Dec. 9, 2015
Hilton Fort Collins
Registration open at 9:30AM

10:30 - 11:15
Obesity Mini-Session
Dr. Kimberley Bruce, Dr. Sreejayan Nair & Dr. Marc-Andre Cornier

11:15 - 12:00
Traumatic Brain Injury Mini-Session
Dr. Julie Dunne, Dr. Paco Herson, & Dr. Kim Gorgens

12:00 - 3:00
Poster Session, Vendors, and Lunch
12:30 - 1:45 Odd Posters; 1:45 - 3:00 Even Posters

3:00 - 4:00
Award-Winning Student Presentations
M Allie Holschbach (Colorado State University)
Brian Heister (Universityof Colorado Denver)
Elizabeth Woodruff (University of Colorado)

4:00 - 4:30
Coffee Break

4:30 - 5:30
Keynote Lecture
“Glia - a new frontier for understanding the brain”

5:30 - 6:30
Awards, Prizes, and Reception

KEYNOTE SPEAKER
Philip G. Haydon, PhD
Professor and Chair,
Department of Neuroscience
Tufts University
http://sackler.tufts.edu/Faculty-and-Research/Faculty-Research-Pages/Philip-Haydon

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Cover Page: Designed by Ashley Leek
Scientific images provided by Allie Holschbach (Colorado State University), Liz Woodruff (Univ Colorado Boulder), and Brian Hiester (CU Denver-Anschutz). Details will be in their oral presentations. The FRNG website (http://FRNG.colostate.edu) was created by Leif Saul in 2005 – see more images on our website.

Special Thanks!

Special thanks to all of you that submitted abstracts for oral and poster presentations! We particularly thank the judges for the poster contest!! – and to Shane Hentges for managed the herculean task of organizing the judging operation for the meeting – no easy task!!!

Special thanks to the vendors listed in this program. These companies have declared by their contributions both in dollars and prizes that they value Front Range Neuroscience Group business. We encourage you to buy from these vendors that support you.

Special thanks to our Platinum Level Industry Supporters: DSM Nutritional Products, Olympus America, and Sigma Aldrich. In addition, special thanks to the Hilton Fort Collins for stepping up to a platinum level of support in providing the ideal venue and extra contributions, and to Laura Joy in particular for help in making this all possible.

Special thanks to the University departments and programs that provided financial support to help make the meeting possible; in particular Colorado State University, the University of Wyoming, the University of Colorado at Boulder, and finally the parent Society for Neuroscience.

Special thanks to the graduate student organizing committee for creating and polishing the program and fixing the details, and in particular for creating the program book. This includes Ashley Leek, Emily Maverick, and Nathan Byers from CSU, Dori R. Pitynski and Paige Dingess from Univ Wyoming, John Soltys from UC Denver Anschutz Health Science Campus, Annie Miller and Lauren Chun from UC-Boulder, Stephanie Stout from DU, and Jonna Jackson from Univ Northern Colorado. And additionally to Graduate Student Advisors Erin Bisenius (Biomedical Sciences, CSU) and Sara Neys Biomedical Engineering, CSU) and the first year MCIN students for helping with attendee registration.

Special thanks to you, the attendees, for making this a meeting that we can be proud to hold on a regular basis, and for forming Front Range Neuroscientists into a vibrant and interactive Community!

Stay tuned for information on our FRNG Website that helps us communicate position openings, course offerings, seminars and a whole lot more!!!

Sincerely yours,

The Front Range Neuroscience Steering Committee,
Shane Hentges, Kim Hoke, Qian-Quan Sun, Serge Campeau, Kimberly Gorgens, Mark Basham, Sondra Bland, Mark Thomas and Stuart Tobet.
Sreejayan Nair, PhD  Professor of Pharmacology, University of Wyoming.

Dr. Sreejayan Nair is a Professor of Pharmacology at the University of Wyoming where he is currently the Director of both the Biomedical Sciences PhD Program and the Center for Cardiovascular Research and Alternative Medicine (C-CRAM). Dr. Nair’s research focuses on the emerging problem of Type-2 diabetes. His lab is interested in understanding the molecular mechanisms that lead to insulin resistance. His lab has characterized novel low molecular-weight chromium complexes that can alleviate insulin resistance by augmenting insulin signaling. In addition to insulin resistance, he is also interested in cardiac complications associated with insulin resistance. His recent studies have unraveled roles cathepsin K and endothelin-receptor-A in obesity associated cardiometabolic dysfunctions.

Kimberley Bruce, PhD  Assistant Professor, University of Colorado Denver.

Dr. Kimberley Bruce is an Assistant Professor at the University of Colorado Denver. She was a research fellow at the University of Southampton and a Research Associate at the Scripps Research Institute. Dr. Bruce’s research focuses on metabolic syndrome in animal models. Some of her recent work includes in utero risk factors for Type-2 diabetes, the alteration of ovarian morphology and gene expression due to diet-induced obesity, and epigenetic priming of the metabolic synodrome.

Marc-Andre Cornier, MD  Professor of Medicine, University of Colorado Denver.

Dr. Marc-Andre Cornier is a member of the Anschutz Health and Wellness Center. Dr. Cornier has also been on staff at Denver Health Medical Center in Denver, CO since 1999 as a clinical endocrinologist and educator. He has been an active volunteer in important health-related associations, such as the American Diabetes Association, American Heart Association, the Endocrine Society and the Obesity Society. Dr. Cornier is an active clinical and translational investigator with a primary interest in understanding the complex regulation of food intake and body weight.
Kimberly A. Gorgens, PhD  Clinical Associate Professor, Graduate School of Professional Psychology, University of Denver.

Dr. Gorgens received her BA at Roger Williams University in Bristol, RI. She then received her MA and PhD from Southern Illinois University in Carbondale, IL. Dr. Gorgens completed her postdoctoral fellowship in clinical neuropsychology and is board certified in Rehabilitation Psychology. Currently, Dr. Gorgens serves as a full-time clinical associate professor in the graduate school of professional psychology at the University of Denver and is also the founding Executive Director of the Center for Professional Development, also at the University of Denver.

Dr. Gorgens engages in leadership and advocacy around disabilities and traumatic brain injury awareness, and has previously served as the Chair of Colorado Traumatic Brain Injury Trust Fund. Additionally, she was involved in drafting and supporting the 2011 concussion law for the State of Colorado. Kimberly has focused much attention to research surrounding TBI including violence within patients with TBI as well as TBI rehabilitation.

Paco S. Herson, PhD  Professor and Associate Chair, Neuroscience, Dept. of Anesthesiology, University of Colorado Denver.

Dr. Paco Herson is a Professor and Associate Chair for Neuroscience at University of Colorado Anschutz Medical Campus. Dr. Herson is also the director of the Neuronal Injury Program. His research focuses on the brain response to and recovery mechanisms for a variety of neurotoxic insults at a variety of molecular tiers ranging from immunologic to metabolic.

Julie A. Dunn, MD  Medical Director of Trauma Research & Education, University of Colorado Health, Medical Center of the Rockies.

Dr. Dunn completed medical school at East Tennessee State University/James H Quillen College of Medicine. Currently, she is the medical director of Trauma Research and Education at the Medical Center of the Rockies in Loveland Colorado.

Dr. Dunn and her research team study a variety of trauma. Recently, research was performed analyzing bike accidents from the MCR and Poudre Valley Hospital trauma registry and compared this data with the national trauma data bank. This study was performed in hopes to uncover information that would lead to greater bicycle safety within the northern Colorado area.

