SOCIETY FOR NEUROSCIENCE
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MEETING
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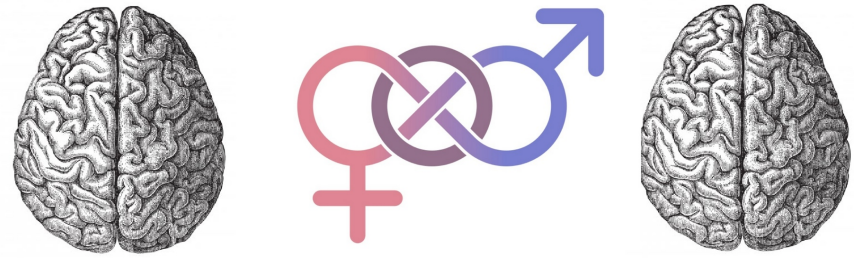
EDGEFIELD WINERY, TROUTDALE, OREGON



PROGRAM

SCHEDULE

Friday, April 12

12:00 Registration and Poster Set-Up

Mini-Symposium on the Neuroscience of Sex Differences

1:00 Charles Roselli, Oregon Health & Science University
Nature's Choice: How Brain Differentiation Influences Sexual Partner Preferences

1:30 Dena Dubal, University of California, San Francisco
Why do women live longer? Dissecting sex differences in aging and neurodegenerative diseases

2:00 Matthew Ford, Oregon National Primate Research Center
Sex differences in alcohol drinking: a tale of neuroactive steroids in mice and monkeys

2:20 Brie Paddock, Southern Oregon University
Sexual dimorphism in curcumin's effectiveness as an antioxidant in Drosophila melanogaster

2:40 Break

4:45 Appetizers and Poster Session I

6:00 Dinner

7:00 **Keynote Address**
Larry Cahill, University of California, Irvine
Sex influences on brain and body: An issue whose time has come

Saturday, April 13

7:30 Breakfast

9:00 Sarah DuBrow, University of Oregon
Memories, together and apart: How the brain segments and connects our experiences

9:30 Meredith Kelleher, Oregon National Primate Research Center
Intra-amniotic Ureaplasma *infection results in fetal brain inflammation that can be reversed by maternal antibiotic and anti-inflammatory treatment*

9:45 Alec Peters, Oregon National Primate Research Center
The novel CEMIP hyaluronidase is elevated in the dentate gyrus in a model of multiple sclerosis: A potential role for hyaluronan catabolism in cognitive dysfunction

10:00 Mollie Marr, Oregon Health & Science University
The trajectory of maternal stress across pregnancy is associated with newborn amygdala functional connectivity and infant negative affect development over the first two years of life

10:15 Kiera Degener-O'Brien, Oregon Health & Science University
Diffuse microglial dystrophy in aging human white matter hyperintensities

10:30 Mei-Ching Lien, Oregon State University
Make you look: An electrophysiological study of attention capture in the aging brain

11:00 Break and Poster Session II

12:00 Lunch

12:15 **Brown Bag Discussion**: How to be an advocate for neuroscience

12:45 Frederick Gallun, Portland VA Medical Center and Oregon Health & Science University
Assessing auditory processing abilities in human listeners: Lessons from auditory neuroscience

1:15 Awards ceremony

1:30 Meeting adjournment

Invited Speakers

Dr. Larry Cahill is a Professor in the Department of Neurobiology and Behavior at the University of California, Irvine. He first became interested in brain and memory as an undergraduate at Northwestern University. After working for two years at Searle Drug Company in Illinois on memory enhancing drugs, he earned his Ph.D. in Neuroscience from the University of California at Irvine in 1990, then conducted post-doctoral research in Germany for 2 years. He returned to UC Irvine to extend his research to studies of human subjects, which in turn led to his discoveries about sex influences on emotional memory, and to his current general interest in the profoundly important topic of sex influences on brain and body function.

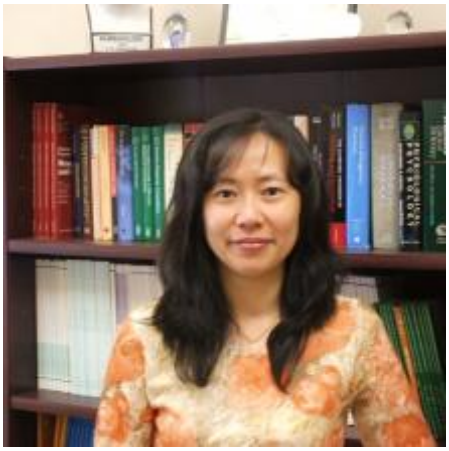
He is a long-standing leader in the area of brain and memory, and more recently has become an influential leader on the topic of sex influences on the brain. In 2017 he edited the first issue of any neuroscience journal devoted to the topic (in The Journal of Neuroscience Research). He is an internationally regarded investigator and speaker whose work has been highlighted often in the press, including in the New York Times, London Times, Washington Post, Frankfurter Allgemeine Zeitung, PBS, CNN, Scientific American and 60 Minutes.

He considers his greatest career honor to be that the seniors at UC Irvine have twice voted him the best professor in the school.

Dr. Dena B. Dubal, MD, PhD, is an Associate Professor of Neurology at the University of California, San Francisco and holds the David A. Coulter Endowed Chair in Aging and Neurodegenerative Disease. She received her MD and PhD degrees from the University of Kentucky College of Medicine and completed a medical internship and neurology residency at UCSF, where she also served as chief resident. She directs a laboratory with a research focus on longevity and brain resilience – and has discovered that "aging suppressors" such as the hormone klotho can boost brain function and counter neurologic diseases. Her lab also studies the roles of sex chromosomes in female longevity. Her work integrates genetic and molecular investigation of human populations with a range of mouse model systems to dissect causative pathways of resilience against neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Dr. Dubal see patients at the Zuckerberg San Francisco General Hospital at UCSF. Among her recent honors, Dr. Dubal received the NIA/American Federation for Aging (AFAR) Paul Beeson Career Development Award for Research in Aging, Glenn Award for Research in Biologic Mechanisms of Aging, AFAR Junior Faculty Award for Aging Research, and the Grass Award in Neuroscience, ANA.

Dr. Charles Roselli is a Professor in the Department of Physiology and Pharmacology and the Department of Anesthesiology at the Oregon Health & Science University. He received his Ph.D. from Hahnemann University in 1981. His lab is focuse on the actions of sex hormones in the brain. In mammals, brain tissue, particularly that which is involved in neuroendocrine function and reproductive behavior, contains high levels of cytochrome P450 aromatase. This enzyme converts circulating testosterone into estradiol. Although estradiol is generally considered a female hormone, aromatization seems to constitute a complementary signal pathway for androgen action in certain brain tissues. Estrogens are known to exert profound feedback effects on the brain that regulate reproduction and, more generally, are able to induce synaptic connections and protect neurons from injury. Locally-synthesized estradiol appears to play a role in male physiology that augments effects exerted by circulating testosterone. His current research uses sheep to address the question of how testosterone acts during development to organize male-typical features of brain morphology and neurochemistry to produce associated sex-differences in reproductive behaviors. Sex differences in brain development are important to understand because they underlie robust neurological differences between healthy males and females and contribute to sex biases in the incidence of certain brain diseases and psychiatric conditions.

Dr. Sarah Dubrow is an Assistant Professor in the Department of Psychology at the University of Oregon. She received her Ph.D. from the Department of Psychology at New York University. Research in the DuBrow lab seeks to understand how we learn the structure of our environments and how we use that structure to organize our memories and guide our decisions. Using neuroimaging methods, Dr. Dubrow investigates how neural representations can mirror the true structure of the external world, and, at the same time, distort that structure to achieve behavioral goals. By mapping between the brain and behavior, she hopes to shed light on fundamental organizing principles in human cognition.



