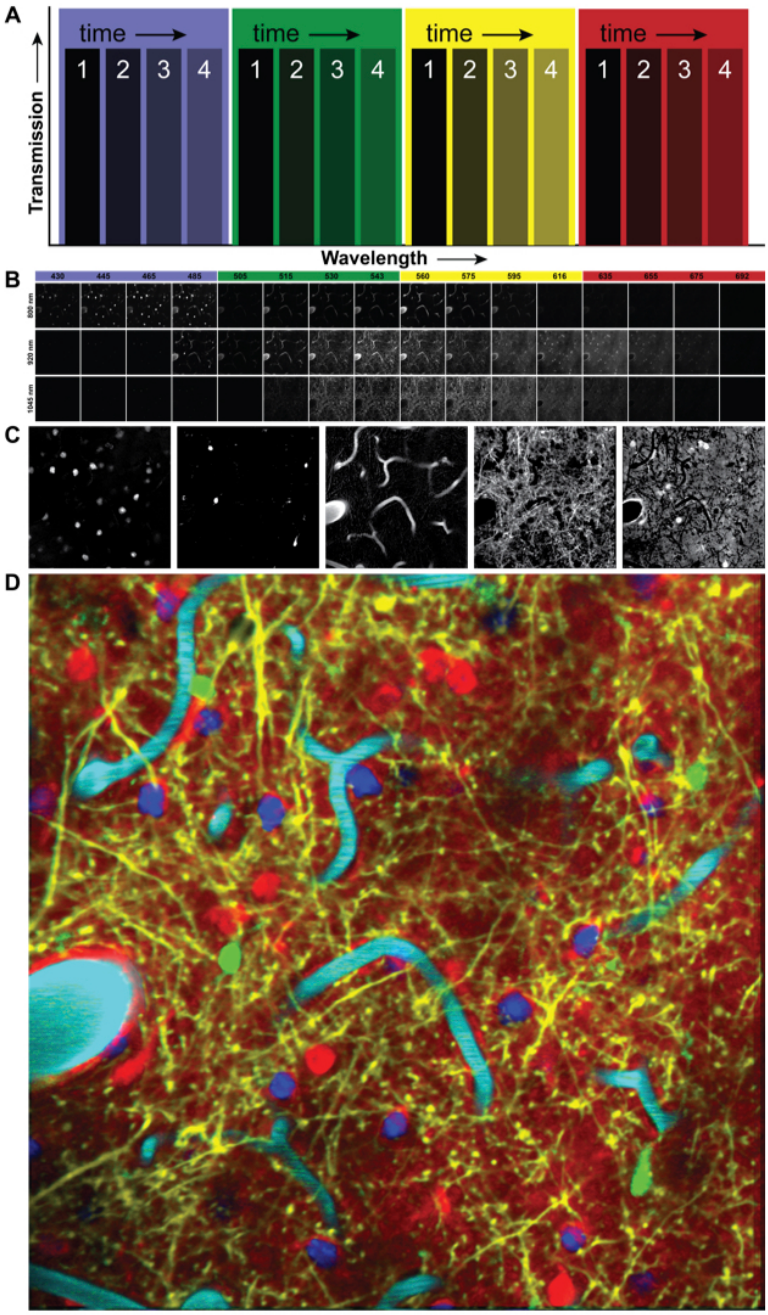
Authors: ***A. J. BARES**, M. A. PENDER, M. A. MEJOOLI, S. TILLEY, K. E. CHEN, J. DONG, P. C. DOERSCHUK, C. B. SCHAFFER;
Meinig Sch. of Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Studies of normal and disease state physiological processes in the brain would benefit from direct imaging of the dynamics and interactions of multiple cell types in live animal models. Nonlinear microscopy enables sufficient depth penetration and spatial resolution for such studies, but is typically limited to imaging only a few cell types at a time because of the inability to cleanly separate the emission from multiple, spectrally-overlapping fluorescent labels. We developed a hyperspectral multiphoton microscope (HMM) that provides 48 channels of excitation/emission information to enable clean separation of labels, while retaining the imaging depth and resolution of standard nonlinear imaging.

Emitted fluorescence is separated by dichroics into four broad color channels, each with an angle-tuned bandpass filter (ATBF). A four-channel image is collected for four successive angles of the ATBFs (**Fig.1A**), producing a 16-channel image that spans the visible spectrum. To leverage differences in excitation cross-section of different fluorophores, this is repeated across three excitation lasers (800 - 1035 nm) producing a 48-channel image for every plane in an image stack (**Fig. 1B**). These data are linearly unmixed to yield an image for each fluorescent label (**Fig. 1C**).

We imaged the cortex of a live mouse that had pyramidal neurons labeled with yellow fluorescent protein (YFP), microglia labeled with green fluorescent protein (GFP), nuclei and astrocytes labeled with topically applied Hoechst and Sulforhodamine 101 (SR101), respectively, and blood vessels labeled by intravenously-injected FITC and Cascade Blue (**Fig. 1B**). After unmixing, **Fig. 1C** shows from left to right: Hoechst nuclei (blue; colors refer to composite image in **Fig. 1D**), GFP microglia (green), FITC/Cascade Blue vasculature (cyan), YFP neurons (yellow), and SR101 astrocytes (red).

The HMM enables in vivo imaging of the dynamics and interactions of many cell types simultaneously, despite overlapping fluorescent labels.



Disclosures: A.J. Bares: None. M.A. Pender: None. M.A. Mejooli: None. S. Tilley: None. K.E. Chen: None. J. Dong: None. P.C. Doerschuk: None. C.B. Schaffer: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.22/LLL10

Topic: I.04. Physiological Methods

Support: Swiss Nationalfonds (SNF)

Novartis Research Foundation

Boehringer Ingelheim Fonds Fellowship to P.R.

Title: Remote z-scanning with a macroscopic voice coil motor for fast 3D calcium imaging in the zebrafish olfactory system

Authors: *P. RUPPRECHT[1], R. W. FRIEDRICH[1], C. WYART[2], A. PRENDERGAST[2]; [1]Rainer Friedrich Lab., Friedrich Miescher Inst., Basel, Switzerland; [2]Inst. du Cerveau et de la Moelle Épinière (ICM), Paris, France

Abstract: We developed a focusing technique for fast axial scanning based on a remote movable mirror that is conjugate to the specimen plane and translated by a voice coil motor. We constructed cost-effective z-scanning modules (<2500 $) from off-the-shelf components that can be mounted onto standard multiphoton laser scanning microscopes to extend scan patterns from 2D to 3D. These systems were designed for large objectives and provide high resolution, high speed and a large z-scan range (>300 μm). We used these systems in adult zebrafish for 3D multiphoton calcium imaging in the olfactory bulb (OB) and telencephalic area Dp, the homolog of olfactory cortex. We measured activity patterns across up to >1500 neurons with single-neuron resolution and high signal-to-noise ratio in response to slowly changing odor stimuli. The results revealed neuronal dynamics in mitral cells of the OB and in somata in Dp that evolved both on short (<1 sec) and long (>10 sec) timescales.

Disclosures: P. Rupprecht: None. R.W. Friedrich: None. C. Wyart: None. A. Prendergast: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.23/LLL11

Topic: I.04. Physiological Methods

Title: A robust and simple adaptive optics module for multiphoton fluorescence microscopy of the brain

Authors: *I. A. CHEN[1], W. SUN[1], Y. LIANG[1], D. MILKIE[1], T. BIFANO[2], N. JI[1];
[1]Ji Lab., Howard Hughes Med. Inst., Ashburn, VA; [2]Boston Univ. Photonics Ctr., Boston, MA

Abstract: As advances in multiphoton imaging techniques continue to push neuronal imaging deeper into the brain *in vivo*, correcting for sample-induced aberrations become vital in recovering diffraction-limited imaging performance at depth. As such, a plethora of adaptive optics (AO) techniques have emerged for biological imaging. However, these methods are often difficult to integrate into existing systems, only correct the aberration within a small field of view, or requires multiple specialized hardware to measure and correct the wavefront aberration. Here, we describe a simple AO module using a multiplexed aberration measurement method and apply it to *in vivo* two-photon imaging of the brain. We compare two different modulation techniques used for multiplexed measurement of aberration, characterize the effects of feature size on their performance, and apply them to in vivo imaging of zebrafish larvae and mouse brain. Because of its simplicity and independent software control, we expect our AO module to be easily integrated into existing custom or commercial laser scanning microscopes.

Disclosures: I.A. Chen: None. **W. Sun:** None. **Y. Liang:** None. **D. Milkie:** None. **T. Bifano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Bifano acknowledges a financial interest in Boston Micromachines Corporation. **N. Ji:** None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.24/LLL12

Topic: I.04. Physiological Methods

Title: A modular system for transcranial optical imaging in behaving mice

Authors: *B. R. MULLEN[1], J. D. MAYO[2], S. L. HILL[1], A. FONG[1], J. B. ACKMAN[1]; [2]Molecular, Cell, Developmental Biol., [1]UC Santa Cruz, Santa Cruz, CA

Abstract: Brain waves are spontaneous or sensory evoked patterns of neural activity that signal collective action within and across groups of neurons. Understanding the flow of cerebral waves across circuits during resting or active behavior is crucial for gaining insight into the mechanisms underlying self-organization in the developing brain. To this end, we've developed a relatively low cost and modular system for imaging genetically encoded calcium indicators expressed throughout the cerebral cortex. Our system allows for simultaneous imaging of neural activity across cortical regions with mesoscale spatial and temporal resolutions (less than 100s of μm and 100s of ms) and provides flexibility when integrating multiple functional signals together with experimental manipulations and tests of behavioral function. The system allows for wide field, single sensor monitoring of brain activity and motor behavior not typically available with conventional microscopes and the acquired signals are suitable for testing the organization of large-scale brain networks during behavior. We show how dynamical wave patterns in developing mouse neocortex are structured both in an areal and behavior dependent fashion and illustrate how assessments of distributed functional architecture may be used to better understand the nature of neurodevelopmental disease.

Disclosures: B.R. Mullen: None. J.D. Mayo: None. S.L. Hill: None. A. Fong: None. J.B. Ackman: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.25/LLL13

Topic: I.04. Physiological Methods

Support: Paul and Jody Allen

Title: Application of industrial product development principles to intrinsic signal imaging systems

Authors: *C. FARRELL[1], D. SULLIVAN[2], R. DIETZMAN[2], C. SLAUGHTERBECK[2], T. KEENAN[2], J. PERKINS[2], N. GAUDREAULT[2], A. BERNARD[2], M. GARRETT[2], F. LONG[2], L.

NG[2], K. ROLL[2], W. WAKEMAN[2], J. PHILLIPS[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Intrinsic signal imaging (ISI) is a well-established methodology for delineating stimulus-evoked functional areas of the brain. The method leverages differential absorption of red light by oxy- vs. deoxy-hemoglobin to distinguish active centers of brain activity. Broader use of intrinsic imaging is hindered by the lack of viable, commercial products capable of generating high-quality ISI maps with sufficient flexibility, standardization, and precision to meet the needs of a broad range of experimental paradigms. Here we describe how application of industrial product development principles enabled the adaptation of a highly-customized, academic research tool into a generalized, robust, standardized, and highly-automated intrinsic signal imaging platform.

Disclosures: C. Farrell: None. D. Sullivan: None. R. Dietzman: None. C. Slaughterbeck: None. T. Keenan: None. J. Perkins: None. N. Gaudreault: None. A. Bernard: None. M. Garrett: None. F. Long: None. L. Ng: None. K. Roll: None. W. Wakeman: None. J. Phillips: None.

Poster

270. Optical Methods Development

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 270.26/LLL14

Topic: I.04. Physiological Methods

Support: University of Oslo

Research Council of Norway Grant No. 217920

Title: Photonflow: an open-source fluorescence microscopy simulator

Authors: *S.-A. DRAGLY[1], M. WIGESTRAND[2], A. DEVOR[4], A. MALTHE-SØRENSSEN[1], G. T. EINEVOLL[5,1], T. HAFTING[3], M. FYHN[2];
[1]Dept. of Physics, [2]Dept. of Biosci., [3]Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway; [4]Dept. of Neurosciences, UCSD, La Jolla, CA; [5]Dept. of Mathematical Sci. and Technol., Norwegian Univ. of Life Sci., Ås, Norway

Abstract: Fluorescence microscopy is increasingly used for measuring neural activity *in vivo*. Arrival of miniaturized microscopes has opened for deep imaging of brain tissue in freely moving animals. The power of these experiments is the possibility of simultaneous recordings of the activity from a large number of neurons. However, this makes it hard to maintain image quality and resolution for each individual neuron, which complicates data analysis.

Although many software tools for data analysis exist, it is challenging to evaluate their accuracy because the ground truth of the data is mostly unknown. Using simulated microscopy, however, the ground truth is known and could be used to test the accuracy and weakness of analysis software and also guide experiments.
Here we present Photonflow, an open-source fluorescence microscopy simulator made to test the performance of analysis software and identify the limits of experimental configurations. Photonflow simulates photon transport to create realistic microscopy images of brain tissue. Typically, the quality of microscopy analysis software is determined by comparing it to manual inspection. For instance, software that identifies neurons from a series of images can be compared with manual outlining of regions of interest. While this is useful, it may be inaccurate because the analysis is idiosyncratic and the ground truth remains unknown. This is overcome in Photonflow by generating a set of simulated microscopy images based on a tailored ground truth. These can then be used to test the analysis tools.
Photonflow may also be useful in pilot experiments and make it possible to understand the potential and limitations of the planned experimental setup. For instance, Photonflow can be used to determine the optimal level of fluorescence to avoid excessive contamination by light from surrounding tissue.
Photonflow is intended to be easy to use. It provides a graphical user interface for exploring the imaged neurons. The simulator is written in C++ and is based on open-source libraries and methods that are in common use both to simulate photon transport and for creating realistic photography. Photon transport simulation can produce realistic images similar to those found in experiments. Our simulator tracks a large number of photon packets that are emitted from light sources, such as fluorescent cells, and are scattered throughout the tissue before they reach the camera sensor. The simulator registers where on the sensor the photon packets arrive and uses this to render the final image.
With Photonflow, we aim to further improve the confidence in fluorescence microscopy and the corresponding data analysis.