Dr. Dunn’s research center brings together physicians, basic science researchers, and entrepreneurs in hopes of finding cures, ideas and solutions to various aspects of trauma.
1) Allie Holschbach, Postdoctoral Fellow at Colorado State University

**Peripartum Plasticity in the Serotonergic Dorsal Raphe: Implications for Postpartum Socioemotional Behavior and Physiology**

*Allie Holschbach, JS Lonstein. From the Neuroscience Program, Michigan State University.*

Postpartum rats are highly maternal and show high aggression and low anxiety compared to nulliparous rats. To promote these dramatic behavioral changes in behavior, new mothers experience intense endocrine changes that elicit widespread neural plasticity. This neural plasticity includes cell birth and death in several regions of the peripartum forebrain, but such plasticity has never been reported in the dorsal raphe (DR), a midbrain site that provides most of the forebrain’s serotonin. Because DR serotonin is critical for postpartum physiology and is involved in caregiving, aggression, and anxiety, I hypothesized that motherhood alters DR plasticity and serotonin synthesis/metabolism to support postpartum changes in socioemotional behaviors. To test this hypothesis, I examined effects of reproductive state and maternal experience on DR cell proliferation, newborn cell survival, cell death, and many aspects of the serotonin synthesis/metabolism pathway, then tested postpartum social and emotional behavior after lesioning the serotonergic DR. I discovered that although an equal number of cells are born in the DR of virgin, pregnant, and postpartum rats, fewer cells survived into the late postpartum period compared to cells surviving into the early postpartum period and this low cell survival required parental contact with offspring. These late postpartum females also had the highest levels of cell death within the DR and lowest levels of serotonin’s precursor (5-HTP) and metabolite (5-HIAA) than early postpartum rats. To begin to test the functional significance of these changes in neuroplasticity and neurochemical function, I performed serotonin-specific DR lesions using a saporin-conjugated toxin targeting the serotonin transporter. Lesioning the DR altered numerous postpartum behaviors, including nursing and licking of pups and aggression toward a male intruder. These data demonstrate that although an equal number of cells are born in the DR of virgin, pregnant, and postpartum rats, fewer cells survived into the late postpartum period compared to cells surviving into the early postpartum period and this low cell survival required parental contact with offspring. These late postpartum females also had the highest levels of cell death within the DR and lowest levels of serotonin’s precursor (5-HTP) and metabolite (5-HIAA) than early postpartum rats. To begin to test the functional significance of these changes in neuroplasticity and neurochemical function, I performed serotonin-specific DR lesions using a saporin-conjugated toxin targeting the serotonin transporter. Lesioning the DR altered numerous postpartum behaviors, including nursing and licking of pups and aggression toward a male intruder. These data demonstrate that the DR is a site of significant peripartum plasticity that occurs concomitantly with parallel changes in serotonin synthesis and metabolism. These neurochemical changes may guide postpartum behavioral adaptations because lesioning the DR of new mothers had numerous effects on postpartum social behaviors. Taken together, these data suggest that the DR is an integral part of the maternal neural network that guides the initiation, modulation, and regression of postpartum behaviors.

**Keywords:** serotonin, plasticity, cytogenesis, cell death, social behavior, emotional behavior

2) Brian Heister, Postdoctoral Fellow at University of Colorado Denver

**L-type voltage-gated calcium channels regulate the fusion of recycling endosomes in neuronal dendrites and spines**

*Brian G. Hiester, Brooke L. Sinnen, Ashely M. Bourke, Emily S. Gibson and Matthew J. Kennedy. From the Department of Pharmacology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO.*

The number and identity of ion channels and receptors displayed on the dendritic surface determines fundamental cellular properties of neurons and is tuned by neuronal activity. Activity-coupled surface expression of important channels and receptors is mediated by fusion of recycling endosomes (REs) with the dendritic plasma membrane (PM). Despite the established importance of RE trafficking for neuronal function, how synaptic activity is coupled to RE fusion with the PM remains largely unexplored. Here we demonstrate that RE fusion in dendrites and spines depends on NMDA receptor activation and a rise in intracellular Ca2+. Surprisingly, NMDA receptors were not the sole source of postsynaptic Ca2+ driving RE fusion. We found that L-type voltage-gated Ca2+ channels (L-VGCCs) play a critical role in RE trafficking by regulating the mode of vesicle fusion. When L-VGCCs were blocked, REs partially fused with the plasma membrane but failed to deliver their membrane-bound protein cargo to the dendritic surface. Conversely, potentiating L-VGCC activity enhanced RE fusion in response to synaptic activation. Together, these experiments reveal an unexpected cooperation between NMDA receptors and L-VGCCs in governing the mode of activity-triggered RE fusion in dendrites and spines.

**Keywords:** Confocal microscopy, Synaptic plasticity, Live-cell imaging, Primary neuron culture
3) Elizabeth Woodruff, Graduate Student at University of Colorado

**Knockdown of prefrontal cortex Period1/Period2 gene expression impairs diurnal-dependent conditioned fear extinction learning**

Elizabeth R. Woodruff1, Lauren E. Chun1, Nicolas M. Varra1, Laura R. Hinds1, Benjamin N. Greenwood2, Colleen McClung3, Robert L. Spencer1. From the 1Department of Psychology and Neuroscience, University of Colorado Boulder, 2Department of Psychology, University of Colorado Denver, and 3Department of Psychiatry, University of Pittsburgh Medical Center

Circadian rhythms are highly conserved 24h fluctuations in physiology and behavior. Optimal organismoal health relies on the integrity of this system. Anxiety disorders such as post-traumatic stress disorder (PTSD) are often associated with impaired circadian functioning as well as poor conditioned fear extinction learning. We have previously shown that conditioned fear extinction but not conditioned fear acquisition is modulated by both time of day and the presence of endogenous glucocorticoids (CORT) in adult male rats. Here we examined the relationship between auditory conditioned fear extinction learning, circadian phase, and prefrontal cortex (PFC) core clock gene (Per1/2) expression. Rats maintained on a 12h light:dark cycle were trained and tested across 4 sessions (conditioned fear acquisition, extinction, extinction recall, and fear renewal) that were administered either during the rats’ active (zeitgeber time 16—ZT16) or inactive (ZT4) circadian phase. One week prior to testing rats received a microinjection in the infralimbic region of the PFC of either adenoassociated virus (AAV) containing a PER1/2-specific shRNA (PER1/2 KD) construct or a scrambled sequence shRNA (SCR). Neither PER1/2 knock down nor time of day had any effect on conditioned fear acquisition. However, rats trained and tested at ZT16 extinguished conditioned fear faster than those trained and tested at ZT4, regardless of PER1/2 status. Interestingly, SCR ZT16 rats showed superior extinction recall than SCR ZT4 rats, but this superior extinction recall was absent in PER1/2 KD ZT16 rats. In addition, PER1/2 KD rats trained and tested at ZT4 exhibited high freezing levels throughout most of the extinction recall session, suggesting impaired additional extinction learning compared to the other treatment groups. During session 4 (fear renewal) PER1/2 KD ZT4 rats showed much higher freezing and thus greater fear renewal compared to all other groups of rats suggesting an inability of these rats to generalize extinction to a novel context. In general, optimal conditioned fear extinction depends on the time of training/testing as well as normal clock gene expression within the infralimbic PFC. These results emphasize the importance of incorporating a consideration of circadian effects in fear conditioning research and psychotherapy for anxiety related disorders like PTSD.

**Keywords:** Circadian, PFC, Period1, Period2, conditioned fear extinction, AAV viral vector
1) Musical Neglect Training for Unilateral Visual Neglect in Right Hemispheric Stroke Patients
Kyurim Kang MM and Michael H. Thaut.

2) Differential impact of 2-AG on social behaviors in male and female rats: implications of parvalbumin interneurons in the PFC
Jonscher, R., Fontenot, J., Boxer, E., Audrey, A., Loetz, E., Matthew, I., Bland, S.