Dr. Mei-Ching Lien is a Professor in the School of Psychological Science at Oregon State University. She was born and raised in Taiwan, where she attended Taipei Teacher's College and was an elementary school teacher for three years before moving to the United States in 1993. She completed her BA in Psychology at Cleveland State University in 1995 and MA in Experimental Psychology at the same university in 1997. After receiving her Ph.D. in Cognitive Psychology, specializing in Human Factors, from Purdue University in 2001, she continued her post-doc training at NASA Ames Research Center in Moffett Field, California, with a National Research Council fellowship support. In 2004, she was appointed as an Assistant Professor in Department of Psychology at Oregon State University. She later was promoted to Associate Professor and granted indefinite tenure in 2009. She was promoted to Full Professor in the School of Psychological Science at Oregon State University in 2016. Her research focuses on understanding visual/spatial attention, emotion processing, cognitive control, and examining how these mechanisms change with age using both behavioral measures and electrophysiological measures (e.g., EEG brain potentials). In 2004, she was awarded the Young Investigator Award in Experimental Psychology: Human Perception and Performance from the American Psychological Association.

Dr. Frederick J. Gallun, Ph.D. is a researcher at the VA RR&D National Center for Rehabilitative Auditory Research, and Associate Professor in Otolaryngology/Head and Neck Surgery and the Neuroscience Graduate Program at Oregon Health and Sciences University. His research focuses on how both aging and injury to the brain have the potential to produce a wide range of impairments, some of which we now believe to result in difficulties with auditory processing. The Gallun Lab explores 1) the types of auditory dysfunction that might be present in various patient populations, 2) the diagnostic tests that are used and could be used for identifying auditory impairments, and 3) the evidence that those who have experience aging and/or suffered TBI are more likely to present with auditory processing dysfunction than would be expected based on their audiograms. Patients with auditory processing difficulties are drawn from a range of sources, including older adults with normal and impaired hearing as well as younger and middle-aged adults with military exposure to high-intensity blasts and/or injury from sports concussion, motor vehicle accidents, and falls. Currently funded work involves both the development of portable, automated, rapid testing (PART) measures for diagnosing auditory processing dysfunction and the development of an auditory training game to be played on a portable device that could potentially improve the ability to make sense of complex auditory signals like speech and music.

Mini-Symposium on the Neuroscience of Sex Differences

Friday, April 12

Nature's Choice: How Brain Differentiation Influences Sexual Partner Preferences

Charles Roselli, Ph.D., Department of Physiology & Pharmacology, Oregon Health & Science University

One of the more controversial questions concerning neurobiology of behavior relates to the mechanisms that control sexual orientation in humans. Although imperfect, tests of sexual partner preferences in animals can be used to model human sexual orientation. Sexual partner preference is one of the most sexually dimorphic behaviors observed in animals and humans. Typically, males choose to mate with females and females choose to mate with males. However, same-sex or sex-reversed preferences occur in individuals of many species. The sheep offers a unique mammalian model in which to study paradoxical same-sex preferences, because as many as 8% of rams exhibit a naturally occurring exclusive sexual preference for other rams. We used sheep to test the hypothesis that same-sex preferences in rams arise from mechanisms related to sexual differentiation of the brain. According to the organizational hypothesis, prenatal exposure of the developing brain to testosterone is responsible for producing both structural and functional sexual dimorphisms. Consistent with this hypothesis, sheep have a sexually dimorphic nucleus (SDN) in the preoptic area that is larger and contains more neurons in female-oriented rams (gynophilic) than in male-oriented rams (androphilic) and ewes (androphilic). Thus, morphologic features of the ovine SDN correlate with a sheep's sexual partner preference. Our research further found that the ovine SDN already exists and is larger in males than in females before sheep are born, suggesting it could play a causal role in sexual preferences. Prenatal exposure of females to testosterone during gestational days 60 to 90 masculinizes their SDN (term pregnancy = 147 days). However, blockade of androgen action in males with a competitive antagonist fails to feminize the brain because the fetal hypothalamic-pituitary-testis axis responds by increasing testosterone secretion and overriding androgen receptor competition. These findings led us to study the development of the central mechanisms involved in the control of testosterone secretion by the fetal testes. This information should allow us to design an alternative strategy for reducing testosterone exposure in male fetuses and testing its effects on brain masculinization and sexual partner preference. Supported by NIH R01OD011047.

Why do women live longer? Dissecting sex differences in aging and neurodegenerative diseases

Dena Dubal

University of California, San Francisco

Women live longer than men – worldwide, across culture and socioeconomic status, and in many neurodegenerative conditions. Female longevity is also observed in the animal kingdom. The pervasive nature of female longevity in humans, even in death during severe epidemics and famine, suggests a role for innate biology in the survival gap between the sexes. We seek to identify intrinsic causes of female longevity in mammalian lifespan – and in the pathophysiology of neurodegenerative disease. To this end, we apply genetic models of sex biology to dissect effects of gonads, sex chromosomes, and sex chromosome dose in health and disease. Using these models in mouse cohorts of aging and models of Alzheimer's disease, we found that the second X chromosome decreases vulnerability to age-induced mortality and counteracts neurodegenerative disease-related mechanisms. The identification and modulation of intrinsic, XX-derived mechanisms of female-advantage could open new pathways to modify and increase healthy aging in both sexes.

Sex differences in alcohol drinking: a tale of neuroactive steroids in mice and monkeys.

Matthew M. Ford[1,2]*, Kathleen A. Grant[1,2], Deborah A. Finn[2]. [1]Division of Neuroscience, Oregon National Primate Research Center and the [2]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR USA.

Women and men differ in the amount of alcohol consumed, drinking pattern, and sensitivity to both the positive rewarding effects and the negative health consequences of alcohol. A host of factors are thought to contribute to these sex differences, including disparities in alcohol metabolism, body composition and alcohol distribution, as well as sexually-dimorphic organization of the brain. Sex-specific differences in neurochemistry and neurotransmission within brain reward circuitry support not only an altered sensitivity to alcohol, but also a divergent responsiveness to regulation by neuroactive steroids. Our earliest work demonstrated that oral alcohol intake during a 2-hr drinking session significantly elevated brain levels of the progesterone (P) metabolite, allopregnanolone (AP), in male mice, but not in females. Subsequent studies revealed that exogenous administration of AP dose-dependently modulated ethanol drinking in males whereas females were largely refractory to treatment. Similarly, alcohol intakes in male mice were significantly reduced following administration of finasteride, an inhibitor for 5α-reductase (rate-limiting enzyme for AP biosynthesis). In contrast, the female drinking response to finasteride was muted. Knockout of 5α-reductase type I *(Srd5a1*) in mice enhanced limited-access alcohol intake by males, but exhibited the opposite effect in females. Deletion of *Srd5a1* eliminated the marked sex difference in ethanol intakes observed in wildtype mice (females consume significantly more). Transient shifts in the plasma levels of P and its centrally-active metabolites (AP, tetrahydrodeoxycorticosterone; THDOC) occur throughout the menstrual cycle, and our model of chronic alcohol intake in female rhesus macaques affords the opportunity to assess the relationship between neuroactive steroid levels and the quantity of alcohol consumed. The first half of the menstrual cycle (follicular phase) is characterized by low plasma P levels whereas the subsequent luteal phase features a pronounced rise and subsequent fall in these steroids. Alcohol intakes following the P peak of the luteal phase were significantly elevated when compared to follicular phase drinking. In macaques the 5β steroid isomers of AP and THDOC mirrored the observed menstrual cycle-related differences in alcohol intake. Collectively, these findings suggest that neuroactive steroids are important *in vivo* regulators of alcohol intake, and that sex differences in alcohol drinking may be attributable, in part, to disparities in steroids levels or responsiveness to their actions within the reward pathway.
Supported by NIH grants AA012439, AA024757, AA013641 and OD011092.