Disclosures: S. Dragly: None. M. Wigestrand: None. A. Devor: None. A. Malthe-Sørenssen: None. G.T. Einevoll: None. T. Hafting: None. M. Fyhn: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.01/LLL15

Topic: I.07. Data Analysis and Statistics

Support: NSF DGE-1144086

NIH 5R01NS047293-12

Title: Crowdsourced label learning for independent components of EEG data

Authors: *L. PION-TONACHINI[1,2], R. MARTINEZ-CANCINO[1], K. KREUTZ-DELGADO[2], S. MAKEIG[1];
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Abstract: Independent component analysis (ICA) has already been shown to be a powerful tool for analyzing electroencephalographic (EEG) data, but there are two inherent difficulties in its usage. The first is data preprocessing, as ICA can be sensitive to spatially non-stereotyped noise/artifact in the data. But while data aggregation, normalization, and feature extraction needed to select data for decomposition are non-trivial and time consuming, they do not present a barrier to working with ICA. The first real obstacle encountered by researchers is the absence of component labels to assist in interpreting the results. As ICA is a form of blind source separation, the estimated independent components (ICs) are not labeled as to their origin (brain or non-brain; if non-brain, ECG, EOG, EMG, line noise, or other). While, given expert knowledge, this ambiguity is not an unresolvable obstacle to using ICA in data analysis, expert knowledge may not be available to beginners nor to automated procedures nor when dealing with large datasets where the time required to obtain detailed expert evaluation is a bottleneck. To address the IC classification problem, we are creating an automated EEG IC classifier to label, probabilistically, the nature of the source processes producing the IC scalp maps and time courses returned by ICA decomposition. Although some IC classifiers are currently available, each has drawbacks we plan to overcome, primarily by using an IC data set that is orders of magnitude larger than those used previously, as well as by employing more complex learning algorithms to make best use of the data. With upwards of half a million ICs in our dataset, seeding the classifier learning process by labeling even a small subset would be difficult to perform individually. Therefore we have turned to crowdsourcing, creating a website and asking researchers on the EEGLAB mailing list to contribute their time to the effort. Others with experience in using ICA decomposition of EEG data are welcome to contribute as well -- visit http://reaching.ucsd.edu:8000/tutorial for details. The major motivation for those contributing is the eventual value to them of such a classifier, as well as gaining some tutorial experience in making these classifications. To date, we have amassed more than 12,000 submitted component labels from more than 50 contributors and are now beginning to process the contributed labels to prepare them for use in the final classifier learning process. The collected labels will be used to generate the most reliable estimated label for each IC shown on the website by comparing which contributors labeled which ICs as what type by using a family of algorithms known as crowdlabeling.

Disclosures: L. Pion-Tonachini: None. R. Martinez-Cancino: None. K. Kreutz-Delgado: None. S. Makeig: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.02/LLL16

Topic: I.07. Data Analysis and Statistics

Support: DFG GA 730/3-1

Title: Adjusting permutation tests for multivariate analysis of neuroimaging data with subclasses

Authors: *H. JAMALABADI[1,2], S. ALIZADEH[1,2], M. SCHÖNAUER[1], S. GAIS[1];
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[2]IMPRS for Cognitive and Systems Neurosci., Tübingen, Germany

Abstract: When multivariate pattern analysis (MVPA) is used for hypothesis testing, a classifier is trained on a portion of a data set to separate data belonging to different classes (e.g. different experimental conditions, different groups of patients, etc.). Then, the classifier is tested on new data, determining the correct classification rate (CCR) using a cross-validation procedure. If this accuracy lies significantly above the level expected by chance (e.g. 50% for a two-class problem), the classifier is able to detect generalizable class-related information in the data, i.e., classes differ significantly.
However, we show that in neuroimaging data with nested subclasses, above chance classification accuracy is possible even when no class-related effect exists. This happens because MVPA is sensitive to any kinds of structure in the data and thus, in such data sets, subclass-specific differences increase classification accuracy beyond what is expected. Subclasses are formed in data if stimuli can be further categorized with respect to a secondary attribute (e.g. physical properties, familiarity, etc.), if stimuli or types of stimuli are presented repeatedly, or if multiple subjects and experimental sessions are included into one analysis. Importantly, however, this subclass-specific information which contributes to the classification is not related to the effect under investigation and their effect should be discarded from analysis. The unexpected above chance classification of data with subclasses has implications when using MVPA for hypothesis testing. In particular, binomial tests as well as trial-wise randomization tests will lead to false positive results. To circumvent this problem, we propose a permutation algorithm that provides exact control of false positives in such data sets. We show if permutation tests are correctly adjusted, false positive rates can be controlled.

Disclosures: H. Jamalabadi: None. S. Alizadeh: None. M. Schönauer: None. S. Gais: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.03/LLL17

Topic: I.07. Data Analysis and Statistics

Support: Sloan Research Fellowship

Title: Neural oscillatory power is not Gaussian distributed across time

Authors: *L. IZHIKEVICH, E. PETERSON, B. VOYTEK;
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Abstract: Neural oscillations are important in organizing activity across the human brain in healthy cognition, while oscillatory disruptions are linked to numerous disease states. Oscillations are known to vary by frequency and amplitude across time and between different brain regions; however, this variability has never been well characterized. We examined human and animal EEG, LFP, MEG, and ECoG data from over 100 subjects to analyze the distribution of power and frequency across time, space and species. We report that between data types, subjects, frequencies, electrodes, and time, an inverse power law, or negative exponential distribution, is present in all recordings. This is contrary to, and not compatible with, the Gaussian noise assumption made in many digital signal processing techniques. The statistical assumptions underlying common algorithms for power spectral estimation, such as Welch's method, are being violated resulting in non-trivial misestimates of oscillatory power. Different statistical approaches are warranted.

Disclosures: L. Izhikevich: None. E. Peterson: None. B. Voytek: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.04/LLL18

Topic: I.07. Data Analysis and Statistics

Support: NIH R01-EB019437

NIH P41-EB015896

NIH S10-RR023043

NIH S10-OD010759

Harvard Society of Fellows

Title: Improving detection and localization of spontaneous oscillatory dynamics in simultaneous EEG-fMRI

Authors: *L. D. LEWIS[1], G. BONMASSAR[2], K. SETSOMPOP[2], J. R. POLIMENI[2], B. R. ROSEN[2];
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Abstract: Simultaneous recordings of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) provide complementary measures of brain function, enabling high spatial and temporal resolution studies of human brain activity. EEG acquired inside the magnet is heavily contaminated by ballistocardiogram artifacts induced by each cycle of the subject's heartbeat. Several cleaning methods have been developed that work well for reducing EEG artifacts in high (>10 Hz) frequency bands, and studies of evoked potentials can further reduce the effects of these artifacts by averaging across trials. However, the effects of these cleaning methods have not been evaluated for a broader range of frequencies. In particular, dynamics in the low (<10 Hz) frequency band can be difficult to recover, especially when studying spontaneous activity that cannot benefit from averaging multiple trials. We sought to identify whether low-frequency activity could be recovered from the EEG without averaging, and test how these signal processing approaches influence the networks identified in EEG-fMRI data. We acquired 256-channel EEG data from three subjects with simultaneous fMRI at 3 Tesla. To induce an ongoing EEG oscillation of known frequency, we presented a radial checkerboard with contrast inverting at a frequency of interest (0.5, 1, 2, 4, 8, 12, or 20 Hz). Subjects wore a custom-constructed reference layer cap to isolate the majority of the channels from the scalp (Chowdhury et al., 2014; Luo et al, 2014). We compared a set of different cleaning methods to determine how each affected EEG content: average artifact subtraction (AAS, Allen et al., 2000), optimal basis sets (OBS, Niazy et al., 2005), reference layer artifact subtraction (RLAS, Chowdhury et al., 2014; Luo et al, 2014), and removal of a subset of independent components (Abreu et al., 2016). We found that while all methods performed well at high frequencies, combined RLAS-AAS achieved the best recovery of the induced EEG oscillations across the full range of frequencies studied, suggesting that experiments seeking to measure lower frequencies would benefit from using an isolated reference layer to directly sample the ballistocardiogram noise. Finally, we applied these methods to resting state datasets from the same subjects to determine how they influenced calculated relationships with the fMRI BOLD signal, and found alterations in measured fMRI correlations depending on the method used. We conclude that commonly used EEG cleaning methods differentially affect signal strength across different frequency bands, and suggest specific ways in which this may influence the results and inferences in EEG-fMRI studies

Disclosures: L.D. Lewis: None. G. Bonmassar: None. K. Setsompop: None. J.R. Polimeni: None. B.R. Rosen: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.05/LLL19

Topic: I.07. Data Analysis and Statistics

Support: James S, McDonnell Scholar Award to JD

Title: Stability of representational similarity analysis across a large range of overall activation levels

Authors: ***S. ARBUCKLE**, A. YOKOI, J. DIEDRICHSEN;
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Abstract: Representational similarity analysis (RSA) characterizes the relationship between the complex patterns of activity recorded using functional magnetic resonance imaging (fMRI), to infer how the brain encodes different classes of information. While the fine spatial organization of the patterns elicited from specific stimuli or tasks can vary widely across individuals, the ratios of relative dissimilarities between patterns (here referred to as representational structure) is often stable across individuals. We recently demonstrated this stability for individual finger movements in primary motor cortex (M1, Ejaz et al., 2015). The great strength of RSA is that the measured representational structure can be compared to predictions derived from theoretical models, allowing inferences about neural processes in different cortical areas.
However, a major concern is that the non-linearity between neural activity and fMRI signal may distort the local pattern of activities, such that the representational structure would depend strongly on activity in the respective region - making the ratios between distances uninterpretable. Here we report the results of a control experiment with 9 participants, in which we increased the overall level of activity in M1 over a large dynamic range by increasing the movement frequency of single finger presses from 0.3Hz to close to performance limit. Average activity in M1 increased approximately linearly with the log of the tapping frequency. We then calculated the crossvalidated Mahalanobis distance as a dissimilarity measure between the 5 fingers for each of the 4 speeds. While the distances between the different fingers increased with increasing activation, the representational structure (i.e. ratio of these distances) remained stable.
We then analyzed all data in a single representational space to determine the relationship between the activity differences between fingers and between speeds. We show that overall

structure is well explained by a linear scaling of the patterns associated with each finger relative to rest plus an additive speed-dependent pattern, which is independent from the individual finger patterns. Importantly, this relatively simple model (20 parameters) can fully describe the representational structure (210 parameters).
These results suggest that RSA using crossvalidated Mahalanobis distances yields a description of the representational structure of activity patterns that remains stable over a large dynamic range of activation levels, and that we can characterize the scaling response of these patterns using a relatively simple model.

Disclosures: S. Arbuckle: None. A. Yokoi: None. J. Diedrichsen: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.06/LLL20

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1R01 MH096914-01A1

Postdoctoral Fellowship from the Simons Center for the Social Brain

Title: Multivariate pattern connectivity

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Abstract: When we perform a cognitive task, multiple brain regions are engaged. Studying how these regions interact is a fundamental step to understand the neural mechanisms at the basis of behavior. Most investigations of interactions between brain regions have focused on the overall univariate responses in the regions. However, in the context of 'static' analyses, important advantages have derived from the application of multivariate techniques considering the fine-grained spatial structure of responses within each region (multivariate pattern analysis - MVPA). In this poster, we introduce and apply a technique to study connectivity in terms of the multivariate relations between patterns of responses within brain regions: multivariate pattern connectivity (MVPC). MVPC characterizes the responses in each brain region as trajectories in region-specific multidimensional representational spaces, and uses multivariate techniques to model the relationship between these trajectories. Considering the fusiform face area (FFA) as a

seed region, we show that MVPC provides novel information about the interactions between regions that goes beyond univariate functional connectivity analyses.

Disclosures: S. Anzellotti: None. A. Caramazza: None. R. Saxe: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.07/LLL21

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant DMS 1557467

NIH Grant P01AA019072

NIH Grant P20GM103645

NIH Grant P30AI042853

NIH Grant R01CA180776

NIH Grant S10OD016366

Brown Institute for Brain Science

Title: Identification of "hot" local brain networks during motor sequence learning

Authors: Y. ZHAO[1], X. LUO[1], E. UPFAL[2], P. BÉDARD[3], *J. N. SANES[3];
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Abstract: Functional magnetic resonance imaging (fMRI) is used to infer brain network activation and interactions, and graph theory methods can identify how networks may mediate behavior. However, current approaches have not focused on identifying local (sub) networks that likely underlie components of behavior, such as the perceptual, cognitive, mnemonic, and motor subprocesses needed to learning new skills. We developed a statistical method to discover significant, task-related subnetworks while participants learned a motor sequence (Sanchez et al. 2010) during continuous acquisition of fMRI signals. We developed an integrated method that addresses three interwoven aims: (1) activation detection, (2) network estimation, and (3) subnetwork identification. In addition, we aimed to develop a method that could scale to large dimensions without incurring substantial computational costs. To achieve these aims, we extended existing frameworks, including general linear models (GLM), sparse inverse

covariance estimation, and "HotNet", a subnetwork identification method originally developed to identify cancer mutation subnetworks (Vandin et al. 2011; 2012). Building on the GLM analysis for activation for each node, we compared existing approaches (e.g., community partition, Rubinov and Sporns 2010) to infer the connectivity network using correlations, inverse covariance estimation, and a recently developed machine learning method for sparse graphical models (Friedman et al. 2008; Liu and Luo 2015). We then applied the HotNet algorithm to identify “hot” subnetworks based on these inferred activations and connectivities using different approaches, and the performance was evaluated using several metrics for subnetwork identification accuracy. We compared the approaches via realistic large scale fMRI simulations and with fMRI data sets obtained during motor sequence learning. Our method identified hot subnetworks with higher accuracy than the community partition algorithms in graph theoretical analysis. Further, the HotNet approach yielded task-related networks in brain regions engaged in visual and motor processing and areas previously identified as becoming activated during motor learning. We anticipate that these methods will become a tool widely used for subnetworks discovery that shifts dynamically during performance of adaptive behavior. References: Friedman et al. (2008) Biostatistics 9: 432-41; Liu and Luo (2015) J Multivar Anal 135: 153-62; Rubinov and Sporns (2010) NeuroImage 52: 1059-69; Sanchez et al. (2010) Psychon Bull Rev 17:790-6; Vandin et al. (2011) J Compu Biol, 18:507-22; Vandin et al. (2012) IEEE Comp March: 39-46.