3) Phases of Systematic Brain Processing Successfully Predict Task-Specific Behaviors
Brittany K. Taylor, PL Davies, and WJ Gavin.

4) Inhibiting ventral pallidum disrupts adaptive salt-seeking behavior in rats
Katherine J. Stansfield, SE Chang, KS Smith.

5) In vivo optogenetic manipulation of dopamine neurons in a novel behavioral economics based food-seeking task
Scott Schelp, Gregory Krystyniak, Katherine Pultorak, Dylan Rakowski, Jeremy Gage, Akshay Kumar, Douglas P. Shepherd, Raibatak Das, Erik B. Oleson.

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Abigail Luman, Andrew Bubak, Jaime Grace, Ken Renner, John Swallow.

7) Effects of chronic caffeine exposure on rat brain serotonergic systems
Arnold MR, Williams PH, McArthur JA, O'Neill, CE, Lowry CA, Bachtell RK.

8) Dopamine Synthesis in the Ventral Tegmental Area in Rams with High or Low Libido
Avery C. Kramer, Kathleen J. Austin, Brenda M. Alexander.

9) Exercise increases mTOR signaling in brain areas involved in cognition and emotion

10) FMRI Investigation of the Accumulation of Categorical Information
Kurt Braunlich and Carol A. Seger.

11) Similar patterns of brain gene expression underlie transgenerational and juvenile developmental plasticity in threespine stickleback
Laura R. Stein, AM Bell.

12) An overview of systematic review evaluating pharmacological treatment outcomes on individuals with traumatic brain injury (TBI) – ACRM Taskforce Project
Patricia Heyn, Joseph Machtinger, Sonya Kim, Zachary Bayer, Amy Herrold, Jennifer Zumsteg, Laurie King, Xiaolei Hu.

13) Methamphetamine intake impairs alcohol consumption in high-alcohol preferring P rats
Madeline C. Winkler, J Stafford, E Greager, S Archuleta, RK Bachtell.

14) Influence of Phonological Neighborhood Density on Word Production in Children: An ERP Study
Doreen Hansmann, William J. Gavin, Stephanie F. Stokes.

15) Age-Dependent Ion Channel Stability in Neurons
Maximiliano Vallejos, Timothy Vernier, Susan Tsunoda. From the Department of Biomedical Sciences, Colorado State University.

16) Effects of MDMA on fear extinction and renewal
Anais Sanchez, Corey P. Simpson, Esteban C. Loetz, Mathew Mondragon, Toni Nicastro, Nathan Gray, Fassi Eyayou, Benjamin N. Greenwood.

17) DREADDing fear: dopamine signaling during fear extinction reduces fear relapse
Courtney A. Bouchet, Esteban C. Loetz, Toni Nicastro, Nathan Gray, Mat Mondragon, Matias G. Saez, Adam Rosberg, Benjamin N. Greenwood.
18) Rats associated with cocaine exposure become cues, thus altering seeking behaviors
Paige Dingess, Morgan J. Deters, Rebecca A. Darling, and Travis E. Brown.

19) Brain response is elevated in adolescent anorexia nervosa to monetary reward receipt and omission when underweight, but only to omission after weight restoration
Marisa DeGuzman, Megan Shott, Guido Frank.

20) Effect of monoacylglycerol lipase (MAGL) inhibition on aggression in female rats after postweaning social isolation
Matt Ishiki, Jazmin Fontenot, Halimah Hamidu, Esteban Loetz, and Sondra Bland.

21) The effect of voluntary exercise during consolidation of auditory fear extinction on renewal and spontaneous recovery of fear
Fassil B. Eyayou, Courtney A. Bouchet, Scott Holmes, Toni M. Nicastro, Esteban C. Loetz, Nathan M. Gray, and Benjamin N. Greenwood.

22) Differences in Frontal, Basal Ganglia, and Limbic Activation during a Word-Emotional Face Stroop Task in Monozygotic Twin Pairs Discordant for Severe Stressful Life Events

23) Test-Retest Reliability of N1 and N2 in Adults During a Flanker Task
Bonnie L. Hogan, HL Hoogs, BK Taylor, MH Lin, WJ Gavin, Patricia L. Davies.

24) Test-Retest Reliability of the P300 in Adults during a Flanker Task
Stephanie The, Alexandra Bicket, Brittany K. Taylor, Mei-Heng Lin, Patricia L. Davies, William J. Gavin.

DEVELOPMENT

25) Reverse Flow is a Key Epigenetic Regulator of Cardiac Valve Development
Neha Ahuja, Molly Zelller, and Deborah Garrity.

26) Prenatal administration of glucocorticoids predicts diurnal cortisol regulation during childhood
MN Edelmann, CA Sandman, LM Glynn, DA Wing and, EP Davis.

27) Characterization of zebrafish models of filamin C related cardiomyopathy
Rasha M. Alnefaie, Deborah M. Garrity. From the Department of Cell and Molecular biology, and Department of Biology, Colorado State University

28) Exercise Produces Neuroplastic Changes Within the Hippocampus During Discrete Periods of Development
Autumn L. Ingalls-Williams, Kevin R. O’Connor, Kristina A. Hulen, Agnieszka Mika, Nicole L. Rumian, Courtney Bouchet & Monika Fleshner.

29) Sensory Gating in Children with High Functioning Autism
Sidney E. Dungan, JE Crasta, PL Davies, WJ Gavin.

30) Developmental trends of performance errors and trial-to-trial variations measured by electroencephalography (EEG)
Mei-Heng Lin, WJ Gavin, and PL Davies.

31) Cellular prion protein promotes axon targeting during adult neurogenesis
Lindsay E. Parrie, Jenna A.E. Crowell, Richard A. Bessen.

32) Exercise restricted to early critical developmental periods can produce long lasting protection against the debilitating behavioral consequences of stressor exposure
Nicole L. Rumian, Aggie Mika, Kristina Hulen, Donald Borchert, and Monika Fleshner.

33) Prenatal Origins of Obesity Risk
Stephanie Stout, Jennifer Hahn-Holbrook, Emma V. Espel, Laura M. Glynn, Curt A. Sandman, Elysia P Davis.
34) Perinatal Maternal Depressive Symptoms and Infant Stress Response  
LD Berger, LD Martinez, DA Swales, MN Edelmann, DA Wing and, EP Davis.

35) Pharmacologic and genetic blockade of NF-κB is neuroprotective in a mouse model of Parkinson’s Disease  
Lindsay Hunt, Sean Hammond, Kelly Kirkley, Evan Richman, Stephen Safe, and Ronald Tjalkens.

36) Generation of Canine Neural Progenitor Cells from Induced Pluripotent Stem Cells  
Lyndah Chow, Kaitlyn McNamara, Jonathan Coy, Dan Regan, William Wheat, Stephanie McGrath, Rebecca Packer, Ray Whalen, Saiphone Webb, Peter Koch, and Steven Dow.

37) Increased mortality of Huntington’s disease mice with Toxoplasma gondii infection: a possible role of elevated Indoleamine-2,3-dioxygenase  
David Donley, Andrew Olson, Merl Raisbeck, Jason Gigley, and Jonathan H. Fox.

38) Creation of a mutant cofilin conditional knock-in mouse to examine the role of cofilin-actin rods in neurodegenerative disorders  
Alisa E. Shaw, JR Bamburg.

39) Scavenging of reactive aldehydes prevents cognitive dysfunction associated with epileptogenesis  
Jennifer N. Pearson, L. Jackson Roberts II, and Manisha Patel.

40) Nurr1 activation prevents neurotoxic injury in the MPTP model of Parkinson’s Disease  
Sean Hammond, Katriana Popichak, Pranav Damale, Evan Richman, Lindsay Hunt, Stephen Safe, and Ronald Tjalkens.