Sexual dimorphism in curcumin's effectiveness as an antioxidant in *Drosophila melanogaster*

Paddock BE, Esquivel AR, Videau P

Oxidative stress, occurring from the imbalance of reactive oxygen species (ROS) and endogenous and exogenous antioxidants, promotes aging and contributes to the pathogenesis of neurodegenerative disorders. Curcumin, a yellow pigment derived from turmeric, has antioxidant properties as an ROS scavenger, but more recent evidence suggests that it influences the regulation of genetic elements in endogenous antioxidant pathways. To investigate the role of curcumin in sex-specific *in vivo* responses to oxidative stress, *Drosophila* were reared on 0.25 mM, 2.5 mM, and 25 mM curcumin and oxidative stress was induced with hydrogen peroxide treatment. High levels of curcumin exhibit two sex-specific effects; protection from this oxidative stressor and changes to brain size. Curcumin treatment additionally increased the expression of dFoxO in *Drosophila* larvae. Current research is quantitatively assessing oxidative stress utilizing a TBARS assay. Taken together, these results suggest that curcumin's role as an antioxidant relies on changes in gene expression, and that significance sexual dimorphism exists in the *in vivo* response to curcumin.

Acknowledgements:
The authors would like to thank Southern Oregon University for supporting this work.

Poster Session I

NW Noggin: Synapses Stories and Song!

GRIESAR, W.S.* **, LEAKE, J.* ** ***

Science needs investment and diverse perspectives, and integrating arts in STEM (STEAM) encourages more people to get involved.

Nonprofit NW Noggin (nwnoggin.org) organizes undergraduates and graduates to collaborate, build community networks and inspire people about neuroscience and art. Volunteers benefit from work across disciplines and institutions, serve as "near peer" role models, gain skill explaining research, and think creatively about careers. We've met over 25,000 K-12 students since 2012!

There is tremendous need to develop approaches to assist academic priority K-12 students, and offer access to social and economic benefits of education. Students struggle with academic achievement. The dropout rate for Native Americans is particularly high.

Oregon has a rich Native culture and history, yet only in 2017 did the Legislature mandate a statewide K-12 curriculum for 'tribal history,…sovereignty, culture, treaty rights, government, socioeconomic experiences, and current events.'

In 2017 we received a grant from Spirit Mountain Community Fund for outreach with tribal majority schools, aimed at making connections between animal figures in Grande Ronde and Siletz stories and neuroscience research. In 2018 we engaged over 300 K-12 students through brain research, storytelling, music and art!

We spent spring 2018 collaborating with Oregon Pacific Area Health Education Center (OPAHEC) visiting students, teachers and staff at Siletz Valley Early College Academy, Amity and Willamina High Schools. In summer, we joined award-winning Native storyteller Esther Stutzman at her annual camp in Curry County, to hear Coos and Kalapuya tales, and discuss relevant brain research.

In fall, Noggin volunteers from Portland State University and OHSU, along with Native storyteller Fish Martinez and puppeteer Ana Mello, spent three days telling stories, answering questions, holding brains, and connecting in classrooms through drumming, music, poetry, research and art!

Intel's Native American Employees Network (INAN) prepared a "Noggin Brain Box" filled with outreach project instructions, pens, paper, 3D printed brain models, and pipe cleaners for crafting brain cells. OPAHEC and Noggin also brought students to OHSU for a tour of MRI imaging facilities, to expand knowledge of clinical and research career options.

Building excitement and awareness of discoveries, educational options and careers through arts-integrated outreach across institutional, state, federal and generational lines trains new scientists to collaborate, engages more communities, and increases awareness and support for investment in brain research and the arts.

* Department of Psychology, Portland State University; ** NW Noggin (nwnoggin.org); *** Department of University Studies, Portland State University

K-12 students want to learn about neuroscience!

Eisen, A., Howard, C., Benefiel, J., Schmidt, J., Chapek, M., Lerner, M., Garduno, R., Uriarte-Lopez, J., Hamilton, H., Kiersarsky, A., Sumrall, L.

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The Neuroscience Club at Portland State University actively works to enrich local communities with taxpayer supported neuroscience research. Our members devote their time and effort into bringing education, art, and enthusiasm to our community. A primary focus of our outreach is directed at K-12 public schools with the objective of giving students opportunities to interact with science in ways they might not necessarily have had. We base our outreach on open inquiry and tie in elements of philosophy for children. The students have the opportunity to guide their own learning, which captures their enthusiasm and engagement for the subject. We have found the students maximize their learning when given the opportunity to ask open-ended questions in a supportive and understanding environment, prompting forth questions filled with both complexity and creativity that could baffle even the most experienced neuroscientists. Students engage the world around them and through the exploration of neuroscience, often draw upon personal experience to connect with others and stimulate thought. Through this process they can better understand themselves and the world around them. Students use the medium of art to support their understanding of advanced concepts guided by their own creativity and physical engagement with the content. Students screen-print works of neuronal art and twist pipe cleaner neurons to life, teaching them ways to engage art with science further while enhancing their understanding of the subject in a hands-on fashion. This poster was inspired by questions collected during an outreach event at North Middle School in Grants Pass, OR. These questions are representative of common student inquiries from all our community outreach events. Although the Neuroscience Club initially focused on students, the club has since expanded its reach to include more diverse populations. The Neuroscience Club's objectives include nourishing the future generation of scientists and artists whilst allowing education to be fun and engaging and have a place to flourish in diverse communities.

Sources:

NW NOGGIN: Neuroscience outreach group (growing in networks) – Building networks in the community through neuroscience education and art. (2019). Retrieved April 1, 2019, from https://nwnoggin.org/

Time course of changes in parvalbumin neurons and perineuronal nets in the rat medial prefrontal cortex after activation of a cocaine-associated memory

Angela E. Gonzalez, Emily T. Jorgensen, John H. Harkness, Jordan M. Blacktop, Deb Hegarty, Sue Aicher, Travis E. Brown and Barbara A. Sorg

Perineuronal nets (PNNs) are specialized extracellular matrix structures that wrap primarily around parvalbumin-containing, fast-spiking interneurons (PV-FSIs) in the medial prefrontal cortex (mPFC). We previously found that PNNs in the mPFC were necessary for the consolidation and reconsolidation of cocaine-associated memories and more recently that cocaine exposure alters the intensity of PNNs and PV in the mPFC. Here we examined the time course of changes in PV and PNN intensity following reactivation of a cocaine-associated memory. Rats were initially trained for cocaine-induced conditioned place preference (CPP) with three injections of saline (1 mL/kg, intraperitoneal, ip) alternating with three injections of cocaine (12 mg/kg, ip). Re-exposure occurred 1 day later in a drug-free state, and rats were sacrificed at either 30 min, 2 hr, 6 hr, or 24 hr later. A separate cohort of rats was sacrificed prior to any re-exposure as a baseline control (t = 0). Brain slices were stained and quantified for PNNs using *Wisteria floribunda* agglutinin (WFA) or PV. WFA intensity was significantly increased at 6 hr. Intensity of PV decreased at 30 min after re-exposure to cocaine CPP context and was maintained to 24 hr. Excitatory and inhibitory puncta analysis was done by staining tissue with GAD 65/67 and VGlut1. The ratio of inhibitory (GAD 65/67) to excitatory (VGlut1) puncta was decreased at 24 hr after memory reactivation. Slice electrophysiology studies in FSIs in the mPFC surrounded by PNNs (likely containing PV) showed that re-exposure to the cocaine CPP context reduced the number of action potentials 30 min and 2 hr post-memory reactivation and returned to control levels by 24 hr. The attenuation in action potential firing was accompanied by an increase in the average miniature excitatory postsynaptic event amplitudes and frequency, but no change in the average miniature inhibitory events. Collectively, our results show that cocaine memory reactivation alone produces relatively long-term changes (up to 24 hr) in PV FSIs, which profoundly control the excitatory and inhibitory balance to modulate mPFC output neurons that drive cocaine-seeking behavior.