Disclosures: Y. Zhao: None. X. Luo: None. E. Upfal: None. P. Bédard: None. J.N. Sanes: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.08/LLL22

Topic: I.07. Data Analysis and Statistics

Support: AMED Grant 16K01469

Title: Cortical current source estimation improves detection of sensorimotor rhythm in resting-state: Validation through simultaneous EEG-fMRI recording in humans

Authors: *S. SHIBUSAWA[1], S. TSUCHIMOTO[1], S. KASUGA[1], K. KATO[3], E. YAMADA[4], H. EBATA[4], M. LIU[3], J. USHIBA[2];
[1]Grad. school of Sci. and Technol., [2]Fac. of Sci. and Technol., Keio Univ., Kanagawa, Japan; [3]Dept. of Rehabil. Med., Keio Univ. Sch. of Med., Tokyo, Japan; [4]Saiseikai Kanagawa-ken hospital, Kanagawa, Japan

Abstract: Electroencephalogram (EEG) is often used for cortical activity recording in humans because of its advantages of noninvasiveness, low movement constraints, and high temporal resolution. However, EEG spatial resolution is low, and signal contaminations from cortical regions are often observed. Cortical current source estimation, a numerical method for solving ill-posed inverse problems on EEG electrophysiology, has been therefore widely used to overcome these issues, but yet its methodological efficacy is not sufficiently tested using physiological data. The present study used simultaneously recorded EEG and functional magnetic resonance imaging (fMRI), and tested the goodness-of-fit of estimated cortical current source signals to fMRI blood oxygen level-dependent (BOLD) signals. Resting-state EEG and fMRI were simultaneously recorded for continuous 10 minutes in 14 neurologically normal participants. To construct individual brain models, T1 weighted structural MR images and EEG electrode positions were acquired from each participant. Cortical current source was estimated by solving the inverse problem using Variational Bayesian Multimodal Encephalography (VBMEG) in this study. Correlation of the estimated cortical current source signals to fMRI BOLD signals of Somatomotor Network (SN) was calculated to see its estimation quality. For quantitative assessment of signal localization, a binary classification test was involved as follows. First, SN in the fMRI space was defined as 'TRUE' region where SMR signal comes from. Other cortical regions except SN in the fMRI space were defined as 'FALSE' regions. Second, the classification performance of the estimated cortical current signal from EEG sensorimotor rhythm (EEG-SMR) recorded over the left sensorimotor cortex was assessed using Receiver Operating Characteristic (ROC) curve analysis. Third, the same classification test was performed with EEG-SMR as a reference, and calculated improvement of signal localization by current source estimation. Area Under the Curve with the current source estimates was significantly larger than that obtained with EEG signals (Wilcoxon signed-rank test, $p<0.001$). The optimal cutoff-value with the current source estimates was also significantly larger than that calculated with EEG signals (Wilcoxon signed-rank test, $p<0.001$). We considered that the estimated current source can successfully segregate cortical signals from EEG. These results suggest that current source estimation successfully segregate cortical signals from EEG, resulting in the improvement of spatiotemporal detection of the sensorimotor cortical activity.

Disclosures: S. Shibusawa: None. S. Tsuchimoto: None. S. Kasuga: None. K. Kato: None. E. Yamada: None. H. Ebata: None. M. Liu: None. J. Ushiba: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.09/LLL23

Topic: I.07. Data Analysis and Statistics

Title: Optimal resolution for searchlight classification of BOLD fMRI data?

Authors: H. MANDELKOW[1], J. DE ZWART[2], *D. PICCHIONI[1], J. DUYN[2];
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Abstract: Conventional univariate BOLD fMRI analysis relies on a fixed a-priori model of the spatio-temporal hemodynamic response to predefined stimuli. An alternative, the Searchlight (SL) method [1] makes less assumptions by inferring local information content from multivariate BOLD signal statistics. It has been hypothesised that higher spatial fMRI resolution would increase SL sensitivity allowing better discrimination between different stimuli. Here we investigate the effect of fMRI resolution on the sensitivity of SL analysis using several machine-learning (ML) classifiers.

Four healthy volunteers underwent fMRI and simultaneous eye tracking while watching one 5-minute scene from a popular action movie repeatedly. For each experimental run fMRI data were acquired either at a high (1.2mm) or a low (2mm) fMRI resolution on a 7T MRI scanner. ML classifiers known as Nearest Neighbour (NN), Gaussian Naïve Bayes (GNB) and Linear Discriminant Analysis (LDA) were used to determine the classification accuracy (CA) in identifying each of the 150 time points (300s/TR) in the movie. A searchlight map was formed by computing this classification analysis separately for each voxel location using a neighbourhood of 1-5 voxels in each direction as input features. SL maps computed separately from high-resolution (HR) and low-resolution (LR) data in each subject were compared by taking the difference HR-LR (Figure 2). The SL analysis was repeated for different classifiers and with different levels of Gaussian smoothing (3-9mm FWHM).

As expected, searchlight CA decreased with decreasing SL volume yielding mostly insignificant CA for SL radii <(6mm)3 for both HR and LR data. For SL volumes >(1cm)3 CA in most cortical locations along the ventral visual stream increased with increasing voxel size. Gaussian smoothing had a similar effect. These findings are consistent with previous results, which showed a maximum in CA for classifiers operating on 25% of all visually activated voxels across the entire ventral visual stream and at a moderate fMRI resolution of 2-3mm [2]. For intermediate SL volumes (6-9mm)3 CA and fMRI resolution effects varied systematically between brain regions, subjects and experimental runs:

a) Searchlight CA maps showed hotspots in early visual areas V1-4 and V5/hMT.

b) Positive resolution effects CA(HR)-CA(LR) were mostly observed in primary visual cortex V1-4.

c) Positive resolution effects were more widespread in subjects with more consistent (correlated) eye gaze patterns.

The aforementioned differential effects were independent of the choice of classifier, even though absolute CA levels differed substantially.

Disclosures: H. Mandelkow: None. J. de Zwart: None. D. Picchioni: None. J. Duyn: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.10/LLL24

Topic: I.07. Data Analysis and Statistics

Support: NEI 1R01EY019684-01A1

NSF/CRNS IIS-1208203

Title: Improving predictive models using non-spherical Gaussian priors

Authors: *A. O. NUNEZ-ELIZALDE, A. G. HUTH, J. GALLANT;
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Abstract: Predictive models for neural or fMRI data are often fit using regression methods that employ priors on the model parameters. One widely used method is ridge regression, which employs a Gaussian prior that has equal and independent variance for all parameters (i.e. a spherical multivariate Gaussian). However, a spherical prior is not always appropriate: there are many cases where expert knowledge or hypotheses about the structure of the model parameters could be used to construct a better prior. Non-spherical Gaussian priors can be employed using a generalized form of ridge known as Tikhonov regression. Yet Tikhonov regression is not (explicitly) used very often in neuroscience. Here we show that Tikhonov regression with non-spherical Gaussian priors can improve several predictive models for fMRI data. We also show that linearized ridge regression (i.e., linearly transforming the regressors before regression) can be formulated as Tikhonov regression.
Our first result is based on an fMRI language experiment in which subjects listened to several hours of natural stories. A predictive model in which the features were indicator variables for each unique word (a spherical prior) provided poor predictions of held-out data. However, performance improved by using a non-spherical Gaussian prior in which words that have similar meanings are likely to be assigned similar weights. Our second result comes from an fMRI vision experiment in which subjects watched several hours of natural movies. A predictive model in which low-level structural and high-level categorical features were combined (a spherical prior) provided poor predictions. However, performance improved by using a non-spherical Gaussian prior in which the two feature spaces have unequal variance. We also show that non-spherical priors can improve the estimation of temporal kernels. Finally, we demonstrate a computationally efficient method for incorporating non-spherical priors into regression models.

Disclosures: A.O. Nunez-Elizalde: None. A.G. Huth: None. J. Gallant: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.11/LLL25

Topic: I.07. Data Analysis and Statistics

Support: NSERC

Title: Correlations between myelin water fraction, ratio of T1- and T2-weighted images, and diffusion tensor imaging measures in human brain white matter

Authors: *M. UDDIN[1], K. G. SOLAR[2], A. SHATIL[3], S. YOUNAS[3], S. M. COURTNEY[4], C. R. FIGLEY[1];
[1]Dept. of Radiology, [2]Physiol. and Pathology, [3]Biomed. Engin., Univ. of Manitoba, Winnipeg, MB, Canada; [4]Psychological and Brain Sci., Johns Hopkins University, , MD, USA, Baltimore, MD

Abstract: Microstructural changes in human brain tissue can be measured noninvasively using myelin water imaging (MWI), diffusion tensor imaging (DTI), and other quantitative MRI methods. These measures can provide novel insights into demyelinating diseases, such as multiple sclerosis. Myelin water fraction (MWF) obtained from MWI (multicomponent T2-relaxation) has excellent specificity for myelin content. Although not a pure measure of myelin, signal intensity ration maps of T1- to T2-weighted (T1w/T2w) images appear to show increased sensitivity and lower variability to myelin content compared to magnetization transfer ratio. On the other hand, DTI metrics such as fractional anisotropy (FA) and mean diffusivity (MD) are considered to be indicators of white matter (WM) microstructure owing to their sensitivities to cellular density, axon diameter, and myelin content. In this study we examined the relationships between MWF, T1w/T2w ratio, FA, and MD in several WM structures using 3 T MRI data from 32 neurologically healthy human subjects. Image analyses were performed using MRIStudio and SPM12, and each subject's images were normalised to ICBM space using non-linear warping to facilitate group comparisons. To assess correlations between the MRI metrics in well defined regions-of-interest (ROIs), we extracted data from 25 WM ROIs listed in the JHU_MNI_SS ("Eve") atlas. Significant correlations were found between MWF and T1w/T2w for several ROIs, including the cerebellum, splenium of the corpus callosum (CC), fornix, inferior fronto-occipital fasciculus, posterior thalamic radiation, cerebral peduncle, anterior internal capsule and external capsule. T1w/T2w correlated significantly with FA in both the genu and splenium of CC, the external capsule, and the fornix, while MD showed negative correlations (as expected) with T1w/T2w as well as with FA for several other WM structures. Perhaps surprisingly, MWF did not show any significant correlations with either FA or MD in any single ROI; however, linear regression of the data combined across structures revealed a highly significant correlation

between MWF and FA ($r = 0.75$; $p < 0.001$), as did T1w/T2w vs. FA ($r = 0.49$; $p < 0.001$). Furthermore, MWF vs. FA and T1w/T2w vs. FA had slopes 3.05 and 2.31 respectively, suggesting that these measures might be more sensitive than FA to microstructural differences between ROIs and subjects. Overall, however, our results indicate that MWF, T1w/T2w, and DTI measures are all correlated, and that findings from one modality can be interpreted, to some extent, within the context of the others (e.g., using previous DTI findings to support novel results based on T1w/T2w or MWF, etc.).

Disclosures: M. Uddin: None. K.G. Solar: None. A. Shatil: None. S. Younas: None. S.M. Courtney: None. C.R. Figley: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.12/LLL26

Topic: I.07. Data Analysis and Statistics

Title: Combining fMRI and ECoG using common naturalistic stimulus

Authors: *A. C. CONNOLLY[1], J. HAXBY[2], B. C. JOBST[3];
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Abstract: fMRI measures the blood-oxygen level dependent (BOLD) signal, which follows the hemodynamic response to local neural activation, which is orders of magnitude slower than the underlying neural fluctuations which occur in milliseconds. Electrocorticography (ECoG) affords superior temporal resolution; however, while the spatial resolution for ECoG may be better than scalp for EEG, the increase in temporal resolution comes at a cost of decreased ability to localize activation compared to fMRI. Because of volume conduction, the voltage changes measured with ECoG contain mixtures of signals from multiple sources whose locations cannot be easily determined. We address these temporal shortcomings of fMRI and the spatial shortcomings of ECoG through a novel a cross-subject/cross-modality functional connectivity paradigm that integrate brain recordings across subjects and across modalities. Based on brain activity recorded while subjects (5 ECoG patients, and 10 healthy fMRI subjects) watched four 15 minute segments of a nature documentary, we report results of a cross-subject/cross-modality functional connectivity analysis. The “seed” signals were derived from electrophysiological recordings from electrodes in epilepsy patients undergoing monitoring for epilepsy surgery. These signals were preprocessed and transformed into spectral power time-courses and then convolved with a canonical hemodynamic response function and down sampled to the sampling frequency of

fMRI (0.4Hz). Our results show that the ECoG signal can be decomposed and related to functional activity recorded using fMRI in other subjects. Furthermore, different frequency bands from the same electrode are correlated with different, but logical anatomical sources: low-frequency bands correlated best with primary sensory regions both auditory and visual depending on the location of the electrode, and high frequency bands recorded in the occipital lobe correlated with high-level vision. We predict that repeating these analyses with a larger sample of subjects and a large number of electrodes will result in a functional mapping of ECoG electrodes to brain regions that will be very useful for all researchers conducting brain mapping using ECoG. It will also provide a means to test the time-courses of activity and causal processes for interconnected functional regions for which the temporal resolution has been lacking in fMRI.

Disclosures: A.C. Connolly: None. J. Haxby: None. B.C. Jobst: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.13/LLL27

Topic: I.07. Data Analysis and Statistics

Support: ITDP IUPUI internal grant

NIH grant R01MH108467

Title: Association of gray matter imaging markers with alcoholism incorporating structural connectivity information: a regularized statistical approach

Authors: *J. HAREZLAK[1,2], M. KARAS[1], M. DZEMIDZIC[2], J. GONI[3], B. G. OBERLIN[2], D. A. KAREKEN[2];
[1]Biostatistics, Indiana Univ. RM Fairbanks Sch. of Publ. H, Indianapolis, IN; [2]Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Purdue Univ., West Lafayette, IN

Abstract: The majority of multimodal neuroimaging studies are analyzed separately for each modality. Importantly, statistical methods that simultaneously assess multimodal data provide a more integrative and comprehensive understanding of the brain. Here we model associations between alcohol abuse phenotypes and regional grey matter measures while incorporating a priori structural connectivity (SC) information. We utilize cortical thickness and integrated rectified mean curvature obtained from FreeSurfer, to predict alcoholism-related phenotypes while incorporating the SC density of connections between 66 Desikan-Killiany atlas regions.

The analyzed sample included 148 young (21-35 years) social-to-heavy drinking male subjects from several alcoholism risk studies. A functional linear model with a penalty operator quantified the relative contributions of cortical thickness and curvature as predictors of drinking frequency, with age and number of years since regular drinking started as covariates. Model parameters were estimated by a unified approach directly incorporating SC information into the estimation by exploiting the joint eigenproperties of the predictors and the penalty operator. The best predictive imaging markers of drinking (number of drinks per week) were the average thickness of the left lateral orbitofrontal cortex, majority of the cingulate gyrus subdivisions (left anterior rostral and caudal, bilateral posterior and isthmus), bilateral paracentral gyrus, and the left inferior temporal gyrus. These regions all showed significant negative associations with the outcome, with no significant positive associations present. Introducing a priori information minimized spurious findings by assigning penalty weights in such a way that highly connected regions associated with the outcome were less penalized than other regions that had no association with the outcome. Future work will incorporate functional connectivity and finer cortical parcellation to discover even more subtle associations.