41) The Return-to-Play (RTP) recovery time for female concussion sufferers at a military academy  
Christopher D’Lauro, Brian R. Johnson, Emily Willson, Jonathan Jackson, C. Dain Allred, Gerald McGinty, and Darren Campbell.

42) Activation of the nuclear receptor NUR77 by a novel diindolymethane analog supresses inflammatory gene expression in primary astrocytes  
Katriana A. Popichak, SL Hammond, SH Safe, RB Tjalkens.

43) Extracellular matrix genes are involved in prion susceptibility  
Matthew Sabel, Julie A. Moreno, Vadim Khaychuk, Jifeng Bian, and Glenn C. Telling.

44) Using a novel ELISA to detect prions in infected cell cultures  
Mahmoud Elkady, Hae-Eun Kang, and Glenn C. Telling.

45) Adaptation of mouse prions in gene targeted mice  
Michael C. Young, Julie A. Moreno, Jifeng Bian, Jeffrey R. Christiansen, Crystal Meyerett-Reid, Sehun Kim and Glenn C. Telling.

46) Characterization of moose CWD in transgenic and gene-targeted mice expressing elk or deer prion proteins  
James E. DiLisio, Jeffrey R. Christiansen, Julie A. Moreno, Sehun Kim, Glenn C. Telling.

47) Monoacylglycerol lipase (MAGL) inhibition differentially alters phosphorylation of mTOR in medial prefrontal cortex neurons and astrocytes in adolescent rats  
EC Loetz1, D Tauber, R Jonscher, E Boxer, Z Narrowe, H Mamo, B Greenwood, & ST Bland.

48) Emerging Roles of Synaptotagmin: Modeling Neurogenic Disease in Drosophila  
Mallory Shields, Matthew Bowers, Maddi Bollig, Rita Horvath, Roger Whittaker, Alysia Mortimer, Noreen Reist.

49) Ca2+-Dependent and –Independent Inhibition of GABA Release onto POMC Neurons by Inhibitory GPCRs  
Reagan L. Pennock & Shane T. Hentges.
50) New approach to assess the ratio of functional channels in the AIS
Laura Solé, EJ Akin, MM Tamkun.

51) Inhibition of neuronal excitability by post-synaptic mu opioid receptors (MORs) overshadows pre-synaptic disinhibition through a GABAergic synapse
Philip D. Fox, RL Pennock, ST Hentges.

52) Effects of dopaminergic D2 receptor activation on layer I and layer V evoked excitatory synaptic responses in mouse medial prefrontal cortex
Jonna M. Leyner-Jackson, Mark P. Thomas.

53) Early life maternal care programs the neurosteroid/GABAergic system in female offspring: A rodent model of premenstrual dysphoric disorder
Amanda P. Borrow, Senem Donuk, Nicole M. Cameron.

54) Gonadotropin Releasing Hormone Stimulates Histone Citrullination to Mediate LHβ Expression in Gonadotrope Cells
Shaihla A. Khan, Coleman H. Young, John Diller, Paul R. Thompson, Kenneth L. Jones, Brian D. Cherrington, Amy M. Navratil.

55) Characterization of the gonadotropin-releasing hormone receptor in Aplysia californica
Scott I. Kavanaugh and Pei-San Tsai.

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ABSTRACTS

COGNITION AND BEHAVIOR

1) Musical Neglect Training for Unilateral Visual Neglect in Right Hemispheric Stroke Patients
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The purpose of this study was to examine the immediate and longer-lasting effect of Musical Neglect Training (MNT) which is part of Neurological Music Therapy (NMT) on unilateral visual neglect. A single-subject design was used, as participants served as their own control. Two individuals with right hemispheric stroke (2.5 years and 10 years post-stroke) participated in this study. Participants underwent two weekly 30-minute individual sessions over a time period of three weeks, for a total of six MNT sessions for each participant. Two standardized assessments (Albert’s and Line Bisection Tests) were used. The assessments were administered immediately before and after each of the 6 MNT sessions to assess the immediate effect of MNT. During the training, participants played a set of horizontally arranged tone bars tuned to ascending triads and scales. At the endpoint of each sequence a cymbal was positioned and played to provide a strong audiovisual target in the left visual field for the participants. The experimenters provided a chordal accompaniment on the keyboard to provide harmonic-rhythmic pacing and to cue continuous playing to the end of the sequence. Follow-up testing was done one week after the 6th session to examine the longer-lasting effects of MNT. Paired t-tests were used to test for statistical improvement between pre- and post-test of interventions (immediate effects). Also, nonparametric statistics (Wilcoxon Signed Rank Test) were calculated in parallel with the paired t-tests due to the small sample size and possible violations of normal distribution. For the longer-lasting effects, raw data were compared between the average of 6 sessions’ pre-test and follow-up test since there was only one follow-up test. For both tests, lower scores mean better performance. Both participants showed statistical improvement with Albert’s Test in the immediate effect (Participant 1: p=.02, Participant 2: p=.01). Results for the immediate effect of MNT on the Line Bisection Test were not significantly different, but means were lower for post-test (Participant 1: M=24.17%, Participant 2: M=9.16%) compared to pre-test (Participant 1: M=25.65%, Participant 2: M=10.39%), indicating positive improvement. Although not statistically significant for the longer-lasting effect, participant 2 had a lower score (score=7) compared to averaged pretest scores of the 6 treatment sessions (M=9.5), indicating a positive outcome, while scores of participant 1 remained the same at follow-up (score=14) compared to the pretest average (M=14.5) in the Albert’s Test. Moreover, participant 1 showed increased deviation percentages from the averaged pre-test (M=25.65%) to follow-up test (deviation=27.18%), indicating no positive effect for the longer-lasting effect in the Line Bisection Test. Participant 2 showed a decreased deviation in follow-up score (deviation=7.70%) compared to averaged pre-test score (M=10.39%) for the Line Bisection Test. The study indicates that MNT is a potential positive intervention for clients with unilateral visual neglect. Future research should employ music-based intervention with clients in subacute recovery stages post-stroke. Furthermore, developing the intervention protocol with increased duration and a higher number of sessions may result in stronger results. Based on the results from this study and previous studies, research focusing on the underlying neural mechanism and tailoring the intervention protocol appropriately to the clinical situation is warranted. Keywords: Neurological Music Therapy (NMT), Musical Neglect Training (MNT), Unilateral Visual Neglect, cognitive rehabilitation technique, right hemisphere stroke

2) Differential impact of 2-AG on social behaviors in male and female rats: implications of parvalbumin interneurons in the PFC
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The endocannabinoid system has been implicated in many important bioregulatory processes, particularly emotional modulation. The present study demonstrates that enhanced 2-arachidonylglycerol (2-AG) signaling through administration of the 2-AG breakdown inhibitor MJN110 elicits dose-dependent and sex-specific alterations in social behavior with regionally distinct differences in neural activation. We show that increased 2-AG signaling elicits play enhancing effects in male but not female rats at a dose of 1mg/kg, while a 5mg/kg dose decreases social interaction and rearing in both sexes. Double-label immunohistochemistry was performed in the infralimbic (IL) and prelimbic (PL) subregions of the prefrontal cortex (PFC) to assess the expression of the protein Fos (marker of neuronal activation) in parvalbumin (PV) expressing GABAergic interneurons. Adolescent rats show sex and regionally specific differences in the percentage of activated PV cells, in males we saw dose-dependent decreases in activation of PV
cells in the IL at a 1mg/kg and both regions at a 5mg/kg; in females these decreases were observed in both regions at a 1mg/kg. These effects were independent of total regional activation. These results suggest sex-specific regional differences in 2-AG’s action on PV cells may underlie the differential behavioral effects of MJN110. We hypothesize that the play enhancing effects are only seen in males due to decreased activation of PV cells in the IL at 1mg/kg, and that both genders show the same behavioral effects at 5mg/kg due to decreased activation of PV cells in both regions at the same dose (1mg/kg in females and 5mg/kg in males). This study suggests that 2-AG based pharmaceutical therapies may be more effective in rectifying adolescent sociobehavioral abnormalities in males than in females. **Keywords:** Immunohistochemistry; Endocannabinoid; 2-arachidonylelglycerol; Sex differences; Social behavior; Play; Parvalbumin; Prefrontal cortex