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Neuronal dystroglycan regulates CCK/CB1R interneuron development

Dystroglycan is a transmembrane glycoprotein that links the extracellular matrix to the actin cytoskeleton through its two subunits, a heavily glycosylated extracellular subunit alpha dystroglycan (a-DG), and the membrane spanning beta-dystroglycan (b-DG). Glycosylation of a-DG is essential for its ability to bind extracellular ligands that contain Laminin G (LG) domains. Dystroglycan is highly expressed in muscle and brain, and mutations in multiple glycosyltransferases required for its function cause a form of congenital muscular dystrophy characterized by muscle weakness, brain malformations, and cognitive deficits. In the brain, dystroglycan is expressed in both glia and neurons, but its specific function in neurons has remained elusive.

Recently, dystroglycan was found to be required for formation of cannabinoid receptor 1 (CB1R) synapses arising from cholecystokinin (CCK) interneurons, one of the basket cell populations that provide inhibitory input to pyramidal cells in the cerebral cortex and hippocampus. However, the mechanism by which dystroglycan orchestrates development and formation of CCK interneuron-derived inhibitory synapses remains unknown. In the present study, we use a variety of mouse lines, including a hypomorphic mouse model of dystroglycanopathy (*B3gnt1*^{M155T}), conditional deletion of *dystroglycan* from pyramidal neurons (*DG*$^{F/-}$*; NEX*^{Cre}), and a mouse lacking the intracellular signaling domain of dystroglycan (*DG*$^{\Delta cyto}$) to determine how dystroglycan regulates CB1R+ synapse formation. Following conditional deletion of *dystroglycan* from pyramidal neurons, we find that CCK-derived presynaptic CB1R+ terminals disappear during the first postnatal week after birth. We also find that maintenance of CB1R+ terminals does not require full glycosylation of a-DG or the intracellular signaling domain of b-DG. Other basket interneuron populations require BDNF/TrkB signaling for their proper development. In contrast, we found that presynaptic CB1R+ terminals were present in *TrkB* knockout mice at postnatal day 14, suggesting that TrkB-mediated neurotrophin signaling is not required for CCK+/CB1R+ synapse formation. Collectively, these data point to a critical role for dystroglycan in mediating CB1R+ synapse formation during early postnatal development.

Funding:
RO1: NS091027-01 (to K.W.)

Exploring the mechanism and cellular specificity of glycine transporter 1 for the treatment of epilepsy.

Sadie B. Baer*, John M. Cook, Landen Weltha, Wakaba Omi, Kerry-Ann Bright, Detlev Boison, and Hai-Ying Shen

Dow Neurobiology Department, Legacy Research Institute, Legacy Health, 97232 Oregon
*presenter of the poster

Abstract
We have demonstrated previously that astrogliosis and the overexpression of hippocampal glycine transporter 1 (GlyT1) are a pathological hallmark in patients with temporal lobe epilepsy (TLE) and rodents with TLE modeling. In the forebrain, GlyT1 regulates the availability of glycine, a major inhibitory neurotransmitter throughout the CNS. Correspondingly, augmentation of glycine via GlyT1 inhibition suppresses acute and chronic seizures in mice. While glycine can bind to glycine receptors (GlyR) to provide an inhibitory effect, it also acts as a co-agonist of N-methyl-D-aspartate receptors (NMDAR) for neuronal excitation. In this study, we aim to *(i)* understand the mechanisms for GlyT1 manipulation in seizure suppression, and *(ii)* dissect the contribution of GlyT1 inhibition in different cell types, i.e., astrocytes vs. neurons, on seizure activity. We used two established transgenic mouse lines with deletion of GlyT1 in the forebrain of either neurons and astrocytes (Emx1-GlyT1 KOs) or neurons only (CamKIIα-GlyT1 KOs). GlyT1 knockouts (KOs) and their wild-type (WT) littermates were tested for suppression of acute seizures with pentylenetetrazole (PTZ) and maximal electroshock (MES) models, and chronic seizure development utilizing the intrahippocampal kainic acid (IHKA) model. Additionally, GlyR agonists and GlyT1 antagonists were used to mechanistically investigate glycine actions on seizure suppression. Our data demonstrates that *(i)* Emx1-GlyT1 KOs increased seizure PTZ thresholds versus WTs, whereas CamKII-α GlyT1 KOs showed a similar PTZ seizure threshold to their WT littermates, suggesting a major role of astrocytic GlyT1 on seizure suppression induced by GlyT1 inhibitors; *(ii)* the pretreatment of GlyR agonist, taurine, increased PTZ seizure threshold to a similar level of Emx1-GlyT1 KOs, indicating that the activation of GlyR contributes to GlyT1 inhibition-mediated seizure suppression; *(iii)* in addition, the pretreatment of GlyT1 antagonists (ORG24598, LY2365109, SSR504734, and RG1678) protected mice from tonic hind limb extension in MES testing; *(iv)* Emx1- and CamKIIα-GlyT1 KOs showed additional evidence of cellular contribution of GlyT1 in IHKA seizure development. In conclusion, our data suggests that GlyT1 inhibition effectively suppresses acute seizures via activation of GlyR and that astrocytic GlyT1 may play a major role in glycine-based seizure suppression in mice.

Acknowledgements:
This work was supported by NIH grant R01NS084920

Do Highly Conserved Protein Sequences in Non-Mammalian Vertebrates Modulate Hair Cell Regeneration in Zebrafish?

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Abstract

Hair cells can be damaged from a variety of sources, including excessive exposure to noise or ototoxic drugs, and mammalian hair cells are unable to regenerate after damage. In contrast, non-mammalian vertebrates exhibit robust and often rapid regeneration of hair cells. Our goal is to identify genes that are responsible for hair cell regeneration in innately regenerative species. Previously, we characterized amino acid-level differences between regenerative and non-regenerative vertebrates and identified conserved protein sequences in regenerators that are divergent in mammals and may therefore contribute to differential regenerative capacity. Using our bioinformatics analysis we identified several novel targets as potential modulators of hair cell regeneration, including the catecholamine metabolism protein catechol-O-methyltransferase (COMT), the metabolic regulator inositol hexakisphosphate kinase 2a, the E3 ubiquitin ligase SHRPH, and the telomere-binding protein TERF1. We used CRISPR-Cas9 technology to individually knock out these target genes in transgenic Brn3c:mGFP or myob6:EGFP zebrafish, then determined the effects on hair cell development and regeneration in the lateral line. Zebrafish embryos were co-injected with sgRNA targeting tyrosinase, a gene responsible for pigmentation, to provide a phenotypic readout for CRISPR efficacy. Preliminary results suggest that COMT may influence lateral line development. Ongoing experiments will examine the effects of COMT and the other target genes on hair cell regeneration. We are also using pharmacological manipulations of the target proteins to further investigate their role in hair cell regeneration. Collectively, these studies enhance understanding of the variety of genetic regulators responsible for hair cell regeneration in non-mammalian species and present new targets to stimulate regeneration in the cochlea.