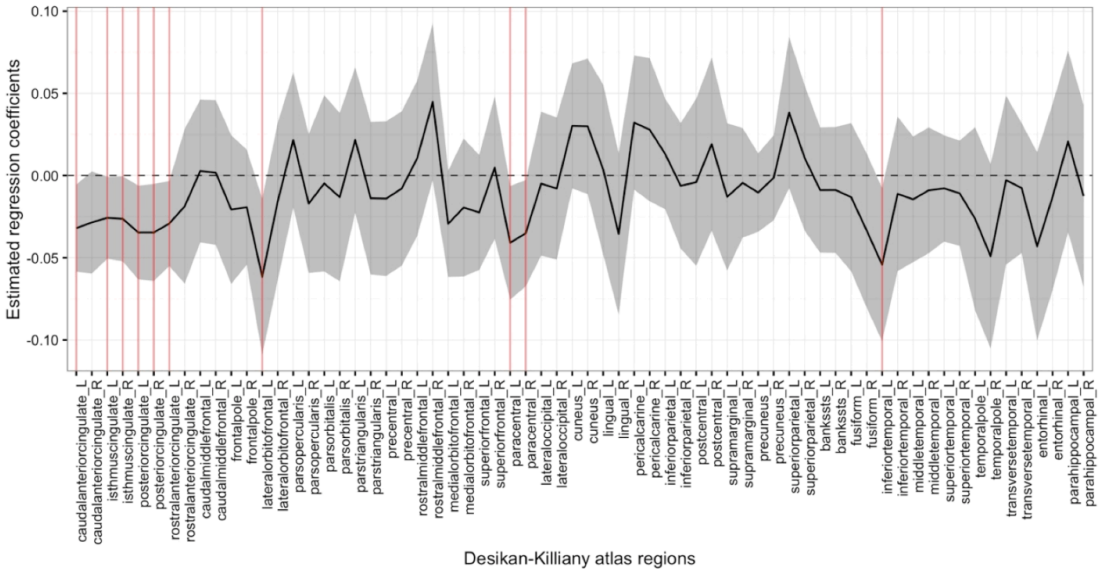

Figure 1: Estimated regression coefficients (black line) and their respective 95% confidence intervals (grey shaded area) based on the structural-connectivity-informed regularization. Vertical red lines indicate coefficients of average cortical area thickness which are significantly different from zero.

Disclosures: **J. Harezlak:** A. Employment/Salary (full or part-time): Indiana University. **M. Karas:** None. **M. Dzemidzic:** None. **J. Goni:** None. **B.G. Oberlin:** None. **D.A. Kareken:** None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.14/LLL28

Topic: I.07. Data Analysis and Statistics

Support: ONR 60744755-114407-UCB

NEI 1R01EY022454-01A1

Title: Supervised and blind voxel-based state space methods recover task-related states from fMRI responses

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Abstract: Purpose: Task-dependent brain responses are complex and difficult to understand. The problem can sometimes be simplified by modeling population responses as a dynamical process that reflects the task. Here we adapt a recent dynamical systems modeling approach developed in neurophysiology (Mante et al., Nature, 2013) and apply it to fMRI data recorded from subjects who performed an attention task and an open-ended video game task. **Methods:** In the attention task, five subjects viewed 1,800 s of natural movies repeated across cue conditions. Subjects fixated on a central point, and were cued to attend to either humans or vehicles in a balanced, randomized order. Humans, vehicles, both, or neither category were equally likely. Attentional state was defined as the cued category. In the open-ended task, one subject played a computer game for 90 minutes. States were labeled using nine categories defined by two people familiar with the game. In both experiments, whole brain MRI data with a 2.24x2.24x4.1mm voxel size was acquired on a 3 T Siemens scanner at UC Berkeley. We tested both supervised and blind voxel-based state space recovery methods. We first used principal component analysis to denoise responses. In the supervised method, we interpreted the weight vector of each state variable across voxels as the state direction and constructed a task state space from these vectors. We projected responses into this space to recover state. In the blind method, we applied independent component (IC) analysis to the responses and projected them into the space spanned by the ICs. The ICs were interpreted retroactively. **Results:** In the attention task, the supervised method recovered axes for attentional state and for the presence of each category in the movie. In the open-ended task, the supervised method found significant shifts for each state along the corresponding axis. State classification accuracy on separate validation data was above chance in both tasks (attention accuracy = 0.58 ± 0.09, chance = 0.25; open-ended accuracy = 0.56 ± 0.03, chance = 0.40). The blind method recovered two axes from the open-ended task. One axis reflects degree of physiological arousal, and the other distinguishes between opposing state

categories of "engage target" and "explore." **Conclusions:** We developed both supervised and blind voxel-based state space recovery methods for fMRI data. The supervised method recovers dimensions for all labeled states. The blind method recovers physiological arousal and a continuum from target engagement to exploration.

Disclosures: T. Zhang: None. J. Gao: None. J. Gallant: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.15/LLL29

Topic: I.07. Data Analysis and Statistics

Support: NIDCD R01 DC007683

NIDCD R01 DC007683

NIMH P50 DC01027

Title: Anatomical evaluation of Colin 27 against a database of labeled brain scans

Authors: *A. J. WORTH[1], J. A. TOURVILLE[2];
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Abstract: The Colin 27 average brain is a stereotaxic registration model from the Montreal Neurological Institute (see Holmes et al. 1998). This T1 weighted MRI scan has very high signal-to-noise ratio and as a result shows fine anatomical details. Because it is of such high quality and is readily available, it is often used in research (e.g. Strangman et al. 2013). While this is a very valuable resource, it is a representation of the anatomy of a single individual. It may be tempting to use this model as a template to represent a generic brain, but before doing that it is important to understand how it compares with other brains. Following Ono et al. 1990, we present a quantitative comparison of both topology and topography of the cortical anatomy of the Colin 27 scan against our database of labeled MRI brain scans. The famous Colin 27 scan was obtained from a 27 year old individual who was scanned 27 times over a period of 3 months. Individual MRI brain scans were automatically registered to a common space in which they were subsampled and intensity averaged. To understand the anatomy in detail, every voxel of the brain was given a neuroanatomical label: 72 regions comprehensively covered the brain including: cerebral and cerebellar gray and white matter, ventricles, brain stem, accumbens, amygdala, caudate, hippocampus, pallidum, putamen, and thalamus according to the "General

Segmentation" protocol defined by the MGH Center for Morphometric Analysis, and the cortex was parcellated into 51 units based on 36 sulci according to the BrainColor protocol. After the initial labeling, an anatomist with years of labeling experience checked all results and the technician made corrections as necessary.
We compared the Colin 27 scan with similarly labeled scans of 113 subjects using methods described in the Ono atlas, which gives common topographic variants of cortical anatomy and reports the incidence rate of each variant. Quantitative measures include sulcus position, replication, interruptions, side branching, and connections to other sulci. Position with respect to other sulci is used to identify common patterns of local sulcal topography.
The resulting images, plots, and statistics show which regions in the Colin 27 template are anatomically similar to their counterparts in the larger group and those regions that are not. In particular, we noted atypical sulcal topography in, and around, Broca's area in Colin 27 relative to the larger population. Our results demonstrate a high degree of local variability in cortical anatomy. We conclude that templates derived from individual subjects are insufficient for accurately identifying and/or localizing a specific brain region.

Disclosures: A.J. Worth: A. Employment/Salary (full or part-time): Neuromorphometrics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuromorphometrics, Inc. **J.A. Tourville:** F. Consulting Fees (e.g., advisory boards); Neuromorphometrics, Inc..

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.16/LLL30

Topic: I.07. Data Analysis and Statistics

Support: James S. McDonnell Foundation Collaborative Award #220020387

Kiwanis Foundation

1 P50 MH094258-04A1

Title: LesionWarp: An automated approach to lesion mapping through the use of nonlinear registration techniques from popular neuroimaging pipelines

Authors: J. BRUSS[1], M. SUTTERER[1], D. WARREN[2], J. HESKJE[1], *D. TRANEL[1];
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Abstract: Neuropsychological studies of patients with focal brain injuries often draw conclusions about structure-function relationships based on the common anatomical components of patients' lesions. A key challenge for the human "lesion method" is the registration of each patient's brain and brain lesion to a common atlas space. The importance of valid registration of individual patient lesion territory to common atlas space has only increased in recent years due to the development of mass univariate and multivariate statistical approaches to interpreting structure-function relationships. Common automated brain registration techniques assume healthy brain tissue and normal T1-weighted image intensities, but the MRI signal obtained from patients with focal brain injury is often distorted or missing in the lesioned area. One traditional approach to lesion transfer relied on precise manual lesion transfer to an atlas brain by an expert in neuroanatomy, but this approach was time-consuming and difficult to standardize. Recent advances in automated nonlinear registration algorithms may provide an opportunity for automated registration of a patient's native space lesion mask into common anatomical space while improving replicability and standardization of the lesion transfer. In order to validate the application of automated registration techniques for purposes of lesion mapping, we performed a comprehensive evaluation of nonlinear registration packages applied to patients with focal lesion damage. In the present study, we compared 50 atlas-space lesion masks created using a manual lesion transfer method (MAP-3) to the masks representing the same lesions created by 1) tracing the lesions in native space and 2) warping those masks to atlas space using a non-linear algorithm; we call this novel approach LesionWarp. Notably, automated non-linear registration has been implemented in several unique software distributions, each of which employs a somewhat different algorithmic approach. In order to sample the accuracy of these different approaches, we compared the results of automated non-linear warping produced by several widely-cited neuroimaging software packages including AFNI, ANTs, Freesurfer, FSL and SPM. We found high spatial similarity (as measured by Dice's coefficient) between the nonlinear registration and manual lesion transfer approaches. Nonlinear registration outcomes between software packages were highly similar. These findings indicate that native space lesion-mapping followed by nonlinear registration to atlas space provides an empirically supported alternative to traditional, manual lesion-mapping approaches.

Disclosures: J. Bruss: None. M. Sutterer: None. D. Warren: None. J. Heskje: None. D. Tranel: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.17/LLL31

Topic: I.07. Data Analysis and Statistics

Support: National Institute on Deafness and Communication Disorders R01 DC006287.

NIH Grant Number 8UL1TR000055

Advancing a Healthier Wisconsin Research and Education Program

National Center for Advancing Translational Sciences

Title: Method to account for spatial bias in multimodal neuroimaging

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Abstract: Fusing functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) brain maps offers the potential for reconstructing neural networks with high spatial and temporal resolution. One approach for fusing the neuroimaging maps is to use fMRI activation foci as priors for EEG source localization, however with the caveat that activity across neuroimaging modalities may not be fully coupled. We propose a technique to fuse fMRI and EEG brain maps that allows for spatial biases and does not rely on a priori assumptions of coupling between the modalities. This is done by projecting the volumetric fMRI maps onto an inflated cortical surface, and modeling the projected map as an EEG signal by passing it through the forward and inverse matrices constructed for EEG source localization. In this way, the spatial bias of the EEG analysis is applied to the fMRI. Preliminary results in 13 subjects tested with simultaneous fMRI/EEG in an auditory oddball task suggest that fMRI activity directly accounts for approximately 73% of the EEG activity, and the EEG activity accounts for approximately 71% of the fMRI activity. Furthermore, much of the non-overlapping fMRI activity is in areas adjacent to the areas of overlap. Furthermore, areas of overlap across subjects are consistent with those implicated in the auditory oddball task. The proposed method for fusing fMRI and EEG maps has important implications for interpreting multimodal neuroimaging data and revealing substrates and dynamics of sensory and cognitive neural networks.

Disclosures: N.J. Heugel: None. S. Beardsley: None. E. Liebenthal: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.18/LLL32

Topic: I.07. Data Analysis and Statistics

Support: Collaborative Grant from Biogen

Imperial College EPSRC Institutional Sponsorship Grant

Title: Cortical parcellation for functional brain "fingerprinting"

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Abstract: Recent studies show that cortical functional parcellations vary between individuals [1-2]. We tested whether cortical functional parcellations can be used to "fingerprint" [3] and identify individuals.
We analysed resting-state fMRI scans from 832 subjects provided by the Human Connectome Project [4]. Each of the subjects completed two resting-state fMRI sessions on separate days. The sessions on the first and second days are denoted as the Day1 and Day2, respectively. An individual cortical parcellation was generated for each subject using the Day1 data and the group-wise K-means algorithm, designed for simultaneously parcellating the full cortices across a large group. The group-wise regularisation in the algorithm ensures the parcellations across the group are similar, but allows for individual variability. A distribution of parcellations was calculated from the Day1 data and used as a prior for a second, identification step using the Day2 data. For this, the regularised K-means algorithm was used to generate parcellations based on the prior distribution. Parcellations were then compared quantitatively between Day1 and Day2 results in a blinded fashion to identify the strongest correspondences. Depending on the choice of adjustable regularisation parameter, 828-830 of the Day2 brains could be matched correctly (identified). Our studies suggest that functionally defined patterns of fMRI activity at rest are highly unique and may be able to be used to explore elements contributing to human variation.
[1] Nie, Lei, et al. "Inferring individual-level variations in the functional parcellation of the cerebral cortex". Submitted to *Transactions on Biomedical Engineering*.
[2] Wang, Danhong, et al. "Parcellating cortical functional networks in individuals". *Nature Neuroscience*.18.12, (2015):1853-1860.
[3] Finn, Emily S., et al. "Functional connectome fingerprinting: identifying individuals using patterns of brain connectivity". *Nature Neuroscience*. 18. 11, (2015):1664-1671.
[4] David C., Van Essen, et al. "The WU-Minn Human Connectome Project: An overview". *NeuroImage*. 80, (2013):62-79.

Disclosures: L. Nie: A. Employment/Salary (full or part-time): Imperial College London. **P.M. Matthews:** A. Employment/Salary (full or part-time): Imperial College London. **Y. Guo:** A. Employment/Salary (full or part-time): Imperial College London.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.19/LLL33

Topic: I.07. Data Analysis and Statistics

Support: This work was supported by Swiss National Science Foundation Grant 320030_143989

Title: Using PLSC method to detect hyperacusis condition based on audiogram and fMRI data of tinnitus patients.