3) **Phases of Systematic Brain Processing Successfully Predict Task-Specific Behaviors**

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Researchers are working to develop biomarkers in an attempt to better understand and address unique needs in human development and dysfunction. Some researchers have started to investigate event-related potentials (ERPs) as potential biomarkers. ERPs, obtained via electroencephalography (EEG), represent a continuous stream of phases of stimulus-response processing in the brain, and consist of positive and negative voltage deflections called components. Components are shown to have functional associations beginning with sensory processing, then detection processes, followed by evaluation and anticipation. Functional associations of ERPs are often studied via univariate analyses. However, such simple analyses cannot capture the complex nature of brain processing. Additionally, these analyses often only reveal weak brain-behavior relationships which are inadequate for use in understanding the dynamic inter-relationships of neural processing and behavioral performance. The problem is compounded when examining data collected from children, who tend to have more variability in their brain responses. As the field moves toward developing biomarkers of development and dysfunction, establishing strong brain-behavior relationships is critical. Predictive models of the full process of the ERP may relate better to behaviors than single components. However, few studies have considered the inter-relationship among ERP components. The purpose of the present study was to develop a model of the systematic phases of brain processing that would successfully predict ERP task-specific behaviors (e.g., accuracy). EEG data were collected from 51 children age 8-to-12 years while performing a visual Go-NoGo task during two separate sessions. Using structural equation modeling (SEM), a latent variable model was derived using ERP component measurements from the two sessions. Four latent variables illustrating phases of brain processing were defined: Sensory, Detection, Evaluation, and Anticipation. A fifth latent variable of Task Behavior was defined using accuracy and reaction time measurements of performance on the ERP tasks from each session. Results indicated that each phase of brain processing successfully predicted the next in a chronological order (Sensory à Detection à Evaluation à Anticipation), demonstrating that ERPs can be modeled as systematic phases of neural processing ($\beta’s = .62 - .87$, all $p’s < .0005$). Additionally, the stream of neural processing successfully predicted Task Behaviors ($\beta = .63$, $p < .0005$). The current model approaches the requirements for good fit according to SEM standards, ($\chi^2(89) = 139.22$, $p < .001$; RMSEA = .11; CFI = .80), even with a relatively small sample of children. With more data, the model can be further elaborated to include more measures (e.g., ERP components from multiple scalp sites), thereby stabilizing model fit. The data indicate that latent variable models of ERPs depicting the full stream of stimulus-response processing can successfully demonstrate the complex nature of neural processing while simultaneously predicting task-specific behaviors. Thus, researchers may consider latent variable models of neural processing as a promising foundation for robust biomarker identification. **Keywords:** electroencephalography (EEG); event-related potentials (ERPs); biomarkers; human development; brain-behavior relationships; structural equation modeling (SEM)

4) **Inhibiting ventral pallidum disrupts adaptive salt-seeking behavior in rats**

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Much of reinforcement learning involves a process of trial-and-error in which ongoing behavior is iteratively updated when outcomes are received. However, prior experience is not always required for changes in reward seeking. Such non-incremental learning is illustrated by the “salt appetite” phenomenon: animals that have learned to associate a cue with aversively concentrated salt taste will, if deprived of sodium, immediately orient to the cue and seek out salt
before the salt has ever been tasted in the sodium-deprived state. The ventral pallidum (VP) plays an important role in aspects of reward learning, motivation, and hedonics, and represents salt appetite in neural dynamics. In a homeostatic state, VP activity occurs to cues associated with pleasant sugar tastes, but not to cues for aversive salt tastes. Yet, immediately after the sodium deprivation, VP neurons become activated by salt-paired cues similarly to sugar-paired cues. The present study investigated the causal influence of VP on adaptive salt seeking in rats using optogenetics. Half of the rats received infusions of halorhopdopsin (Group Halo) or a control virus (Group Control) into the VP. All rats received bilateral intra-VP fiber implants. Rats then underwent 8 days of place preference training in which they learned to associate one context with a pleasant flavored sucrose solution (4 days) and another context with a distinctly flavored salt solution (4 days). Rats were given a baseline test session in which they were given access to both contexts for the first time with the sucrose and salt removed to measure their baseline preference. Following 2 retraining days in each context, rats were sodium-depleted through systemic injections of furosemide. After 48 hours, rats were placed into the chamber with access to both contexts without sucrose or salt to measure their preference. All rats received yellow laser stimulation (3 s on/off pulses) throughout the entire test session. Thus, VP was inhibited for rats in Group Halo but not for rats in Group Control. We found that rats in Group Control showed a normal “salt appetite”, spending more time in the salt-paired context than the sucrose-paired context following sodium depletion (a form of “unconditioned place preference”). In contrast, rats in Group Halo were indifferent and spent equal amounts of time in the contexts. VP inhibition did not affect general motor activity nor salt/sucrose consumption when the solutions were made available. Thus, inhibiting VP selectively impaired the ability of rats to use environmental cues to guide adaptive salt-seeking behavior, highlighting a critical role for this area in adaptive reward seeking. Keywords: Optogenetics, Incentive learning & memory, Ventral pallidum

5) In vivo optogenetic manipulation of dopamine neurons in a novel behavioral economics based food-seeking task
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The mesolimbic dopamine system is strongly implicated in motivational processes. Currently accepted theories suggest that transient mesolimbic dopamine release events are involved in assessing the value of reward predictive stimuli and/or in generating motivated action sequences directed toward obtaining reward. During the pursuit of reward, critical associations are formed between the reward and otherwise neutral stimuli that begin to predict reward availability. Through these experiences, dopamine neurons, which initially represent the receipt of reward, begin to represent its earliest conditioned predictor (i.e., cue). The resulting concentration of dopamine release scales proportionally to the magnitude of reward predicted. Here, we are investigating the role of cue- and reward-evoked dopamine release on cue-motivated food seeking. To address this research question we developed a novel behavioral economics food-seeking task. In this task, food is provided to rats across 10 different unit-prices (i.e., response requirement/reward magnitude). Importantly, in this task, multiple pairings (>10/price/session; unlike with progressive ratio schedule) occur between each unit-price, reward and its predictive cue. Using fast-scan cyclic voltammetry we first determined that the concentration of accumbal dopamine time-locked to cue presentation decreases as a function of unit-price in this task. We next sought to assess the effect of optically augmenting release both at reward delivery and cue presentation. We selectively activated channelrhodopsin-2 expressing dopamine neurons within the ventral tegmentum during either cue or reward presentation (order counter balanced across animals). Preliminary data reveal that optically facilitating dopamine release at the cue decreases motivation for food; whereas, facilitating release at reward delivery increases motivation for food. Interestingly, optically augmenting release at either cue presentation or reward delivery decreased response latency, consistent with an invigoration of responding that might be dissociable from value-based changes in motivation. It is possible that augmenting cue-evoked dopamine release decreases motivation in our task because we are violating the animal’s expectation (i.e., the animal receives less than expected) and vice versa. Together these findings suggest that cue- and reward-evoked dopamine release play a causal role in action initiation, yet oppositely influence motivation in value-based behavioral economics based tasks. Keywords: Dopamine, mesolimbic, motivation, behavior, optogenetics, voltammetry
6) Effects of Social Isolation of Aggressive Behaviors in Stalk-eyed Flies