Focal manipulation of adenosine kinase using gene therapy approaches: Effects on seizure activity in a mouse model of TLE

Stacie L. Ong, Jesica E. Reemmer, Sadie B. Baer, Detlev Boison, Hai-Ying Shen
Dow Neurobiology Department, Legacy Research Institute, Legacy Health, 97232 Oregon

Epilepsy is an incapacitating neurological disorder that affects over 50 million people worldwide. Because current available anti-epileptic drugs (AEDs) are still ineffective for approximately one third of patients with temporal lobe epilepsy (TLE), there is an urgent need to develop novel treatments for TLE. Previous research has shown that augmentation of adenosine, an endogenous anticonvulsant, can suppress seizure activity. Specifically, targeting adenosine kinase (ADK), a metabolic adenosine removal enzyme that is overexpressed in astrocytes of the epileptic brain, has been proposed as a potential therapeutic strategy for TLE. Due to off-target effects of systemic ADK inhibition, we developed genetic tools that allow us to investigate the effects of focal, selective manipulation of astrocytic ADK on seizure activity in an intrahippocampal kainic acid (IHKA) mouse model of TLE. To investigate the efficacy of different viral strategies, we developed two approaches: *(i)* Cre-lox recombination to knockout ADK in transgenic *Adk*-floxed mice and *(ii)* miRNA viral-mediated knockdown of ADK in wild-type mice. AAV8 vectors were introduced to the hippocampus of wild-type or *Adk*-floxed mice at 4 weeks post-IHKA injection and EEG evaluation of seizure phenotypes was performed at 3 and 6 weeks post-virus injection. Our results showed *(i)* successful ADK deletion in the hippocampus via AAV8 vectors containing the astrocyte-specific promoter gfa2 to deliver Cre recombinase or miRNA, *(ii)* reduction of ADK in the AAV8-Cre recombination approach compared to the miRNA approach, validating a higher efficacy of ADK reduction in the knockout versus knockdown strategy, and *(iii)* preliminary evaluation of viral-mediated miRNA knockdown of ADK on seizure burden through EEG analysis. Conclusion: We used viral strategies to manipulate astrocytic ADK in the hippocampus to effectively reduce focal ADK expression and evaluate their effects on seizure activity in mice with TLE.

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Sarcosine delays epileptogenesis in a rat model of temporal lobe epilepsy with altered hippocampal transmethylation

John M. Cook*, Sadie B. Baer, Landen Weltha, Wakaba Omi, Stacie L. Ong, Detlev Boison, and Hai-Ying Shen
*presenter of the poster

Epigenetic modifications have been evidenced as a pathological feature in the epileptic brain that is linked to the development and progression of seizures. The overexpression of glycine transporter 1 (GlyT1) is also identified as a pathological hallmark in the hippocampus of patients with temporal lobe epilepsy (TLE) and rodents with modeled TLE. Accordingly, modification of the glycine signaling pathway is proposed for therapeutic intervention against epilepsy. In the present study we aim to evaluate the effect of sarcosine, i.e., methyl glycine, on epileptogenesis and its possible underlying mechanisms with a focus on the aspect of hippocampal transmethylation. We used an electric kindling rat model of TLE with seizure score monitoring to evaluate the effect of sarcosine effects on epileptogenesis. Specifically, rats were kindled for 5 consecutive days, then after a 5-day non-kindling period animals were tested for their status of epileptogenesis. Sarcosine (3g/kg, daily i. p.) was administered systemically to animals during the kindling period. Immunohistochemistry and Western blot assay were performed for evaluation of pathological and epigenetic changes in the epileptic brain, including 5-methylcytosine (5-MC), 5-hydroxymethylcytosine (5-HMC), and enzymes involved in transmethylation processing, such as methylcytosine dioxygenase TET1 (TET1), DNA-methyltransferase 1 (DNMT1) and DNMT3a. We demonstrate *(i)* sarcosine treatment (3g/kg, i.p. daily) suppressed seizure development in the kindled rats; *(ii)* the sarcosine induced antiepileptogenic effect was accompanied by a reduced level of hippocampal 5-MC and increase of 5-HMC; *(iii)* in addition, sarcosine treatment caused different changes on the expressions of TET1 and DNMTs. In conclusion, sarcosine can effectively suppress the epileptogenesis in rats that was linked to altered transmethylation processing.

Acknowledgements:
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KEYNOTE PRESENTATION

Sex influences on brain and body: An issue whose time has come

Larry Cahill
University of California, Irvine

Neuroscience witnessed a remarkable shift the past 15-20 years, changing from a general belief that sex influences, if they exist at all, are restricted to directly reproductive function/regions, to increasingly recognizing that sex influences are extremely widespread, occurring at essentially all levels of brain function, often in completely unexpected ways. This talk will explore this development so important in particular for understanding brain function in females, with particular emphasis on the speaker's domain of emotional memory.

General Meeting Presentations

Saturday, April 13

Memories, Together and Apart: How the Brain Segments and Connects our Experiences

Sarah Dubrow
University of Oregon, Department of Psychology

Experiences unfold continuously in time, yet this continuity is not always preserved in memory. Instead, we tend to segment our experience into distinct episodes. This segmentation process brings some memories together while pulling others apart. In this talk, I will present a series of studies employing behavioral, neuroimaging and neuropsychological methods with a focus on hippocampal processes. I will show how changes in our mental states segment our subjective experience and address how we are able to link distinct episodes together to preserve the continuity of experience. The goal of this work is to understand how we reflect and impose structure in our learning in order to behave adaptively.

Intra-amniotic *Ureaplasma* infection results in fetal brain inflammation that can be reversed by maternal antibiotic and anti-inflammatory treatment.

Meredith A. Kelleher[1], Victoria H.J. Roberts[1,2], Terry K. Morgan[2], Antonio E. Frias[1,2], Larry S. Sherman[3]

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INTRODUCTION: Intrauterine infections caused by bacteria are considered the major cause of early preterm birth, with 85% of births that occur earlier than 28wks gestation associated with infection and inflammation. *Ureaplasmas* are the microbes most commonly associated with preterm birth being identified in 47% of placentae after preterm labor and strongly associated with histological chorioamnionitis. Intra-uterine infection is also associated with fetal inflammatory response syndrome (FIRS) that may contribute to perinatal brain injury linked to preterm birth. The objective of this study was to assess fetal brain inflammation and development following intra-amniotic *Ureaplasma* infection and the efficacy of *in utero* antibiotics and anti-inflammatory treatments to treat fetal neuroinflammation.

METHODS: Time-mated pregnant rhesus monkeys were chronically catheterized and assigned to Control [n=7]; intra-amniotic inoculation with *Ureaplasma parvum* (10^7 CFU/ml at 123 ± 6.15d gestation) [IAI, n=6]; and IAI plus maternal Azithromycin therapy (12.5 mg/kg, every 12 h, intravenous for 10 days) [AZI, n=6] and Azithromycin combined with Dexamethasone and indomethacin [ADI=7]) treatment groups. Cord blood IL-6 was measured by ELISA. Immunohistochemical staining of the fetal brain (Iba1, GFAP, MBP, Olig2, Caspase-3, HABP) was performed and placental histology assessed after preterm C-section delivery.

RESULTS: Fetal cord blood IL-6 was elevated in the IAI group and reduced by AZI and ADI treatment. By histology, chorioamnionitis, chorionic plate inflammation and funisitis were present with IAI but less frequently observed in the AZI group. Decidual inflammation was present in all IAI animals and was not reversed with AZI treatment. Fetal brain microglial activation was significantly increased in the periventricular white matter in fetal brains from the IAI group and normalized to control levels following AZI and ADI treatment. In the sub-cortical white matter region ADI treatment was more effective that AZI alone at reducing microgliosis. MBP staining was also increased in the AZI group compared to the IAI animals. Hyaluronic acid staining co-localized with areas of microgliosis in IAI animals and was reduced with treatment.