Authors: *N. GHAZALEH[1], W. VAN DER ZWAAG[2], R. MAIRE[3], M. SAENZ[4,1], D. VAN DE VILLE[5,1];
[1]École Polytechnique Fédérale De Lausanne (EPFL), Lausanne, Switzerland; [2]Spinoza Ctr. for Neuroimaging, Royal Netherlands Acad. for Arts and Sci., Amsterdam, Netherlands; [3]Dept. of Otorhinolaryngology, Head and Neck Surgery, Lausanne Univ. Hosp., Lausanne, Switzerland; [4]Dept. of Clin. Neurosciences, Lausanne Univ. Hosp., Lausanne, Switzerland; [5]Dept. of Radiology and Med. Informatics, Univ. of Geneva, Geneva, Switzerland

Abstract: Intro
Tinnitus is the chronic perception of ringing or other phantom sounds. Some tinnitus patients additionally suffer from loudness hyperacusis. We used audiogram measurement and fMRI data of auditory activity for detecting hyperacusis patients using partial least square correlation method.
Method
We recruited ten patients with unilateral hearing loss and tinnitus, among which five also have loudness hyperacusis. For every patient, the audiogram was acquired by measuring the audible thresholds for 9 frequency presentations in the range 125Hz-8kHz. The fMRI data was recorded on a 7T MRI. The experimental paradigm was according to the tonotopic mapping experiment by (Dacosta et al., 2011) which consists in presenting a sequence of 15 pure frequency tones in 14 cycles (88Hz- 11340Hz). Voxels from the auditory cortex are then extracted and then amplitudes of voxels with the similar preferred frequency are averaged resulting into 15 fMRI features/subject. We then use partial least-squares correlation (Kirshnan et al, 2011) to establish the link between the audiogram measures and the fMRI tonotopy responses. We make PLSC model in two different forms to find out which set of these data can discriminate better the other set of data. In the 1st form we take audiogram measurement as the behavioral data and in the 2nd form, the fMRI data is taken as behavioral data. By maximizing correlation in a multivariate way, PLSC identifies a set of latent variables that each contain a saliency vector for the main data and two saliency vectors for the behavioral data (one for each group; i.e., hyperacusis and

non-hyperacusis).
Result
In each form of applying PLSC method, every subject has three features. In form1, the measurements for the three frequency tones (4,6 and 8 kHz) can discriminate fMRI data of the two groups. In form 2 it is just the first fMRI saliency (voxels with the frequency label of 88Hz) that can discriminate the audiogram data of the two groups.
Conclusion
This is the 1st study that clearly establishes a link between audiogram and fMRI tonotopy responses. We see that taking audiogram measurement as behavioral data, its salience reveals the differences between two groups for more measurements than taking fMRI response as behavioral data the reason could be that the functional changes of hyperacusis in respond to different frequency tones in the cortex level is more general than in the ear because of the homeostatic plasticity and lateral inhibition that maintains the overall level of activity. Our
results do not only show that it is possible to detect hyperacusis patients, but it might also open new avenues to study the neural mechanisms that underlie this condition.

Disclosures: N. Ghazaleh: None. W. Van der Zwaag: None. R. Maire: None. M. Saenz: None. D. Van DE Ville: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.20/LLL34

Topic: I.07. Data Analysis and Statistics

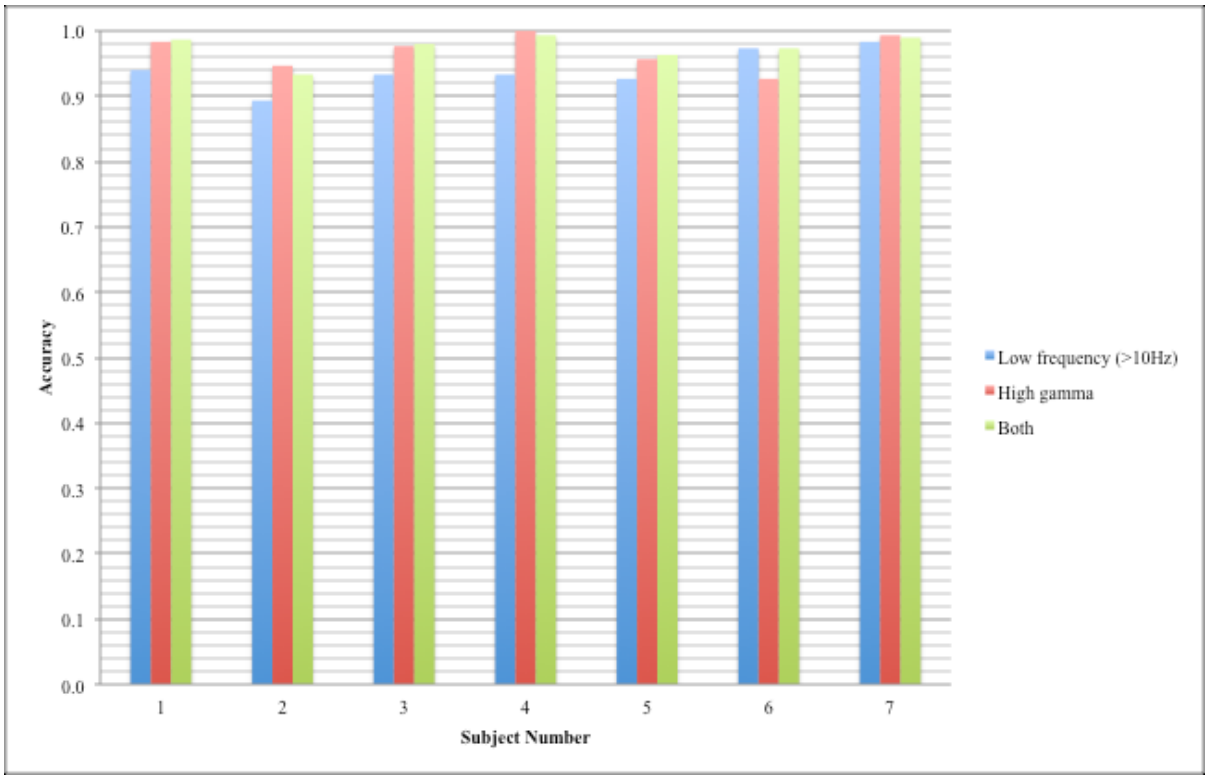
Title: Classification of visual stimuli from electrocorticographic recordings using stacked autoencoders

Authors: *S. CANCINO, J. F. DELGADO SAA;
Electrical and Electronics Engin., Univ. Del Norte, BARRANQUILLA, Colombia

Abstract: In this work, we propose a deep-learning-based algorithm for classification of visual stimuli from Electrocorticographic (ECoG) signals. In the experiment performed, a random sequence of grayscale images from houses and faces are presented to 7 subjects, and the electrical potentials from their ventral temporal cortical surface are recorded. The proposed approach makes use of stacked auto-encoders and a soft-max layer to perform classification of the two types of visual stimulation. Our experimental results show the advantages of the proposed method over state of the art techniques based in spectral decomposition, achieving an average accuracy of 97% (+/- 0.02%) across subjects. Contrary to traditional approaches, the

proposed method has the advantage of requiring minimum prior information about which features need to be used. Furthermore, the analysis of the final structure of the network can provide insight about common patterns generated by the brain during the visual stimulation presented to the subjects.

Figure 1. Classification results using the proposed stacked auto-encoders and different frequency ranges of the signal, low frequency components, high gamma and both



Disclosures: S. Cancino: None. J.F. Delgado Saa: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.21/LLL35

Topic: I.07. Data Analysis and Statistics

Title: Exploring conductive materials for low-cost EEG phantom devices

Authors: *W. HAIRSTON[1], A. B. YU[2], B. BURKE[2], G. A. SLIPHER[3];
[1]Real World Neuroimaging, [2]Human Res. and Engin. Directorate, [3]Vehicle Technol. Directorate, US Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Despite electroencephalography (EEG) being commonly used for decades in medicine and as the basis for a myriad of neuroimaging research, the EEG community as a whole has yet to adopt a single device or approach for calibrating and validating EEG data acquisition equipment or related algorithms. While so-called "phantom" devices are used in MRI, CT, PET, and other imaging devices for testing and calibration, to date no such device has been widely adopted for EEG. To be useful, such a device would need to be (1) electrically conductive in a manner roughly analogous to the human head (brain, skull, scalp), (2) consistent and predictable, and (3) relatively cost-effective in order to be accessible across the community. A major challenge to all three of these has been finding a suitable material which can satisfy all of these needs. Here, we discuss exploring the electrical conductive properties of potential materials for use in creating a cost-effective, easily producible EEG phantom. In particular we characterize the conductive properties of ballistic gelatin, which can be easily formed and mixed with additional solutes to change its conductance across the frequency spectrum typically used for electrophysiology, including both the real and imaginary components. Ballistic gelatin samples were formed at a range of concentrations (10-20%) relative to water, and salt (NaCl) was added at up to 10% by mass to improve conductance. A custom U-shaped frame including removable sides of sheet acrylic was fabricated to allow forming of disc-shaped samples with flat contact surfaces. Impedance magnitude and phase angle were collected for the gelatin samples using a network analyzer, and interrogated between 5Hz and 1500Hz using a parallel plate electrode configuration with the gelatin samples sandwiched in the middle. Results support viability and tenability for use as a phantom, with a dramatic (>100x) nonlinear reduction in impedance magnitude with increased NaCl, with a functional maximum around 10% NaCl by mass; beyond this point the material does not consistently solidify. Critically, the response is also non-linear across frequency, similar to biological tissue Significant phase shifts as well as non-linear trends in phase are also observed. The similarity to biological tissue and tunability allows a wide range of scalp conditions to be simulated on a phantom EEG device in an inexpensive and repeatable manner. A remaining challenge however is the limited lifespan of gelatin due to its organic nature. Ongoing work focuses on alternative, stable materials such as carbon nanofibers within elastomeric substrates in order to fabricate devices with greater long-term stability.

Disclosures: W. Hairston: None. A.B. Yu: None. B. Burke: None. G.A. Slipher: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.22/LLL36

Topic: I.07. Data Analysis and Statistics

Title: Getting ahead of ourselves: Fabricating an inexpensive EEG phantom

Authors: *A. B. YU[1], G. A. SLIPHER[2], W. D. HAIRSTON[3];
[1]Integrated Capability Enhancement Br., [2]Autonomous Systems Div., [3]Real-world Soldier Quantification Br., United States Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Phantoms are tools for testing and calibrating imaging devices to specific standards over time and across installation sites. Electroencephalography (EEG) is an imaging approach that has excellent temporal resolution but lacks spatial precision due to volume conduction effects. It is also highly susceptible to artifacts in the form of environmental and non-neural electrical noise. Despite their potential usefulness, phantoms do not widely exist for EEG. We describe a simple approach to EEG phantom head fabrication that is inexpensive and easily reproduced across individual laboratories. A three-part mold was designed using a scalp surface extraction from the T1-weighted MRI scan of a volunteer. The mold was 3-D printed and made watertight using silicone-based sealing material. The phantom head was formed within the mold using ballistic gelatin mixed with NaCl serving as an ionic source in aqueous solution. Dipole sources were placed within the phantom head volume using tubular guides and terminated in leads for signal input via function generator. Standard waveforms (sine wave, square wave, colored noise, etc.) can serve as input, or pre-recorded EEG can be played back. This degree of control provides a rare opportunity within EEG to know the ground truth of the source signal. Both the phase angle and impedance vector magnitude were characterized over a range of frequencies relevant to EEG in order to derive the full complex electrical impedance profile for a range of NaCl loadings. Current-voltage phase angle is an important parameter directly influencing localization accuracy that is often neglected. We show that NaCl loading in ballistic gelatin can be used to broadly match the electrical properties of human tissue, including both impedance magnitude and phase angle. Due to its inexpensive and highly reproducible nature this approach has the potential to achieve widespread adoption within the EEG community.

Disclosures: A.B. Yu: None. G.A. Slipher: None. W.D. Hairston: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.23/LLL37

Topic: I.07. Data Analysis and Statistics

Support: SFN GRFP DGE-1313667

Title: Graph models and recursive feature elimination identify biomarkers of schizophrenia

Authors: *D. MASTROVITO[1], S. HANSON[2], C. HANSON[2];
[1]Rutgers Univ., Newark, NJ; [2]Psychology, Rutgers Unviersity, Newark, NJ

Abstract: Differences in resting-state functional connectivity between schizophrenics and healthy controls have been found across the brain as well as in specific functional networks. We have characterized these differences in brain dynamics associated with schizophrenia by creating graph theoretic models of unconstrained brain activity measured from schizophrenic patient populations. Using standard machine learning algorithms, we are able to classify patients from healthy controls with 99% accuracy. Furthermore, we have evidence that the scale-free properties of the connectivity profiles of these patient populations diverge from those of healthy subjects and of theoretically efficient network configurations. Finally we identify the specific changes in brain organization that underly this disorder, by means of recurrent feature elimination.

Disclosures: D. Mastrovito: None. S. Hanson: None. C. Hanson: None.

Poster

271. Data Analysis and Statistics: Human Data II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 271.24/LLL38

Topic: I.07. Data Analysis and Statistics

Support: Kahn Neurotechnology Award

NIH NS059957

Title: Development of a targeted proteomics approach to unravel the molecular etiologies of synaptic pathologies

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Abstract: Synapses are the most abundant and distinguishing feature of the brain, providing enormous functional diversity and plasticity to neural circuits. Human genetics has recently unveiled mutations associated with a large number of brain disorders. These mutations are highly prevalent in genes that encode synaptic proteins, leading to the emerging realization that many complex neurodevelopmental and psychiatric disorders are synaptopathies. It is estimated the synapse carries the greatest disease burden of all subcellular structures in the brain. Thus, it is increasingly apparent that novel methods are needed to analyze the molecular alterations of synapses associated with neuronal dysfunction. Here we describe the development of a high-throughput targeted proteomic assay enabling the quantitative analysis of hundreds of synaptic proteins associated with brain disorders from any mouse model. This novel approach is expected to significantly advance our understanding of synaptic abnormalities associated with multiple neurological disorders.

Disclosures: T.W. Bradshaw: None. A. Uezu: None. E.J. Soderblom: None. S.H. Soderling: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.01/LLL39

Topic: I.07. Data Analysis and Statistics

Title: Sophion Analyzer as a tool for biophysical and pharmacological characterization of eight Na_v subtypes evaluated in parallel on Qube

Authors: A. LINDQVIST[1], *P. CHRISTOPHERSEN[2], M. P. G. KORSGAARD[1], H. L. OLSEN[1], L. D. LØJKNER[1], T. BINZER[1];
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Abstract: Drug discovery on ion channels is a slow and complicated process and demands a high throughput system with high data quality, but also with a flexible design and easy-to-analyze data. Sophion Qube is a next-generation giga-seal automated patch clamp (APC) screening instrument, capable of testing thousands of compounds with a single click on a button.

Data analysis is as important as data acquisition. In HTS the need for powerful analysis with efficient quality filtering is evident in order to handle the vast amount of data generated on an electrophysiological platform.. Qube is capable of testing up to 16 different cell lines or cell clones in one experiment. This can be utilized to test a different panel of cell lines or for selecting the best suited cell clone before embarking on a HTS campaign. The integrated analysis software, Sophion Analyzer, ensures analysis to keep track of all the results and whenever another QChip is assayed the analysis is done with the same set of user defined criteria. Here we demonstrate the power of automated analysis by exploring three types of experiments executed on eight different Nav channel subtypes; 1) TTX sensitivity, 2) IV-relationship for activation and inactivation and 3) pulse train suitable for screening for use dependent sodium channels blockers. For every run Nav1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 were tested in parallel on a QChip. Recorded ion channel whole-cell currents were automatically analyzed for IV-relationships for activation and inactivation (V½ and Boltzmann fit and time constants) and concentration-dependent drug effects (Hill fit and IC50) were analyzed using the Sophion Analyzer. If preferred, data can by a click be exported and calculated by other programs as Spotfire, Genedata screener or implemented in in-house software. For each subtype, the experiments identified the expected pharmacology for use- and state-dependent drugs as well as biophysical properties. The findings determined the differences between the different subtypes as expected and also that post experiment analysis can be performed with minimum of effort when using integrated, automated analysis software.