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Aggression is a vital behavior in many animal species as a means of obtaining resources, mating, and overall survival. Therefore, the presence of aggressive behaviors in a species can also be an indicator of evolutionary fitness. Stalk-eyed flies, Teleopsis dalmanni, have elongated eyestalks that protrude laterally out of their heads with eye bulbs residing on each end. Males and females of this species and other related species in the Diopsidae family are often sexually dimorphic – meaning that males and females are morphologically distinct. As stalk-eyed flies exhibit aggressive behavior in competition over access to food and mates, they are a useful model species for studying aggressive behaviors in invertebrates. This study examines the effects that social isolation has on aggressive behavior in stalk-eyed flies. Social isolation implies the removal of a sexually mature fly from a normal population cage into separate housing for the period of 7 days. We hypothesized that socially isolated stalk-eyed flies would exhibit an increased quantity and intensity of aggressive behaviors. This was tested by pairing a size-matched isolate with a control fly in a partitioned fighting coliseum. After a period of 24 hours, the flies were provided with corn medium to encourage fighting and the number and intensity of each behavior was recorded and analyzed. Preliminary data suggests a trend toward isolates exhibiting a larger number high intensity aggressive behaviors and overall wins. We will discuss the results in the context of a larger sample of fights. Keywords: Aggression, Stalk-eye Flies, Social Isolation

7) Effects of chronic caffeine exposure on rat brain serotonergic systems

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Chronic caffeine exposure during adolescence has been shown to induce persistent maladaptive anxiety-like behavioral responses in the adult rat. It is possible that these maladaptive responses are mediated by the serotonergic system. In this study, we investigated the effects of chronic adolescent caffeine exposure on the rat brain serotonin (5-hydroxytryptamine; 5-HT) system. Specifically, we analyzed serotonergic neuron activation in subregions of the dorsal raphe nucleus (DRN), a brainstem region with abundant serotonergic neurons. After a week of acclimatization, rats were randomly divided into four groups in a two-by-two experimental design. Two groups received chronic caffeine (CC) administration in drinking water (0.3 g/L) from postnatal day 28 to postnatal day 56 while the other two groups received drinking water (NC) alone during the same developmental time period. After 28 days of caffeine or control treatment and a 24-hour washout period, rats received an i.p. injection of either 30 mg/kg caffeine (C) or 0.9% sterile saline (S) vehicle, were then replaced in their home cages, and were euthanized 90 minutes following treatment. This was a 2 x 2 design with four treatment groups, NCS, NCC, CCS, and CCC. Using a double immunostaining technique we quantified the immunoreactivity for the acute activation marker c-Fos and tryptophan hydroxylase 2 (Tph2) as a marker of serotonergic neurons. NCC rats, relative to NCS and CCC groups, had higher activation of 5-HT neurons in the rostral DRD and caudal DRD, ventral part of the dorsal raphe nucleus (DRV), DRC, and DRI. These data are consistent with the hypothesis that the DRN is a key structure in promoting the adult pro-anxiety behavioral phenotype following adolescent caffeine exposure.

8) Dopamine Synthesis in the Ventral Tegmental Area in Rams with High or Low Libido

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Dopamine synthesis and release in the ventral tegmental area (VTA) of the brainstem is credited for pleasurable aspects of certain behaviors such as recreational drug use and mating activity, and is central to the reinforcement of those behaviors. It was hypothesized rams lacking overt sexual interest in ewes in estrus would have fewer dopamine synthesizing neurons in the VTA, as well as decreased dopamine 2 (D2) receptor expression in the forebrain. Additionally, we hypothesized that rams lacking sexual interest in ewes in estrus would have a similar stress response to exposure as rams of high sexual interest. Rams characterized as having high (n = 4) or low (n = 3) expression of sexual behavior were exposed to urine from ewes in estrus. A second group of high performing rams (n = 3) were exposed to urine from ovariectomized ewes. Following exposure, rams were exsanguinated and brains were preserved by carotid perfusion of 4% buffered paraformaldehyde. The VTA and forebrain were dissected using surface landmarks, paraffin embedded and sliced at 6 µm. Paraffin embedded slices from the VTA were stained for tyrosine hydroxylase, while slices from the forebrain were stained for D2 receptors; both using immunocytochemistry. Blood samples were collected prior to and following exposure to urine stimulus. A sheep-specific ELISA kit was used
to analyze serum concentrations of cortisol. Following exposure to a putative sexual stimulus, inactive rams had fewer \( (P < 0.05) \) tyrosine hydroxylase positive neurons than sexually active rams. Exposure to ovariectomized-ewe urine reduced \( (P < 0.05) \) numbers of tyrosine hydroxylase-active neurons in sexually active rams and did not differ \( (P > 0.05) \) from sexually inactive rams exposed to urine from ewes in estrus. Sexually inactive rams had decreased expression of D2 receptors in the forebrain \( (P = 0.04) \) when compared to sexually active rams. Differences in D2 receptors were not noted in sexually active rams \( (P = 0.46) \) exposed to either ovariectomized or estrus ewe urine. Cortisol blood concentration did not differ among sexually active and inactive rams prior to or following urine exposure \( (P \geq 0.52) \). Lack of sexual interest in low performing rams, may be, partially a result of decreased tyrosine hydroxylase and hence dopamine synthesis in the VTA and decreased D2 receptor expression in the forebrain leading to attenuated reinforcement of sexual behavior. There was no difference in cortisol concentration between the groups suggesting low sexually performing rams do not experience increased stress and contribute to their lack of sexual interest.

9) Exercise increases mTOR signaling in brain areas involved in cognition and emotion

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Exercise can enhance learning and memory and produce resistance against stress-related psychiatric disorders such as depression and anxiety. In rats, these beneficial effects of exercise occur regardless of exercise controllability: both voluntary and forced wheel running produce stress-protective effects. The mechanisms underlying these beneficial effects of exercise remain unknown. The mammalian target of rapamycin (mTOR) is a translation regulator important for cell growth, proliferation, and survival. mTOR has been implicated in enhancing learning and memory and producing antidepressant effects. Moreover, mTOR is sensitive to exercise signals such as monoamines and metabolic signals. The effects of exercise on mTOR signaling, however, remain unknown. The goal of the present study was to test the hypothesis that exercise increases levels of phosphorylated mTOR \( (p\text{-mTOR}) \) in brain regions important for learning and antidepressant responses. Moreover, it was hypothesized that increases in \( p\text{-mTOR} \) would be independent of exercise controllability. Rats were exposed to 6 weeks of either sedentary (locked wheel), voluntary, or forced wheel running conditions. At 6 weeks, rats were sacrificed during peak running and levels of \( p\text{-mTOR} \) were measured using immunohistochemistry. Compared to locked-wheel controls, both voluntary and forced wheel running increased the number of neurons expressing \( p\text{-mTOR} \) in the infralimbic and prelimbic regions of the prefrontal cortex (PFC). However, only voluntary running increased levels of \( p\text{-mTOR} \) in the nucleus accumbens core and nucleus accumbens shell. Data from the dorsal striatum, amygdala, and hippocampus are still being analyzed. Results suggest that mTOR signaling is sensitive to exercise. Increases in mTOR signaling could contribute to the beneficial effects of exercise on cognitive function and mental health. Supported by NIH grant 068283 and CU Denver startup funds. Keywords: exercise, stress, depression, anxiety, mTOR