CONCLUSIONS: Increased cord blood IL-6 was present in the IAI group, which also had increased periventricular microgliosis. Accumulation of hyaluronic acid in regions of microgliosis suggests a mechanism by which myelin synthesis could be delayed in the fetal brain exposed to inflammation during gestation. These outcomes may be improved by maternal antimicrobial and anti-inflammatory treatments.

The novel CEMIP hyaluronidase is elevated in the dentate gyrus in a model of multiple sclerosis: A potential role for hyaluronan catabolism in cognitive dysfunction

Alec Peters, Weiping Su, Peter Pham, and Larry S. Sherman

Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR

Oligodendrocyte progenitor cells (OPCs) are present in central nervous system (CNS) demyelinating lesions such as those found in patients with multiple sclerosis (MS), but fail to differentiate into myelinating oligodendrocytes (OLs). This arrest in OPC differentiation has been attributed in part to the presence of increased extracellular levels of the glycosaminoglycan hyaluronic acid (HA). Enzymatic digestion of HA by hyaluronidases produces fragments of HA that inhibit OPC maturation and remyelination *in vivo*. There is a debate as to which hyaluronidase generates these inhibitory products. One promising candidate, CEMIP (CEll Migration Inducing and hylauronan binding Protein), has been implicated in HA digestion and was reported to be expressed in MS lesions. We find that a selective hyaluronidase inhibitor that has been shown to increase functional remyelination and OPC maturation in rodent white matter injury models blocks CEMIP-induced HA degradation. However, we do not observe increased CEMIP expression in demyelinating lesions from mice with experimental autoimmune encephalomyelitis (EAE), a model of MS. Instead, we find that CEMIP expression in the hippocampus is increased in mice with EAE, implicating it in cognitive defects associated with MS. Indeed, we found that HA digestion in the dentate gyrus of the hippocampus leads to aberrant neural stem cell proliferation and delayed neuronal maturation, and that disruption of HA signaling leads to hippocampal learning and memory deficits. Ongoing studies will determine if HA digestion products produced by CEMIP inhibit either OPC maturation and remyelination or neurogenesis in the hippocampus.

The trajectory of maternal stress across pregnancy is associated with newborn amygdala functional connectivity and infant negative affect development over the first two years of life.

Mollie Marr, BFA; Alice M Graham, PhD; Eric Feczko, PhD; Elina Thomas, BA, MS; Darrick Sturgeon, BS; Emma Schifsky, BA; Jerod Rasmussen, PhD; John H. Gilmore, MD; Martin Styner, PhD; Steven G. Potkin, MD; Sonja Entringer, PhD; Pathik D Wadhwa, MD, PhD; Claudia Buss, PhD; Damien Fair, PhD

Objectives: Maternal prenatal stress (MPS) has been linked to increased risk for development of psychiatric disorders in offspring. Most studies primarily consider the overall level or magnitude of MPS as a risk factor; less is known about how the timing and pace of MPS change over pregnancy (the trajectory) affect brain systems and behavioral phenotypes in infancy. This study examined the relationship of MPS magnitude and trajectories to newborn amygdala functional connectivity and negative affect development over the first two years of life.

Methods: Maternal stress was assessed at each trimester and 1 month of infant age using the Perceived Stress Scale, State Trait Anxiety Inventory, and Center for Epidemiological Studies Depression for 115 maternal-infant dyads. MPS trajectories were created for the pre- and perinatal period using the Functional Random Forest, which combines Functional Data Analysis, the Random Forest, and graph theory. Newborn amygdala to anterior insula (Am-aI) and amygdala to ventromedial prefrontal cortex (Am-vmPFC) resting state functional connectivity rsFC MRI (scan age= 25.4 ± 11.3 days) were examined relative to MPS trajectories. These connections, Am-aI (emotional-salience) and Am-vmPFC (emotional-cognition) are relevant to negative affect development and psychiatric disorders across the lifespan. Infant negative affect was assessed at 3, 6, 9, 12, and 24 months using the Infant Behavior Questionnaire-Revised and compared to MPS trajectories.

Results: Four distinct MPS trajectories were identified by our Functional Random Forest algorithm. Our approach also identified a split in MPS by magnitude. The MPS trajectory characterized by peak stress during the 3rd trimester predicted increased rsFC for Am-aI (B=0.471, p=0.02) and Am-vmPFC (B=0.507, p=0.02). The MPS trajectory characterized by increasing stress through pregnancy predicted increased rsFC Am-vmPFC (B=0.441, p=0.03) as well as a smaller increase in infant negative affect from 3 to 24 months (M=-0.927, p=0.04). MPS magnitude predicted higher infant negative affect at 3 months (M=0.279, p=0.03).

Conclusions: Heterogeneity in MPS can be characterized by both magnitude and trajectory over the pre- and perinatal period. This characterization is associated with both newborn amygdala rsFC and infant negative affect development over 24 months of age. These data highlight that the trajectory, in addition to the magnitude, of MPS may contribute to offspring brain and affective development.

Funding

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Diffuse microglial dystrophy in aging human white matter hyperintensities

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Recent experimental studies support that dystrophic microglia contribute to tau-dependent neuronal degeneration and impaired cognitive function. Although microvascular white matter injury (WMI) is commonly coincident with Alzheimer's disease (AD) and contributes independently to cognitive decline, the role of microglial dystrophy in either disease process with respect to these lesions is unclear. We hypothesized that astroglia and microglia would display similar hyper-reactivity in human WMI defined by myelination failure and disrupted extracellular matrix integrity. Subjects enrolled in prospective longitudinal population-based studies received serial cognitive testing to define the progression of cognitive decline or dementia prior to brain autopsy at death. The burden of AD neuropathologic change (ADNC) was similar between white matter hyperintensity (WMH) cases and cases without WMI (nWMI). The majority of subjects were in earlier stages of cognitive decline when evaluated by Mini Mental State Examination and the Cognitive Abilities Screening Instrument. We quantified microglial and astroglial responses by stereology in lesions identified in MRI-defined prefrontal periventricular WMHs and adjacent regions of normal-appearing prefrontal white matter (NAWM). Astrocytes displayed diffuse hypertrophic reactivity and were significantly elevated in WMHs and NAWM ($p<0.001$), whereas activated microglia were less significantly elevated in WMHs ($p<0.05$), but not in adjacent NAWM. However, dystrophic microglia were significantly elevated in both WMHs and NAWM and were nearly 3-5-fold higher relative to activated microglia (****$p<0.0001$). Microglial dystrophy was observed across a broad spectrum of myelination disturbances broadly defined by Luxol-Fast Blue staining intensity. Since different sizes of hyaluronan (HA) can influence microglial activation, we analyzed the HA fragment sizes generated in subjects with microvascular WMI and found significant elevations in both medium and low molecular weight forms of HA. Our findings suggest novel associations between myelination disturbances, disrupted WM integrity and microglial dystrophy that occur diffusely throughout WMHs and in distant regions of NAWM. Our results support that MRI-defined WMHs underestimate the burden of pathologically-defined WMI. Injury in WMHs involves a shift from activated to dystrophic microglia that occurs during early stages of age-related cognitive decline.