Disclosures: A. Lindqvist: None. P. Christophersen: None. M.P.G. Korsgaard: None. H.L. Olsen: None. L.D. Løjkner: None. T. Binzer: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.02/LLL40

Topic: I.07. Data Analysis and Statistics

Support: Howard Hughes Medical Institute

Title: Fast, accurate spike sorting pipeline for large-scale extracellular recordings

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Abstract: Extracellular recording technologies such as silicon probes provide means to simultaneously record from ever increasing number of neurons, and one of the latest exemplary

advancements is the Neuropixels probe containing hundreds of sites (up to 966) on a single shank. Such large-scale extracellular recordings bring a unique set of computational challenges to assign spikes correctly to individual units. In-vivo experimental conditions such as probe drift can necessitate time-consuming manual curation. To address this bottleneck, we engineered a spike sorting pipeline (JRCLUST) to achieve high accuracy, speed and usability by taking advantage of the latest clustering algorithm and GPU computing. Our analysis pipeline involves an initial automated sorting followed by a manual stage that offers efficient means of verifications and corrections. This algorithm scales linearly to the number of channels by spatially dividing the detected spikes according to their center positions. Event timing is assigned at the peak-amplitude site for each spiking event, and low dimensional features are extracted from a fixed-size spatiotemporal window surrounding each event center. Clustering is then performed for the spikes centered at each sites, and including spikes from adjacent sites for spatial merging. We employed a recently developed method of clustering by "fast search and find of density peaks" [Rodriguez and Laio, 2014] that can deal with non-Gaussian cluster shapes often-observed in real recordings. Although this algorithm scales quadratically to the number of spikes due to pairwise distance calculation, we perform this in GPU by running massively parallel threads to minimized its relative impact on the total run-time (~10 min. to sort 83 min. 120-channel recording containing 4.7M spikes). We quantified accuracy of our automated and manual sorting using three types ground-truth datasets obtained from manual-curation [Steinmetz et al, 2016], paired-juxtacellular recordings [Neto et al, 2016] and biophysical simulations [Mitelut et al, 2015]. Our automated sorting achieved 85-95% accuracy in terms of the percentage of correctly assigned spikes depending on the signal-to-noise ratio, spike amplitude and firing rate. The sorting accuracy further improved after efficient manual corrections guided by an intuitive graphic user interface and semi-automated merging and splitting. A probe drift compensation step also significantly improved the clustering performance for the datasets with noticeable drifts. We conclude that JRCLUST offers an accurate and fast means to sort spikes from large-scale extracellular recordings containing hundreds of channels.

Disclosures: J.J. Jun: None. C. Lai: None. T. Harris: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.03/LLL41

Topic: I.07. Data Analysis and Statistics

Support: PSG Brain Canada grant

Title: The methodology of developing interleaved transcranial magnetic stimulation (TMS) with functional magnetic resonance imaging (fMRI) at UBC

Authors: ***R. GE**[1], A. DIPINTO[1], L. BARLOW[1], E. MACMILLAN[1], A. MACKAY[1], M. ALFONSO[2], F. VILA-RODRGUEZ[1];
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Abstract: Transcranial Magnetic Stimulation (TMS) is a non-invasive neurostimulation tool, and in combination with fMRI, we can assess cortical excitability while monitoring intra-cerebral functional connectivity. Through new techniques we can apply these tools simultaneously (interleaved). The present study aimed to develop a TMS/fMRI system at UBC for applying the technique to clinical populations, to gain insight into the underlying mechanisms of the neural response to TMS treatment.
Phase one aimed to implement a standardized setup for interleaved TMS/fMRI at UBC with strategic placement of: MR-compatible TMS coil, high current filter box, and TMS stimulator outside of the MR room. Custom-written software (controlled by the trigger signals from the scanner) was used for interleaving the TMS pulse with the echo planar imaging (EPI) acquisition, thus preventing the TMS pulses interfering with the EPI images.Phase two was acquiring measurements to determine image quality. Using the fBIRN phantom, we determined if the EPI data quality was being affected by the interleaved TMS pulses by measuring with the TMS coil off, and the following stimulation intensities: 0%, 30%, and 90%.
To check for static signal dropout, the following measures were: 1) Signal to Fluctuation Noise Ratio (SFNR), 2) Temporal Fluctuation Noise Images (TFNI), 3) Signal to Noise Ratio (SNR) summary value. For possible dynamic artefacts, we selected the first volume as a benchmark and computed the percent signal change rate (SCR) of the consecutive volumes relative to the benchmark.Phase one was successfully completed. For phase two, 1) the SFNR calculations showed that the EPI data's temporal stability was not affected by the stimulation; 2) TFNI showed that the stimulation conditions with 30% and 90% intensity are very similar to that of free-stimulation condition, and 3) SNR summary value decreased with stimulation intensity increased. However, there was a static signal dropout observed due to the coil being inside the scanner. For possible dynamic artefacts, SCR values showed that although consecutive volumes showed fluctuations relative to the benchmark, these fluctuations were sufficiently small, which indicated that there were no significant dynamic artefacts produced by the stimulation.
With the foundation of interleaved TMS/fMRI completed, future testing experiments will determine whether the system could be applied to human participants safely and noise-freely.

Disclosures: R. Ge: None. A. Dipinto: None. L. Barlow: None. E. MacMillan: None. A. MacKay: None. M. Alfonso: None. F. Vila-Rodrguez: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.04/LLL42

Topic: I.07. Data Analysis and Statistics

Title: DeepNeuron: applying convolutional networks as a web service for neuroscientists

Authors: J. WU, 08540[1], I. TARTAVULL[1], *H. SEUNG[2];
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Abstract: Convolutional networks (ConvNets) have become a popular technique for diverse computer vision tasks like object recognition and image segmentation. ConvNets are being applied to brain images generated by MRI and microscopy. Training often involves datasets of limited size, due to the requirement of ground truth obtained via human annotation. After training, a ConvNet may be applied to extremely large datasets. Here, we report a web service that performs this function, called DeepNeuron. Given 3D image stacks and the ConvNet architecture and parameters as inputs; the service returns the ConvNet output. Computations are performed by Amazon Web Service (AWS) EC2 instances, which can scale with the amount of data elastically. A dataset that is too large to be processed in the RAM available to a single EC2 instance is divided into chunks, each of which is processed on a separate instance. We have tested DeepNeuron to process over 7 billion voxels in 3 days using 5 AWS instances. We illustrate DeepNeuron by applying it to the segmentation of serial section electron microscopy image stacks.

Disclosures: J. Wu: None. I. Tartavull: None. H. Seung: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.05/LLL43

Topic: I.07. Data Analysis and Statistics

Support: NIH grant 5R01NS047293-12

Gift from The Swartz Foundation

Title: Hierarchical general-linear modeling of EEG data in EEGLAB and LIMO extension

Authors: *R. MARTINEZ-CANCINO[1], S. MAKEIG[1], C. PERNET[2], A. DELORME[1]; [1]Swartz Ctr. for Computat. Neurosciences, La Jolla, CA; [2]Div. of Clin. Neurosciences, Univ. of Edinburgh. Brain Res. Imaging Ctr., Edinburgh, United Kingdom

Abstract: Here we present results of integrating into EEGLAB (sccn.ucsd.edu/eeglab) functionality for computing general linear model (GLM) statistics leveraging functionality in the LIMO EEG Toolbox (Pernet et al., 2011), and validating their efficacy using a publicly available data set (Wakeman and Henson, 2015). In the near future, statistical analysis based on hierarchical general linear models (within subjects, between subjects) will be the default approach supported by EEGLAB. In this approach, model parameters are estimated for any selected data measures (ERP, ERSP, power spectra, etc.) independently at each time (or time/frequency) point for each subject and each source process (or other spatial channel combination). Parameters estimated in these first-level (within-subject) analyses are then integrated across subjects into a second-level GLM, similar to the approach used to analyze fMRI data. Analysis of data following decomposition into independent component source processes (or other decomposition) is supported. To effect this integration and the consequent extension of statistical analysis available in EEGLAB, we have developed functions to define and test statistical contrasts, to visualize their results, and to interact with the user via intuitive graphic user interfaces (GUIs). EEGLAB internal data and file structures have been modified to improve computation speed while bearing the burden of increased data storage required for more complete single trial-based data analysis. Existing EEGLAB statistical analysis methods will also continue to be supported. Here we validate the results of the new development using a publicly available EEG data set by qualitatively comparing the results to those returned by the existing EEGLAB statistical analysis. The integration into EEGLAB of hierarchical GLM statistics will allow users to process data collected in more complex statistical designs and to report more robust results.

Disclosures: R. Martinez-Cancino: None. S. Makeig: None. C. Pernet: None. A. Delorme: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.06/LLL44

Topic: I.07. Data Analysis and Statistics

Title: Neurotransmitter identification raman spectroscopy in rat brain

Authors: *R. BELTRAN-RAMIREZ[1], C. VENTURA-MEJIA[2], J. CHAVEZ-GARCIA[2], J. ESPINOZA-JR[2], C. GONZALEZ-SANDOVAL[2], R. MACIEL-ARELLANO[1], R. ZEPEDA-GOMEZ[1];
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Abstract: Epilepsy is a neurological alteration characterized by the recurrent appearance of spontaneous seizures due to neuronal hyperactivity in the brain. Approximately 10% of the general population experience at least one seizure during their lifetime and 1% have epilepsy. [1-2-3]. There are different techniques to identify active substances that are present before, during and after a seizure. HPLC is the preferred method for quantification of biologically compounds concentrations, both in normal and pathological conditions. However, these techniques require a long time and a high level of complexity. Here we describe an alternative method to measure of biologically active compounds. [1-2-3]. Raman spectroscopy is an established and increasingly utilized technique for the rapid and effective discrimination among various compounds. Epilepsy is a brain dysfunction syndrome, recurrent character which is characterized by sustained abnormal synchronous group of brain neurons discharge. Typically epilepsy originates in neural networks under normal synchronous oscillations generate local or long distance. It is also considered that the seizure activity is the result of an imbalance between the excitatory activity which leads to neuron depolarization by increased neurotransmitter Glu, and inhibitory hyperpolarization generated due to a decrease of γ-aminobutyric acid (GABA) (4-5-6). The pathogenesis of epilepsy is associated with abnormal stimulation occurring in a certain region of the brain, causing depolarization of the membrane and which expands to the surrounding cells. Characteristic electroencephalographic (EEG) Epilepsy is the interictal spike-wave discharge which reflects abnormal depolarization events and membrane hyperpolarization, which occur synchronously in many neurons of the epileptogenic region; EEG spike is formed by the sum of paroxysmal depolarization, while the slow wave is formed by the sum of potential inhibitors (7).Microdialysis coupled to HPLC is the preferred method for quantification of neurotransmitter concentrations, both in normal and pathological conditions. However, these techniques require a long time and a high level of complexity Raman spectroscopy is a spectroscopic technique based on inelastic scattering of monochromatic light, usually from a laser source. Inelastic scattering means that the frequency of photons in absorbed by the sample and then reemitted. Frequency of the reemitted photons is shifted up or down in comparison with original monochromatic frequency, which is called the Raman effect.

Disclosures: R. Beltran-Ramirez: None. C. Ventura-mejia: None. J. Chavez-garcia: None. J. ESPINOZA-Jr: None. C. Gonzalez-sandoval: None. R. Maciel-Arellano: None. R. Zepeda-gomez: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.07/LLL45

Topic: I.07. Data Analysis and Statistics

Support: IZKF-Project E15

Title: Advanced perception threshold estimation using neurophysiological and behavioral parameters

Authors: *A. SCHILLING, P. KRAUß, K. TZIRIDIS, H. SCHULZE;
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Abstract: We present a novel, robust and precise method to universally estimate behavioral or physiological thresholds using the example of measurements of acoustic brainstem responses (ABR) and acoustic startle responses (ASR). By definition the threshold defines the smallest possible stimulus strength that evokes a response significantly different from that one of the non-stimulus condition. It is common practice that for estimating thresholds measurements of physiological or behavioral responses to stimulus intensities that are close to the putative threshold are carried out. Unfortunately, the signal-to-noise ratio (S/N) naturally is worst near the threshold, since the intensities of evoked responses are positively correlated with stimulus strength.
Virtually all psychometric and physiological stimulus-response functions resemble sigmoid functions like the logistic function. Here we demonstrate that thresholds may be estimated without any near threshold measurements if data are fitted to a generalized logistic function and an additive term representing the measured response intensity to the non-stimulus condition is added. We demonstrate that the goodness of fit becomes best if the supporting points are located within the area of the logistic function with the highest gradients, also referred to as its dynamic range, i.e. in a range with good S/N. To become independent from the number of measurement repetitions and the absolute noise level we perform stepwise subsampling with increasing sample-size followed by extrapolation and estimation of the asymptote. This method is highly robust against outliers which we proof using bootstrapping of sample data.
We validate our method using extensive numerical simulations and a broad variety of different experimental data. We conclude that our new approach will be beneficial for all purposes aiming to estimate a threshold based on physiological or behavioral parameters.

Disclosures: A. Schilling: None. P. Krauß: None. K. Tziridis: None. H. Schulze: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.08/LLL46

Topic: I.07. Data Analysis and Statistics

Support: FAPESP CEPID Grant 2013/07699-0

CNPq Research Productivity Grant 306251/2014-0

Title: NES: an open-source tool for management of neuroscience experimental data

Authors: *A. C. ROQUE[1], C. D. VARGAS[2], K. R. BRAGHETTO[3], E. S. ROCHA[3], M. RUIZ-OLAZAR[3], S. S. RABAÇA[3], C. E. RIBAS[3], A. S. NASCIMENTO[4];
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Abstract: NES (http://neuromat.numec.prp.usp.br/nes) is a web-based system that provides neuroscience experiments data and metadata record facilities in a secure and user-friendly platform. It was designed to provide a single repository for the experimental data of an entire research laboratory, group, or project. NES is a free software tool, developed using open technologies and tools that can be easily installed and used in any research laboratory. Its modular structure and web interface provide an intuitive use of its data management functionalities. NES was developed to combine experimental data with its provenance information. Examples of provenance information maintained by NES are: information about scientists responsible for experiments and data collection; details about recording protocols or behavioral data collection; details of the experimental protocols used in primary data collection; date and time of data collection start and end; experiment aim; conditions to which experimental subject groups were submitted; laboratory where data was collected; and publications or other results which resulted from the study. The system also allows recording of additional data for experimental volunteers, such as information on clinical history and socio-demographic data. NES requires the experimenter to record in detail each step involved in the experimental protocol before storing collected primary data. The experimental protocol is described as a workflow, which can contain both sequential and parallel steps. NES uses a standardized data model in neuroscience, enabling interoperability with other initiatives for data representation standardization. In its current version, NES is able to manage several types of electrophysiological data and metadata used by the neuroscience community, and the neuroimaging module is under development. NES is an initiative of the Research, Innovation and Dissemination Center for Neuromathematics (NeuroMat), hosted at University of São Paulo, Brazil. It is licensed under Mozilla Public License version 2.0 and its source code and documentation are available at https://github.com/neuromat/nes. One of the goals of NeuroMat is

to establish in Brazil a leading research group in Neuroinformatics, specialized in experimental data curation. With the development of free software tools such as NES, we want to encourage and support the collection of high-quality data and metadata and the creation of more open databases in neuroscience.