10) FMRI Investigation of the Accumulation of Categorical Information

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We used a temporally-extended categorization task to investigate the neural substrates underlying our ability to integrate information over time and across multiple stimulus features. Using model-based fMRI, we tracked the temporal evolution of two important variables as participants deliberated about impending choices: 1) categorical evidence, and 2) confidence (the total amount of evidence provided by the stimuli, irrespective of the particular category favored). Importantly, in each model, we also included a covariate which allowed us to differentiate signals related to information accumulation from other, evidence-independent functions that increased monotonically with time (such as urgency or cognitive load). We found that somatomotor regions tracked the temporal evolution of categorical evidence, while regions in both medial and lateral prefrontal cortex, inferior parietal cortex, and the striatum tracked decision confidence. As both theory and experimental work suggest that patterns of activity thought to be related to information-accumulation may reflect, in whole or in part, an interaction between sensory evidence and urgency, we additionally investigated whether urgency might modulate the slopes of the two evidence-dependent functions. We found that the slopes of both functions were likely modulated by urgency such that the difference between the high and low evidence states increased as the response deadline loomed.
11) Similar patterns of brain gene expression underlie transgenerational and juvenile developmental plasticity in threespine stickleback

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Nongenetic parental effects occur when a parent’s phenotype influences its offspring’s phenotype. Parental phenotypes may therefore act as a cue to offspring about the current environment, resulting in changes to offspring development (“transgenerational plasticity”). It has been suggested that similar mechanisms operate on both within-generation (developmental) plasticity and transgenerational plasticity. Yet, we still know little about whether the molecular mechanisms underlying transgenerational plasticity are similar to those underlying developmental plasticity. We compared juvenile brain gene expression in response to direct exposure to predation risk, and in response to indirect exposure to predation risk (parental exposure to predation risk) in threespine stickleback (Gasterosteus aculeatus), a teleost fish in which the father is the sole provider of parental care. Offspring of predator-exposed fathers were smaller, in worse body condition, and less active than offspring of unexposed fathers. Further, offspring of unexposed fathers exposed to predation risk as juveniles showed these same phenotypes, confirming that phenotypes associated with father’s experience mirrors those associated with direct experience. We found 82 genes shared between transgenerational and developmental plasticity, and these genes were regulated in the same direction under both conditions. Our study suggests that juvenile developmental and transgenerational plasticity in behavior are controlled by similar molecular mechanisms, suggesting that multiple cues can be co-opted to produce similar phenotypes. Keywords: RNA-Seq, behavior, parental effects, developmental plasticity, gene expression

12) An overview of systematic review evaluating pharmacological treatment outcomes on individuals with traumatic brain injury (TBI) – ACRM Taskforce Project

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Background: No definitive evidence has been published regarding the effect of pharmaceutical treatment on outcomes for patients with sustained traumatic brain injury (TBI). The minimal research that is available to the scientific community varies greatly in sample size, degree of TBI, type of pharmaceutical used, and overall design of study. It would benefit the scientific community greatly to generate a comprehensive overview of pharmaceutical effects on TBI outcome in order to simplify the results that have already been observed, as well as make more accessible the proper recommendations for treatment. Furthermore, an overview of the state of pharmaceutical outcomes on TBI could emphasize which areas of future pharmaceutical research on TBI are most promising. This study aims to evaluate the outcome of pharmacological treatments after TBI and provide an overview. Design: Overview of systematic review and meta-analysis studies by data synthesis procedures. Data abstraction protocol designed by the Measurement Networking Applied Cognition TBI Workgroup from the American Congress of Rehabilitation Medicine (PROSPERO ID:CRD42015017355). Setting: According to the original pharmacological intervention review. Participants: Children and/or adults with sustained traumatic brain injury followed by pharmaceutical treatment. Main Outcome Measure(s): Psychological/cognitive, functional/ADL/motor-based, adverse events, physiological/biological, quality of life, and neurobehavioral. Data Extraction: Descriptive and data summaries based on each study characteristics such as methods, setting, sample characteristics, pharmacological treatment characteristics, study outcomes, study/review results and summary, and AMSTAR critical appraisal tool. Preliminary Findings: The quality of the current literature is medium to low. Study results are confusing and undefined. It seems that there is a trend towards timing for administration of pharmaceutical drugs and outcome response after sustained TBI that affects improvement. Conclusion: Future studies need to be of better quality, with improved and more rigorous protocols. Future evidence should follow the high standards of meta-analysis systematic reviews (i.e. Cochrane Library guideline, PROSPERO). Keywords: TBI

13) Methamphetamine intake impairs alcohol consumption in high-alcohol preferring P rats

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Polysubstance use and abuse at subclinical levels (those not meeting DSM criteria) is quite prevalent and an extremely understudied phenomenon. While any combination of substances may be used together, alcohol is commonly one of those substances. Epidemiological studies suggest that the psychostimulant, methamphetamine,
and alcohol are often co-used, especially in heavy alcohol drinkers. The studies presented here therefore sought to determine whether the combination of alcohol drinking and methamphetamine self-administration would produce alterations in the consumption of either or both substances. In experiment 1, adult male high-alcohol preferring (P) rats were surgically implanted with chronic indwelling intra-jugular catheters. Following recovery, two drinking tubes were introduced into each rat's cage. Animals in the alcohol-drinking group received one tube containing a solution of ethanol (10-20% v/v), and another tube containing water. The alcohol-naive control animals had two tubes containing water. The tubes were rotated following daily measurements of fluid intake and body weight. Five days after the introduction of the drinking tubes, rats self-administered saline or methamphetamine (FR1:TO15 sec; 0.05 mg/kg/injection) in daily 2-hr sessions. Experiment 2 was conducted similarly, except methamphetamine self-administration occurred 5 days before the drinking tubes containing alcohol were introduced. The results of both experiments were striking in that methamphetamine self-administration significantly reduced alcohol intake and alcohol preference ratios. Importantly, methamphetamine’s inhibition of alcohol drinking was reversible. Thus, ceasing the methamphetamine self-administration procedures produced a rebound in alcohol intake and alcohol preference ratios. The results suggest that methamphetamine intake may disrupt alcohol consumption and preferences in P rats that have been selectively bred to prefer alcohol. Future studies will continue to characterize this surprising interaction and explore the potential mechanisms of this phenomenon. This work was supported by NIH R01 DA033358.

14) Influence of Phonological Neighborhood Density on Word Production in Children: An ERP Study
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Behavioral studies of picture naming provide evidence that the accuracy and speed of single word production is affected by the linguistic variable of phonological neighborhood density (PND). PND has been implicated in vocabulary development in young children, turning this linguistic factor into a potential predictor of language development. So far, no neurophysiological data exist on whether or not PND exerts an influence on underlying cognitive processes involved in word production. The present study addressed this issue by making use of electroencephalography (EEG) recordings. In two different tasks (immediate and delayed picture naming) the PND level was manipulated to investigate the impact of PND on word production in 7-year-old children.

15) Age-Dependent Ion Channel Stability in Neurons
Maximiliano Vallejos, Timothy Vernier, Susan Tsunoda. From the Department of Biomedical Sciences, Colorado State University.

Ageing typically relates to a decline in cognitive abilities and coordinated behaviors. These changes are likely due to age-related changes in neuronal signaling. Ion channels, which underlie much of neuronal signaling, have been described to undergo age-dependent changes in aggregation and distribution. Little is known about the ageing effects on ion channel stability. Here, we show an age-dependent decrease in Shal/Kv4 ion channel in Drosophila. Shal is a voltage-gated potassium channel involved in locomotion, learning and memory. We report that natural age-dependent decline in locomotion is improved by Shal overexpression. To investigate the mechanisms underlying the age-dependent decline in Shal protein, we quantitated Shal mRNA in young/old flies. Shal mRNA levels remain unaffected, suggesting that transcription is not involved in declining Shal protein. To test if the decline involves loss of a scaffolding protein, we examined SIDL (Shal-interactor of di-leucine), a novel protein identified to interact with a highly conserved C-terminal di-leucine motif required for somato-dendritic targeting. We found that SIDL transcript is significantly decreased with age. To test whether SIDL is necessary for Shal stability, we used an RNAi-SIDL construct resulting in 60% and 25% SIDL mRNA knockdown at larval and adult stages, respectively. This knockdown resulted in a 40% decrease of Shal protein levels in adults, suggesting that SIDL is required for Shal stability in vivo. Interestingly, RNAi-SIDL expressed during development resulted in decreased Drosophila viability. Our results suggest that loss of SIDL during ageing leads to a decline in Shal, and deterioration in behaviors such as locomotion.