Funding sources: NIH

Make you look: An electrophysiological study of attention capture in the aging brain

Mei-Ching Lien
Oregon State University

Some studies have suggested that visual attention (mind's eye) can be captured involuntarily by certain stimuli, such as unique singleton objects (e.g., a red dot among several white dots on the screen) or a flash of an object (e.g., abrupt onsets). However, it is not clear whether these objects have the power to capture our attention regardless of our current task goals and how the capture of attention changes with age. One interesting hypothesis is that, relative to younger adults, older adults will be captured more by stimulus salience and less by their goals because aging is often associated with cognitive control decrements. The present study examined this hypothesis using electrophysiological measures. Experiment 1 assessed the capture by irrelevant abrupt onsets. We found that the abrupt onset did not capture attention away from the current task. Critically, older adults demonstrated the same resistance to capture by the abrupt onset. Experiment 2 extended these findings to irrelevant color singleton. We argue that the ability to attend to relevant stimuli/tasks and resist capture by salient-but-irrelevant stimuli is preserved with advancing age.

Assessing auditory processing abilities in human listeners: Lessons from auditory neuroscience

Frederick Gallun, PhD
Research Investigator, VA RR&D National Center for Rehabilitative Auditory Research
Associate Professor, Oregon Health and Science University

Most diagnoses of hearing function focus on the evidence provided by the audiogram, which primarily evaluates peripheral auditory function. It is increasingly understood that normal audiograms can occur in the presence of auditory processing difficulties due to dysfunction at the level of the auditory nerve, brainstem, auditory cortex, and beyond. Auditory difficulties of older listeners and patients who have suffered traumatic brain injury are clearly at risk of being overlooked by a diagnostic approach that focuses only on the audiogram. Modern auditory neuroscience has provided great insight into the information processing that underlies auditory perception and have allowed behavioral researchers to identify specific stimuli and tasks for the evaluation of central auditory function. So far, however, this information has largely remained in the laboratory. This presentation will describe our work developing and evaluating 1) a set of tests that draw upon modern neuroscientific data and theories of auditory system function and 2) a freely-available mobile application that instantiates a portable, automated, rapid testing (PART) platform that can be used by minimally-trained personnel to collect high-quality measures on a wide range of auditory processing abilities with only a quiet room, an iPad or iPhone, and a pair of consumer-grade headphones.

Initial verification of the PART platform will be discussed as well as the early successes in introducing it into educational, research and clinical settings at minimal cost. The near-term goal is to leverage the low financial barrier, ease of use, and enthusiasm of our clinical research partners to collect large normative data sets that take into account peripheral dysfunction and aging. The long-term goal is to be allow any clinician who suspects auditory dysfunction to quickly perform an evaluation using PART that can then be added to the patient record and thus both form the basis of further referral and provide evidence for the need for (and potential success of) rehabilitation. [Support provided by NIH/NIDCD R01 015051 and the VA RR&D National Center for Rehabilitative Auditory Research]

Poster Session II

Longitudinal brain volumetrics in aged rhesus monkeys: a progress report

Steven Dash[1], Zheng Liu[1], Chris Kroenke[1,2], Henryk Urbanski[1,2], Mary Lou Voytko[3], Steven Kohama[1].

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Studies of the effect of hormone therapy on cognitive function in post-menopausal women have been equivocal, however some studies initiated hormone treatment (HT) long after the onset of menopause. There has been some question of whether or not there is a "window of opportunity" for HT effectiveness, especially with initiation closer to the onset of menopause. We tested a group of aged female rhesus macaques on a spatial working memory and a visuospatial attention task, after ovariectomy (ovx) and HT. On both tests, E-replaced animals performed better than the intact and ovx controls and the E-group supplemented with progesterone, supporting the window hypothesis. Moreover, while the animals continued on treatment (but no further cognitive testing), longitudinal anatomical MRI scans of the brain were collected on a 3T Siemens Magnatom system. T1-weighted MPRAGEs were collected in quadruplicate at each scan time-point to increase the signal to noise ratio, on treatment years 3, 4 and 5. Using a volumetric analysis pipeline, scans were averaged for each animal at each time-point, then masked and skull stripped. We found no change in total brain volume, as a factor of treatment, age or the interaction. Ventricular volume, while suggestive of an age-related increase, just fell short of significance. The volumes of gray and white matter analysis longitudinally across age is currently being performed. We also plan a sub-regional approach to examine the effects of age and treatment. However, in the absence of overt pathology, age-related volumetric changes in the aged macaque brain appears, so far, to be relatively minor.

Support: This work was supported by National Institutes of Health Grants AG-019100, AG-024978, AG-026472, AG-036670, and OD-011092.

Effects of perineuronal net removal prior to and after memory reactivation.

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Memories, key contributors to persistent behaviors such as drug addiction, are dynamic and changeable. When memories are recalled (reactivated), they become labile and vulnerable to disruption. In addition, when memories are reactivated in the presence of novel information, they are more labile than memories reactivated in the presence of already learned information. Perineuronal nets (PNNs) are composed of dense extracellular matrix that when removed impair the acquisition and reconsolidation, which is the recall and updating of drug related memories. Hence, here we test the effects of memory attenuation in a cocaine self-administration model in Sprague Dawley rats by injecting chondroitenase-ABC (Ch-ABC), which degrades the PNNs, prior to or after memory reactivation in the presence of novel information. The results indicate that injection of Ch-ABC prior to memory reactivation attenuates memory, but injection after memory reactivation does not. This result raises many questions about how Ch-ABC disrupts memory. Future experiments will provide more information about Ch-ABC as a potential therapeutic agent.

Supported by NIH Grant DA040965 and the WSU Alcohol and Drug Abuse Research Program

Adenosine kinase regulates adult neurogenesis

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Traumatic brain injury (TBI) is one of the leading causes of disability. Human rehabilitation studies suggest that the injured brain maintains a limited capacity for self-repair in line with studies showing that the adult brain is capable of generating new neurons, especially after brain injury (1, 2). Understanding endogenous repair mechanisms holds promise for the development of novel regeneration-promoting therapies. The nuclear isoform of adenosine kinase (ADK-L) has recently been identified as regulator of the DNA methylome. Here, we explored its functions related to development and regeneration of the brain. We first studied changes in ADK isoform expression during fetal and postnatal murine and human brain development and found a strong association of ADK-L with immature neurons. To study the role of ADK-L in the adult brain, we generated mice that either overexpress (ADK-L^{tg}) or lack ADK-L ($ADK^{\Delta neuron}$) in mature neurons. Under baseline conditions, ADK-L^{tg} mice showed enhanced neurogenesis in the dentate gyrus as evidenced by increases in DCX and Ki67 positive cells. However, three days after a controlled cortical impact to mimic traumatic brain injury, we found a significant increase in neurogenesis in the dentate gyrus of $ADK^{\Delta neuron}$ mice, whereas neurogenesis was attenuated in ADK-L^{tg} mice. These findings suggest a function of ADK-L as context-specific regulator of cell proliferation.

References:

1.Altman J & Das GD (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of comparative neurology* 124(3):319-335.

2.Neuberger EJ, Swietek B, Corrubia L, Prasanna A, & Santhakumar V (2017) Enhanced Dentate Neurogenesis after Brain Injury Undermines Long-Term Neurogenic Potential and Promotes Seizure Susceptibility. *Stem Cell Reports* 9(3):972-984.