Disclosures: A.C. Roque: None. C.D. Vargas: None. K.R. Braghetto: None. E.S. Rocha: None. M. Ruiz-Olazar: None. S.S. Rabaça: None. C.E. Ribas: None. A.S. Nascimento: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.09/LLL47

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 2R01EB016407-09

Title: The real-time experiment interface: a hard real-time closed-loop data acquisition system with sub-millisecond latencies.

Authors: *A. GEORGE[1], Y. PATEL[2], F. ORTEGA[1], D. CHRISTINI[1], A. DORVAL[3], R. BUTERA[2], J. WHITE[4];
[1]Weill Cornell Med. Col., New York, NY; [2]Georgia Inst. of Technol., Atlanta, GA; [3]The Univ. of Utah, Salt Lake City, UT; [4]Boston Univ., Boston, MA

Abstract: To understand causal interactions between neural activity and function, both need to be monitored at the sub-millisecond scale. This requires closed-loop, hard real-time technologies that are capable of controlling stimulation (optogenetic, electrical, thermal, etc.) dependent upon some functional measure (behavior, motor action, etc.) within a deterministic period. While monitoring neural activity at precise time scales is commonplace, there is an important unmet need in a cost-effective sub-millisecond closed-loop real-time framework capable of interacting with neural activity and function. To meet that need, we have developed the Real-Time eXperiment Interface (RTXI) - a versatile interface that enables deterministic closed-loop monitoring, stimulation, and control of single-cell, network, animal, and human electrophysiology experiments.
RTXI is a free and open source platform based on real-time Linux. It runs on standard desktops and contains a built-in driver that uses National Instruments cards for data asquisition. At its core is a plugin framework where users load modules designed to run specific protocols in real-time. RTXI has a set of built-in modules, such as a high-speed oscilloscope, signal generator, and common filters, and it boasts a large library of user-made modules designed to run custom

closed-loop protocols tailored for their experiments. The framework, along with the open-source license, enables users to share resources freely and openly, and it also maximizes reproducibility of experimental setups and results. RTXI is benchmarked to provide deterministic, hard real-time performance for up to 32 16-bit I/O channels and worst-case jitter less than 5 microseconds, and it is currently used in over 65 labs around the world. Addtional information is available on our website (http://rtxi.org), and all source code is available on GitHub (https://github.com/RTXI).

Disclosures: A. George: None. Y. Patel: None. F. Ortega: None. D. Christini: None. A. Dorval: None. R. Butera: None. J. White: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.10/LLL48

Topic: I.07. Data Analysis and Statistics

Support: Natural Science Foundation of China (91232303)

Title: A new method to analyze the synaptic connection between interneuron and pyramidal neuron in 3D mice brain

Authors: *Q. XIA[1], W. CAO[1], Y. DU[1], S. LIN[1], Q. YANG[1], J. LUO[1], J. XU[1], J. XIA[2]; [1]Sch. of Basic Med. Sci., Zhejiang Univ., Zhejiang, China; [2]The Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: Although microscopy offers a method to present the number, location and the chemical nature of synapse in cultured neurons, and gives a way to visualize the spatial information of specific targets in a brain slice, a new method accompanied with microscopy is still in need to accurately analyze the synaptic connections occur in between different neurons of tissue sections in a 3D-way. Here we present a method for quantifying the number, intensity and size of neuronal synapse in the 3D mouse brain sections. We used specific synaptic markers and genetically engineered mice to specifically localize the synapses in inhibitory or excitatory neurons, and analyzed with the Imaris software. Here we show that we are able to analyze the synapses between different neurons as 3D objects from brain section slice which is comparable to the electrophysiological recordings. We also used this method to examine the synapses between interneurons and pyramidal neurons in an autism model mouse, the Neuroligin-3 R451C knock-in mouse, and observed the synaptic formation abnormalities in a 3D-manner. With this method, we can get a better understanding about morphology and anatomy of neurons, which may provide more evidence to the formation, maturation and the activity of synapse *in vivo*.

Disclosures: **Q. Xia:** None. **W. Cao:** None. **Y. Du:** None. **S. Lin:** None. **Q. Yang:** None. **J. Luo:** None. **J. Xu:** None. **J. Xia:** None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.11/LLL49

Topic: I.07. Data Analysis and Statistics

Support: NIH grant 5R01NS047293-12

Gift from The Swartz Foundation (Sag Harbor NY)

Title: LabStreamingLayer: a general multi-modal data capture framework

Authors: ***D. E. MEDINE**[1], C. KOTHE[2], M. GRIVICH[3], S. MAKEIG[4];
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Abstract: We present the Lab Streaming Layer (LSL) software framework (*github.com/sccn/labstreaminglayer/)*. LSL is a system for the synchronized collection of measurement time series in research experiments. It handles both networking and time synchronization, and allows near-real-time access and centralized control of data collection, data reviewing, and data recording functions. LSL consists of a general and cross-platform transport library (*liblsl*) with several language interfaces (C, C++, Python, Java, C#, MATLAB) as well as a constellation of data acquisition applications built on top of the library that can stream data from a wide range of hardware and software devices attached to a local computer network. LSL data streams can be written to a single (xml based) file for offline analysis. The native file format is XDF (*github.com/sccn/xdf/)*. LSL was written by Christian Köthe at the Swartz Center for Computational Neuroscience, UCSD (*sccn.ucsd.edu*), and is already in wide use to collect and record experimental data from multi-modal EEG experiments. LSL is free, open source software and as such can be modified and improved by anyone. Furthermore, LSL has been incorporated into software suites and development kits of several commercial research tools and EEG hardware devices. The LSL framework offers a solution to the problem of synchronizing multiple data streams collected via devices having different hardware clocks without the need to introduce external triggering signals (although those can also be incorporated into any LSL-based experimental setup). Testing experiments have shown that LSL is capable achieving sub-millisecond synchronization accuracy between different data streams, provided the throughput

latency and jitter of the hardware devices are known. These can be learned by simple experiments using the LSL framework itself as a test bed.

Disclosures: D.E. Medine: None. C. Kothe: None. M. Grivich: None. S. Makeig: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.12/LLL50

Topic: I.07. Data Analysis and Statistics

Title: Total conductive flux analysis provides fast and physiologically relevant quantification of amyloid pore toxicity in bilayer recordings

Authors: *A. L. GILLMAN[1], J. PHANEUF[3], J. LEE[1,2], R. LAL[1,2];
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Abstract: Ion channels are membrane protein complexes essential to signaling and communication between cells of living systems. The dysfunction of ion channels has been widely studied and directly related to the pathology and cell death in many disorders, including Alzheimer's disease (AD). The amyloid channel hypothesis of AD theorizes that amyloid β (Aβ) peptides induce ionic leakage of Na^+, K^+, and Ca^{2+}, disrupting membrane potential and cellular homeostasis leading to cell death. Electrophysiological studies of amyloid poration in membranes are typically conducted in model lipid bilayer systems. Unfortunately, traditional analysis of membrane channels is unsuitable for Aβ, and other amyloid peptides. This is due to the variable nature of amyloid activity which introduces challenges for their analysis. Investigation of amyloid toxicity has suffered from the lack of a quantitative analysis tool and technique that directly links changes in ionic conductance to cytotoxicity and provides a benchmark for comparison between peptide species. No current commercial software program is capable of properly analyzing the channel activity of heterogeneous peptides, such as amyloids. While qualitative conclusions can be drawn by observing the traces, without unitary conductance, quantitative assessment is time consuming, labor intensive, and speculative, at best. Comparison of amyloid pore activity is most often accomplished via histograms of the single channel conductance for the ensemble, which could erroneously bias histogram analysis toward prevalent, but short lived, pores that may not hold significance to dysregulation of cellular ionic balance. Instead, we propose that the total ionic flux through the bilayer is the key parameter that should be analyzed with respect to cytotoxicity. To address this issue, we have developed a custom Python analysis program that allows for more accurate understanding of the

characteristics and behavior of amyloid channels. As proof of principle for the program, we analyze our previously published data on pyroglutamate-modified amyloid-β ($A\beta_{pE3-42}$) toxicity. Analysis time was reduced by >90% while demonstrating that $A\beta_{pE3-42}$ exhibits more sustained, less transient, activity and causes cytotoxicity faster than full length $A\beta_{1-42}$. While statistically meaningful bilayer analysis will require many more recording experiments than were previously possible, emerging high throughput technologies provide the means to reach this goal. Proper understanding of amyloid toxicity through this analysis will improve diagnosis, therapeutic development, and ultimately the prevention of amyloid channel diseases and disorders.

Disclosures: A.L. Gillman: None. J. Phaneuf: None. J. Lee: None. R. Lal: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.13/LLL51

Topic: I.07. Data Analysis and Statistics

Title: Quantification of immunohistochemistry on adjacent sections comparing fluorescent and dab markers

Authors: *J. BAUN, C. ZURHELLEN, T. YORK, B. TIPTON, R. C. SWITZER, III; Neurosci. Associates Inc, Knoxville, TN

Abstract: The location of antibody binding sites in brain tissue sections is commonly detected with fluorescent markers or with colored reaction products such as diaminobenzidine. We sought to answer the question: do both types of detection schemes reveal the antibody binding sites to the same extent as judged by density of staining?
Adjacent sections of tissue were stained with several antibodies including GFAP for astrocytes and Iba1 for microglia from rat brains that were unilaterally rendered ischemic by middle cerebral artery occlusion. Sets of adjacent sections were immunohistochemically stained free floating using standard IHC methods. For one set the antibody binding site was detected using a secondary antibody conjugated with a fluorescent molecule. The second set was stained using the sequence of a secondary antibody conjugated with a biotin molecule, followed by an avidin-HRP complex and then reacting with diaminobenzidine and H_2O_2.
Two areas of interest were digitally captured for each detection scheme/stain: an ischemic area in one hemisphere and an unaffected area in the other hemisphere. The same region of interest for each stain was analyzed on adjacent sections to rule out any random differences in brain areas. Images of both the DAB and fluorescent stains were converted to 8-bit grayscale. The grayscale images of DAB stained tissue were modified to a pseudo-green (fluorescence-like) state by

applying the LUT tool in FIJI. Densitometry was performed to determine any differences between the true fluorescent and pseudo-fluorescent images. The percent area in both the ischemic and normal areas of interest were analyzed.
There were dramatic differences observed between the two staining methods. In both the GFAP and Iba1 stains there were obvious variations in staining intensity in both the affected and normal brain regions. This is not surprising because there is considerably greater amplification of the antibody presence using the DAB multiple stage sequence vs. the 'single' stage fluorophore-secondary antibody sequence. Nonetheless, the degree of hypertrophied astrocytes seen with the fluorescent method is meager compared to the image using the DAB sequence.
Antibody staining using fluorophores is required in some applications, for instance if colocalization in the same structure (e.g. cytoplasm) is expected. For reaction products where colocalization is not expected DAB detection seems to be more sensitive and has the added benefit of not being susceptible to signal loss or fading over time.

Disclosures: J. Baun: None. C. Zurhellen: None. T. York: None. B. Tipton: None. R.C. Switzer: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.14/LLL52

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant #1337983

Title: Web-based real-time 3D visualization framework for teravoxel volumes

Authors: *J. KWON[1], A. ASHWINI[1], S. RAGHAVAN[1], Y. CHOE[2], D. MAYERICH[3], T. HUFFMAN[4], M. GOODMAN[4], C. DANIEL[4];
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Abstract: Thanks to high-throughput and high-resolution imaging technologies such as Knife-Edge Scanning Microscopy (KESM), it is possible to acquire three-dimensional neuronal morphology and microvascular system of whole mouse brains at sub-micrometer resolution. To facilitate group efforts from research communities it is imperative to efficiently share the teravoxel volumes. Yet, due to the immense size of the data sets, sharing and managing them have been a big challenge. There have been efforts to address the issue. Vaa3D-TeraFly can

visualize terabyte-sized volumetric images on common laptops and graphic hardware. But this open-source plug-in can be used only for local data sets. The KESM Brain Atlas (KESMBA) visualizes and analyzes neuronal circuits and vascular networks enabling pseudo 3D visualization through stacking semitransparent image slices. But the KESMBA is far from real-time, not real 3D, and the maximum overlay is only 30 layers.We propose a Web-based and real-time (subsecond) 3D visualization framework for teravoxel volumes. The 3D visualization is completely Web-based so that it is independent of the underlying operating system. The image processing pipelines are as follows. A raw data set of 2D images is in the form of stacks and columns. As a preprocessing step, the tissue area from each of the raw images is automatically cropped and saved. From each of the cropped images, noise is removed and image intensity is normalized. Then the images from across columns are stitched in an image sheet. This process repeats for all images. The dimension of the final volume is about 10K^3. As the next step, multiple resolutions for the stack of the stitched images are generated. From the several different resolutions, unit volumes (256^3) are created. The total number of unit volumes are about 75K. Isosurfaces of each of the unit volumes are found using the Marching Cube algorithm and are saved into a 3D mesh STL file. The result of the image processing pipelines is a set of multi-resolution unit volume meshes. A web application manages visualization and interactions with the 3D models along with selections of region-of-interest and zoom in/out levels.The image pipelines were written in C++ with ITK and VTK along with Qt. We utilized the X toolkit; a lightweight web toolkit for scientific visualization.We expect the proposed Web-based and real-time 3D visualization framework to enhance accessibility of teravoxel volumes and help research communities to use the data sets.

Disclosures: J. Kwon: None. A. Ashwini: None. S. Raghavan: None. Y. Choe: None. D. Mayerich: None. T. Huffman: None. M. Goodman: None. C. Daniel: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.15/LLL53

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant DP1DA031386

Title: Multiple statistical comparisons. Is it really a problem?