Enzymatic efficacy of aged chondroitinase-ABC in degrading perineuronal nets

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Perineuronal nets (PNNs) are dense extracellular matrix structures surrounding fast-spiking, parvalbumin-containing GABAergic interneurons throughout the brain. Within the medial prefrontal cortex (mPFC), PNNs are important for the stabilization of memories following learning. Much of our research focuses on fluctuations in the staining intensity of these PNNs, which may reflect their thickness, and in turn, their ability to help form and maintain memories. One method to manually degrade PNNs is by giving an intracerebral injection of chondroitinase-ABC (Ch-ABC), an enzyme that digests chondroitin sulfate proteoglycans, a key structure in PNNs. However, it is not known how long this enzyme remains active once it is dissolved in buffer and stored frozen. Therefore, we determined the period over which dissolved Ch-ABC that was maintained at -20 °C would be enzymatically viable. To test the lifespan of enzymatic activity, we identified aliquots of Ch-ABC that were stored at -20 °C for 0, 0.5, 1, 4, 5, 8, 13, 18, or 20 months. A 0.4 µL volume/hemisphere of Ch-ABC or vehicle was microinjected into the mPFC of adult male rats (n=9). Rats were perfused three days following injections, brains were then frozen, sliced into 40 µm coronal sections, and stained with *Wisteria floribunda* (WFA) to identify PNNs. The intensity and number of PNNs were analyzed for each time period using the PIPSQUEAK program and grouped into three time-bins (0-4 mo., 5-8 mo., 13-20 mo.). While every age of Ch-ABC significantly reduced WFA intensity, Ch-ABC from the 0-4 mo. and 4-8 mo. time points were more effective at reducing the intensity of PNNs compared with Ch-ABC from the 13-18 mo. time point. Additionally, Ch-ABC from the 5-8 mo. time point significantly reduced the intensity of PNNs compared to all other time points. These data indicate that Ch-ABC was the most effective between five to eight months post reconstitution; however, significant enzymatic activity may still occur even after being maintained at -20 C for up to 20 months.

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Differences in saccade task gaze metrics following mild traumatic brain injury

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Mild traumatic brain injury (mTBI), or concussion, is the most common head injury sustained by athletes (one estimate suggests 3.8 million sport-related concussions annually) in the United States. Despite these epidemic proportions, the diagnosis and treatment of mTBI continues to be an issue due to a lack of objective, quantitative measures or biomarkers. Current research indicates that the occurrence of one or more concussions can have lasting impacts – increased risk of psychiatric health issues including anxiety, depression and dementia later in life, and socioeconomic concerns (e.g., loss of employment, lower educational achievement). It is therefore imperative to develop reliable measures, independent of subject self-report, to diagnose concussions and point of recovery. Because concussion is a brain injury impairing neuron function (e.g., excitation, neurotransmitter release, axon conduction) and metabolism, and because neural circuits controlling and coordinating eye and head movements are distributed throughout several brain regions, concussions have been shown to affect certain eye movements when the head is restrained. To test the feasibility of examining head-unrestrained gaze shifts as a diagnostic, we used videooculography (VOG) and head accelerometers to noninvasively examine measurements of visually guided, combined eye-head gaze shifts in college varsity athletes (primarily Women's Soccer) before and after sport-related concussion. Our previous (unpublished) results suggest that gaze peak velocity typically changes following concussion. Because our subjects are free to move their heads, we also tested the hypothesis that head movement metrics differ following concussion. Preliminary results show differences in the head movement component of gaze shifts (head movement peak velocity, head movement contribution to gaze, and post-VOR eye position); however, subjects who completed the fall athletic season without concussion show similar, albeit smaller, differences. This was unexpected, and suggests that a season of practices and competition without concussion may also have cumulative effects on head movement metrics. Further analysis including non-athlete controls may clarify whether head movement metrics may serve as reliable biomarkers of sport-related concussion.

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In vivo reprogramming of astrocytes into new neurons after TBI

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Traumatic brain injury (TBI) is one of the leading causes of disability (1). The hallmarks of TBI include irreversible loss of neurons, formation of a glial scar, and neurological dysfunction. After TBI, astrocytes become reactive and migrate towards the injury site forming glial scars. Although it is beneficial to isolate damages, glial scars are detrimental to axonal regrowth and brain regeneration around the injury site. A therapeutic strategy targeting these reactive astrocytes and shifting their fates into new neurons could, reduce the detrimental effect of glial scars after TBI. Here, we employed gene therapy approach to enforce ectopic expression of Neurogenin2 (NGN2) gene and an antioxidant diet to reduce oxidative stress and optimize cell reprogramming after TBI. Three groups (all n=30) of C57BL/6 mice were given antioxidant diet two weeks before TBI (to maximize the level of antioxidants at the time of virus injection). All groups were exposed to moderate TBI, using control cortical impactor (CCI), and then received intracranial virus injection of either a control green fluorescent protein (GFP) virus or a therapeutic virus (NGN2) three days after TBI. Animals were sacrificed at different time points – 3, 7, 14, and 30 days post injury (DPI) and new cells were tracked after virus injection by 5-bromo-2'-deoxyuridine (BrdU) incorporation. Animal motor functions were also assessed at different time points (baseline, 1DPI, 6DPI, 13DPI, 29DPI). Our results showed a significant increase in the number of new neurons in NGN2 virus groups compared to the control GFP virus groups at 14 and 30 DPI. We also found significant recovery in motor functions of NGN2 14 DPI and 30 DPI groups when compared with their corresponding NGN2 7 DPI and baseline groups. More interestingly, animals treated with antioxidant diet, specifically the 3 DPI group with maximal level of antioxidants, showed significant reduction in glial scarring and activated astrocytes when compared to those without antioxidant diet. These results hold promise for the treatment of brain injuries whose effects are often exacerbated by glial activation and neuronal loss.

References:

1. Taylor CA, Bell JM, Breiding MJ, & Xu L (2017) Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013. *MMWR Surveill Summ* 66(9):1-16.

Probing the Diversity of Raphe Serotonergic Nuclei: The Impact of Perinatal Western-Style Diet

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Mounting evidence from preclinical and clinical models reveals that maternal diet and metabolic state have long-lasting influences on offspring behavior and neurodevelopment. Using a nonhuman primate model, we have previously shown that maternal and post-weaning Western-style diet (WSD) exposure impair behavioral regulation and alter components of the serotonergic system in juvenile offspring. The current experiment utilized fluorescent immunohistochemistry to quantify the presence of the serotonin synthesis protein tryptophan hydroxylase 2 (TPH2) and vesicular glutamate transporter 3 (VGLUT3) in the brainstem raphe nuclei of juvenile Japanese macaques (*Macaca fuscata*). The raphe is the predominant site of central serotonin production and sub-populations of TPH2 positive neurons in the raphe co-express VGLUT3 to varying degrees. To our knowledge this is the first study to examine VGLUT3 distribution in nonhuman primate raphe nuclei.

Raphe tissue was collected from 24 animals at 13.10 ± 0.86 months and using a standard fluorescent immunohistochemistry protocol were subsequently labeled with anti-TPH2 and anti-VGLUT3 antibodies. Animals belonged to one of three unique diet groups, each consisting of 4 males and 4 females. Diet groups were designed to examine the effects of WSD exposure from gestation until necropsy (WSD/WSD) versus early intervention at 7 months of age (WSD/CTR), in comparison to non-diet manipulated controls (CTR/CTR).

Preliminary analysis revealed substantial variation in staining patterns, particularly in the TPH2 channel. This variation was largely due to a number of factors unique to our model of juvenile macaque neurodevelopment. To prevent bias in the data set we are developing a method to empirically identify outlier images for each subject based on their individual expression patterns representing unique stages of development. We believe that possible corrections for extreme outlier images will further elucidate our preliminary results suggesting that macaque dorsal raphe subnuclei possess significantly different TPH2+ and VGLUT3+ staining patterns. These results correspond with literature classifications of raphe subnuclei phenotypes in rodents. In addition, WSD/WSD male offspring expressed significantly altered patterns of VGLUT3+ signal compared to both CTR/CTR males and WSD/WSD females. Given that the neuronal subtypes defined by the relative of abundance of TPH2 and VGLUT3 have unique developmental sublineages, activity signatures, and afferent and efferent connections, these preliminary results give important insight to how altered perinatal diet can impact behavioral regulation.

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