Authors: *V. L. TSIBULSKY;
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Abstract: Every *Statistics* textbook strongly suggests not running multiple statistical tests for significance of the difference between groups. Herein, the validity of this statement is reviewed. Let's assume that we sample from a large pool of a normally distributed parameter. The difference between two groups is then estimated by a t-test. What is the probability finding a *P*-value less than 0.05 or higher than 0.95, for example? Statistical models tell us that both probabilities are equal because the *P* distribution function is flat between 0 and 1. It suggests that *any* result of a single significance test will be insufficient to help us to make a decision about rejecting the null hypothesis because the probability of finding *any P*-value is approximately equal. There are two solutions to this problem. The first one is, contrary to all textbooks, repeating the experiment two or more times. Obtaining $P < 0.05$ again should strongly suggest that sampling was not from the same pool. However, in many cases we cannot repeat the experiment under the same conditions. The second solution is based on using the effect size. There are different approaches to measure the effect size. The idea could be illustrated using ξ (ksi) estimate as a measure of the effect size: $\xi = n^{0.5}\theta/\sigma$ where n = number of observations, θ = difference between means, σ = standard deviation. If we are to measure the probability of finding $P < 0.05$ as a function of ξ we should see that the probability density function is a sigmoid curve. At low $\xi < 1.6$ the probability of a significant difference between groups is very low and grows to 0.2. At $\xi \approx 2.8$, the probability to find $P < 0.05$ after single t-test is about 50%. Then at high $\xi > 4.0$ the probability of a significant difference grows from 0.8 to 1. It could be advisable to repeat the experiment if the effect size is $1.6 < \xi < 4.0$. There is no need to repeat the experiment if $\xi < 1.6$ because the probability of finding $P < 0.05$ again is below 20%. In contrast, there is no need to repeat the experiment if $\xi < 1.6$ because the probability of finding $P < 0.05$ again is above 80% meaning that the effect is highly significant. These ideas are illustrated with statistical analysis of effects of a monoclonal cocaine antibody on the rate of cocaine self-administration in rats. The antibody effect is the strongest on the day of injection. Then it gradually disappears along with the degradation of antibody with a half-life of 5 - 7 days. Multiple t-tests are unavoidable, but still everyday comparison between control and experimental groups is unique because every day the sampling is from different pools. Estimation of the effects size ξ allows determination on what day after antibody injection the effect becomes insignificant.

Disclosures: V.L. Tsibulsky: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.16/LLL54

Topic: I.07. Data Analysis and Statistics

Title: Automated functional analysis of astrocytes from chronic time-lapse calcium imaging data

Authors: *G. YU[1], Y. WANG[1], G. SHI[2], D. J. MILLER[3], Y. WANG[1], G. BROUSSARD[2], Y. WANG[1], L. TIAN[2];
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Abstract: The interest in analyzing astrocyte functional status is greatly evoked by recent discoveries that astrocytes exert proactive regulatory effect on neural information processing and are deeply involved in normal development and disease pathology. The functional analysis is further facilitated by technical advances in modern microscopy and chronic ultra-sensitive genetically encoded Ca^{2+} indicators. However, there is a big gap between the capability of generating huge amount of data and the availability of sophisticated analytical approaches. The current practice relies on eyeballing the time-lapse imaging data and manually picking up regions of interest, which not only limits the analysis throughput but also is at risk of introducing bias and missing important information encoded in the big complex dynamic data. Here, we report a suite of algorithms, called *Functional AStrocyte Phenotyping* (FASP), to automatically analyze and characterize the functional status of astrocytes. Aiming at the complex nature of Ca^{2+} signaling and low signal to noise ratio, FASP is designed to be data-driven and probabilistically principled, to flexibly account for complex patterns and accurately control false discovery rates. We demonstrate the effectiveness of FASP on both synthetic and real data sets. A user-friendly plugin for ImageJ and Fiji was developed and tested. The plugin and its source code are freely available on the internet.

Disclosures: G. Yu: None. Y. Wang: None. G. Shi: None. D.J. Miller: None. Y. Wang: None. G. Broussard: None. Y. Wang: None. L. Tian: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.17/LLL55

Topic: I.07. Data Analysis and Statistics

Support: NSF STC award CCF-1231216

Title: New Data Science tools for analyzing neural data and computational models

Authors: *E. M. MEYERS[1], M. DEAN[2], G. HALE[3];
[1]Brain & Cognitive Sci., [2]Hampshire Col., Amherst, MA; [3]MIT, Cambridge, MA

Abstract: As the amount of data collected by neuroscientists continues to increase (Stevenson et al, 2011), new tools are needed to turn this data into insights into about the algorithms that underlie complex behavior (Brown et al, 2004). Here we present our latest research on computational tools we have developed at Hampshire College and at the Center for Brains, Minds and Machines at MIT. In particular, we describe new tools for neural population decoding including a graphical user interface to the Neural Decoding Toolbox (Meyers 2013), methods for analyzing single neurons, and ongoing work on a parallelized population decoding framework that uses R and Apache Spark™ to greatly increase the speed of population decoding. We also discuss CBaaS, which is a distributed platform that allows one to evaluate the effectiveness of different computational models (such as different versions of deep neural networks). These tools will allow researchers to gain deeper insights from the data they collect, and to better assess whether computational models are acting in similar ways to biological systems.

Disclosures: E.M. Meyers: None. M. Dean: None. G. Hale: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.18/LLL56

Topic: I.07. Data Analysis and Statistics

Title: The allen cortical activity map data processing pipeline

Authors: *F. LONG[1], D. FENG[2], W. WAKEMAN[2], L. KUAN[2], Y. LI[2], T. FLISS[2], N. SJOQUIST[2], F. LEE[2], T. DOLBEARE[2], A. SODT[2], M. CHAPIN[2], C. BARBER[2], S. SHI[2], C. LAU[2], J. ZHUANG[2], J. PERKINS[2], C. THOMPSON[2], S. DE VRIES[2], J. LECOQ[2], M. GARRETT[2], G. OCKER[2], M. BUICE[2], A. BERNARD[2], M. HAWRYLYCZ[2], C. REID[2], J. PHILLIPS[2], H. ZENG[2], C. KOCH[2], L. NG[2];
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Abstract: The Cortical Activity Map (CAM) project is a survey of neural activities in different retinotopically mapped areas of visual cortex, cortical layers, and Cre lines during visual stimulation in behaving young adult laboratory mice. It will provide a resource for understanding how the sensory information is processed through the cortical visual pathway at both the single-cell and population levels. The project collects multiple data streams, including intrinsic signal imaging (ISI) data sequence and two photon time-series of neuron responses. A data processing pipeline involving multiple interconnected modules was built to process these data streams. To visualize functional areas and guide the measurement of neuron responses to a targeted retinotopic location, each intrinsic signal imaging (ISI) experiment data was processed to

generate a sign map. The functional areas in the sign map were then automatically segmented and annotated. Each two-photon time-series of neuron responses was processed in multiple steps. First, to remove the fluorescence leakage between nearby pixels in the two-photon time-series, a regularized Wiener deconvolution method was applied by taking the spatially non-uniform leakage into consideration. A motion correction step based on iterative phase correlation algorithm was then applied to reduce the artifactual fluorescence changes that could contaminate actual cell response analysis. After that, fluorescence from individual cells that fire at different intervals in the two-photon time-series were detected using adaptive morphological operations and classification techniques. These segmented cells define the ROIs. To correct for contamination of the ROI calcium traces by surrounding neuropil, a gradient descent method was used to estimate the contamination ratio and true ROI fluorescence signal in a linear model. Mean fluorescence traces were then extracted from ROIs after subtracting signal from the surrounding neuropil. After that a normalized DF/F trace was computed using a sliding windowed low-passed mode operation. A variety of tuning metrics based on evoked and spontaneous activity of individual ROIs was computed for static and drifting gratings and natural scene stimuli. To find the same cells across different optical physiological experiments, an automated registration and cell mapping module was developed. All data was registered to the ALLEN Mouse Brain Common Coordinate Framework (CCF) in order to integrate CAM data with all other Allen Brain Atlas resources. Data product, API, as well as SDK are made publicly available for the global brain research community.

Disclosures: F. Long: None. D. Feng: None. W. Wakeman: None. L. Kuan: None. Y. Li: None. T. Fliss: None. N. Sjoquist: None. F. Lee: None. T. Dolbeare: None. A. Sodt: None. M. Chapin: None. C. Barber: None. S. Shi: None. C. Lau: None. J. Zhuang: None. J. Perkins: None. C. Thompson: None. S. de Vries: None. J. Lecoq: None. M. Garrett: None. G. Ocker: None. M. Buice: None. A. Bernard: None. M. Hawrylycz: None. C. Reid: None. J. Phillips: None. H. Zeng: None. C. Koch: None. L. Ng: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.19/LLL57

Topic: I.07. Data Analysis and Statistics

Title: Enabling large scale analysis of calcium imaging data on standalone machines

Authors: *A. GIOVANNUCCI, E. A. PNEVMATIKAKIS;
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Abstract: Calcium imaging methods continue to gain traction among experimental neuroscientists because of their capability to monitor in vivo large population of neurons across weeks with centisecond time and single neuron resolution. Advances in genetically encoded calcium indicators and microscopy techniques facilitate imaging larger brain areas with finer time resolution, producing an ever increasing amount of data. We estimate that a standard two photon rig equipped with resonant scanners today can produce data at a rate of 2 TB per week (40 hrs/week), a number that is even higher for alternative imaging techniques (e.g., one photon and lightsheet imaging, or voltage imaging). This sheer amount of data poses significant challenges in terms of both handling and processing. While large scale solutions for these problems are available[1], their use typically requires dedicated or shared computing clusters, thus hindering their diffusion to the average user.

Here we present a suite of open source tools developed in MATLAB® and Python for the analysis of large scale calcium imaging datasets. Our focus has been threefold: First, apply state of the art methods to the universal pre-processing problems (motion artifact correction, source separation/segmentation[2] of overlapping fluorescence signals, and neural activity deconvolution[2]), in an optimized and automated way that requires minimal user intervention. Second, enable processing of large scale datasets on a single multi-core machine by employing parallelization, memory mapping, and downsampling[3] techniques, that harness the computational resources of standalone machines. Third, equip the user with visualization and interactive processing capabilities for reviewing and modifying (if necessary) the results of the analysis.

We demonstrate the usage of our suite on a wide variety of datasets, with sizes that are comparable or even exceed the amount of available memory, including parietal and visual cortices, hippocampus and cerebellum. Even though the focus has been the analysis on a standalone machine, we note that our software can be readily employed in high performance computing clusters, thus enabling the analysis of even larger datasets.

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[3] Friedrich J., et al. "Fast Constrained Non-negative Matrix Factorization for Whole-Brain Calcium Imaging Data." NIPS workshop on Statistical Methods for Understanding Neural Data (2015).

Disclosures: A. Giovannucci: None. E.A. Pnevmatikakis: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.20/LLL58

Topic: I.07. Data Analysis and Statistics

Support: Allen Institute for Brain Science

Title: Bigneuron toolbox for neuron image acquisition, visualization, annotation, reconstruction, analysis, retrieval, and sharing

Authors: *H. PENG[1], Z. ZHOU[1], X. LIU[1], B. LONG[1], H. CHEN[1], Y. LI[1], Y. WAN[2], A. BRIA[3], T. BIGNEURON CONSORTIUM[4];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Janelia Res. Campus, Ashburn, VA; [3]Univ. of Cassino, Rome, Italy; [4]Allen Inst., Seattle, WA

Abstract: Digitization of neuron structures is arguably one of the most important steps in current neuroscience studies. How to perform image acquisition of neurons and their connections effectively? How to manage the many different types and vast amount of neuron image data? How to visualize them and compare the neuron structures quantitatively? How to analyze such data comprehensively? How to search the data and how to share with colleagues? All these have become substantial, although technical, challenges that neuroscientists must have good tools so that they could focus on understanding the biology. The BigNeuron project (Peng, Hawrylycz et al. Neuron, 2015, DOI: 10.1016/j.neuron.2015.06.036; Peng, Meijering et al. NeuroInformatics, 2015, DOI: 10.1007/s12021-015-9270-9) is a global effort to standardize the interfaces of neuron tools and neuron image data, and to provide a common platform to facilitate fair, high-throughput bench testing of various neuron reconstruction methods. We have established a Vaa3D (http://vaa3d.org) based Open Source platform for Neuron Image Acquisition, Visualization, Annotation, Reconstruction, Analysis, Retrieval, and Sharing. Currently, the released suite of BigNeuron Toolbox consists of over 130 plugin tools developed by worldwide contributors. We will highlight in this presentation some of the killer applications of the BigNeuron Toolbox, such as how to develop a SmartMicroscope to efficiently acquire sparsely labeled neurons without imaging the background areas, how to freely surf the terabyte of neuron images in three-dimensions freely, how to edit a complicated neuron structure with tens of thousands of compartments quickly, how to blast-search databases with tens of thousands neuron reconstructions, how to visualize hundreds of neurons simultaneously, and even run analysis with them, and how to share and reuse the data with users with cloud-services.

Disclosures: H. Peng: None. Z. Zhou: None. X. Liu: None. B. Long: None. H. Chen: None. Y. Li: None. Y. Wan: None. A. Bria: None. T. BigNeuron Consortium: None.

Poster

272. Data Analysis and Statistics: Software Tools II

Location: Halls B-H

Time: Sunday, November 13, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 272.21/LLL59

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01 MH104251

Title: Oculomatic: high speed, reliable, and accurate open-source eye tracking for humans and non-human primates

Authors: Y. VAZQUEZ, J. ZIMMERMANN, P. W. GLIMCHER, B. PESARAN, *K. LOUIE; Ctr. Neural Sci., New York Univ., New York, NY

Abstract: Video-based noninvasive eye trackers are an extremely useful tool for many areas of research. Many open-source eye trackers are available but current open-source systems are not designed to track eye movements with the temporal resolution required to investigate the mechanisms of oculomotor behavior. Commercial systems are available but employ closed source hardware and software and are relatively expensive therefore limiting wide-spread use. Here we present Oculomatic, an open-source software and modular hardware solution to eye tracking for use in humans and non-human primates. Our fully modular hardware implementation relies on machine vision USB3 camera systems paired with affordable lens options from the surveillance sector. Our cross platform software implementation (C++) relies on openFrameworks as well as openCV and uses binary image statistics (following Green's theorem) to compute pupil location and diameter. Oculomatic makes use of data acquisition devices to output eye position as calibrated analog voltages for direct integration into the electrophysiological acquisition chain. Oculomatic features high temporal resolution (up to 600Hz), real-time eye tracking with high spatial accuracy (< 0.5°), and low system latency (< 1.8ms) at a relatively low-cost (< 1000USD). We tested Oculomatic during regular monkey training and task performance in two independent laboratories and compared Oculomatic performance to existing scleral search-coils. While being fully non-invasive, Oculomatic performed favorably to the gold standard of search-coils with only a slight decrease in spatial accuracy. We propose this system can support a range of research into the properties and neural mechanisms of oculomotor behavior as well as provide an affordable tool to scale non-human primate electrophysiology further.

Disclosures: Y. Vazquez: None. J. Zimmermann: None. P.W. Glimcher: None. B. Pesaran: None. K. Louie: None.