16) Effects of MDMA on fear extinction and renewal
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Available treatment options for post-traumatic stress disorder (PTSD) are limited to selective serotonin reuptake inhibitors and exposure therapy. Extinction-based exposure therapy is the behavioral therapy of choice for PTSD, but
long-term efficacy is limited due to fear relapse phenomena such as renewal and spontaneous recovery. Renewal is the return of fear in contexts different from where extinction was learned, and spontaneous recovery is the return of fear after the passage of time. Emerging evidence suggests that pairing the psychedelic drug 3,4-methylenedioxymethamphetamine (MDMA) with psychotherapy can reduce symptoms of PTSD in humans for up to one year. Little research has investigated the mechanisms underlying this effect. The goal of the current studies is to begin to investigate whether MDMA paired with fear extinction can enhance fear extinction and reduce the relapse of fear in an animal model that can then be used to investigate underlying mechanisms. On day one, adult, male Long Evans rats were exposed to auditory fear conditioning during which a foot shock was paired with an auditory conditioned stimulus (CS) in context A. On day two, rats were injected i.p. with either saline or MDMA (3 mg/kg) 30 minutes prior to exposure to fear extinction training in context B. The next day, rats were again exposed to the extinguished CS either in the same context B or in a novel context C. MDMA treatment delayed fear extinction learning and impaired fear extinction memory. Although contrary to our hypothesis, these data are consistent with anxiogenic effects of acute increases of serotonin in rats. Future studies will further characterize the effects of MDMA on fear extinction and relapse. Supported by Multidisciplinary Association for Psychedelic Studies.

17) DREADDing fear: dopamine signaling during fear extinction reduces fear relapse
Courtney A. Bouchet1, Esteban C. Loetz2, Toni Nicastro2, Nathan Gray2, Mat Mondragon2, Matias G. Saez2, Adam Rosberg2, Benjamin N. Greenwood2.

Anxiety disorders are the most prevalent mental health disorders in developed countries, yet treatment options are lacking in long-term efficacy. Extinction-based exposure therapy is the most common behavioral therapy used to treat anxiety disorders. Exposure therapy is effective within the context where it is learned; however, extinction memories are highly susceptible relapse in different contexts, a phenomenon termed fear renewal. Exploring the mechanisms underlying fear extinction and identifying novel manipulations to reduce the relapse of fear are of utmost importance to mental health. Using we are investigating a role for dopamine in fear extinction learning and memory. Designer receptors exclusively activated by designer drugs (DREADD) were used to activate a specific population of dopaminergic neurons during auditory fear extinction in male transgenic TH-Cre rats. Relative to wild-type rats, DREADD-induced activation of the nigrostriatal dopamine pathway during auditory fear extinction learning reduced fear (measured by behavioral freezing) during extinction, and also reduced the renewal of fear when tested 24 h later in a novel context drug-free. We further investigated the role of dopamine 1 receptors (D1) in the dorsal striatum in the behavioral effects of DREADD-induced dopaminergic activity. Activation of the D1 receptor in the dorsal striatum during fear extinction also reduced fear renewal, but did not impact fear extinction learning or memory. Together, these data suggest that activation of the nigrostriatal dopaminergic pathway during fear extinction can block the renewal of fear through a mechanism involving D1 receptors in the dorsal striatum. Manipulations that activate the nigrostriatal pathway during fear extinction thus represent a novel strategy for enhancing the efficacy of extinction-based therapies. Keywords: DREADD, fear extinction, dopamine

18) Rats associated with cocaine exposure become cues, thus altering seeking behaviors
Paige Dingess1, Morgan J. Deters1, Rebecca A. Darling1, and Travis E. Brown1,2.

Three primary triggers can induce drug relapse after periods of abstinence: stress, re-exposure to the drug, and drug-associated cues. There are numerous drug-related (e.g. drug paraphernalia) and drug-neutral cues (e.g. street sign) that may become associated with drug taking behaviors and induce changes in drug craving and drug seeking behaviors. Intuitively, it is known that social cues can induce relapse in recovering drug addicts. However, to our knowledge nobody has modeled whether social cues may induce relapse to drugs of abuse. Using a model similar to conditioned-place preference, which is commonly utilized to study drug addiction, we sought to develop a social interaction model to demonstrate that social cues could induce drug seeking behaviors. We initially habituated the rats to a custom-made testing chamber that had three compartments. The two “buddy” animals were separated by a perforated wall from the test animal. The test animal was able to interact with the other two rats through the perforations of the wall, termed nose pokes. Following the initial preference day (IP), we did 20 days of 30 minute pairings with either a “drug buddy” while on cocaine or a “study buddy” after a saline injection. Injections were alternated daily. The test day was performed one day after the final pairing. All three animals were placed back in the custom-made chamber for 30 minutes. The sessions were all recorded for later analysis. During the test, the rats showed a significant increase in nose pokes to the “drug buddy” compared to their initial preference day (IP = 28.0
± 6.2 vs Test = 62.5 ± 8.8, p < 0.05, F (2, 13) = 4.953). There was no significant change in the number of nose pokes observed for the study buddy (IP = 25.3 ± 8.1 vs Test = 42.3 ± 7.4). We conclude that our model was successful at demonstrating that the individual rat can become a salient cue when associated with cocaine exposure and could be used as a model to study social cues in regards to drug relapse.

19) Brain response is elevated in adolescent anorexia nervosa to monetary reward receipt and omission when underweight, but only to omission after weight restoration

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Understanding neurobiological changes associated with anorexia nervosa (AN) is important in treatment development. Previously, adults with AN showed heightened brain response to unexpected reward receipt or omission in taste reward paradigms, a pattern that improves with recovery. Using a similar paradigm with monetary stimuli, we tested whether this sensitized brain reward function is seen in adolescents with AN, generalizes beyond taste, and improves with weight restoration. Twenty-five healthy control female adolescents (age = 14.7±2.3 years) and 23 female adolescents (age = 16.0±1.9 years) diagnosed with AN, and enrolled in a treatment program underwent functional magnetic resonance imaging (fMRI) before and after the treatment program (mean time between the two scans = 42 +/-14.3 days, mean BMI increase = 2.13 +/-0.96 kg/m2). During fMRI, participants learned to associate visual and monetary stimuli. The prediction error evoked when this learned association was violated has been linked with the dopamine function of the brain reward circuit. All images were preprocessed using SPM8. Group by condition first level contrast images were analyzed using a general linear model. Small volume anatomical ROIs at a threshold of p<0.001 & 10 voxels with subsequent FWE-correction (p<0.05) were used to assess activity. TETRAD V software was used to evaluate effective connectivity between the anatomical ROIs with IMaGES and LOFS algorithms. In response to unexpected receipt of monetary reward, AN showed greater activation than controls in the right caudate and right posterior insula before, but not after weight restoration. In response to the unexpected omission of monetary reward, AN showed greater activation in the left and right medial orbitofrontal cortex (mOFC), which persisted with weight restoration in the left mOFC. During unexpected omission of reward, certain connections normalize with weight restoration in AN, while an additional central nucleus of the amygdala and ventral striatum connection seen in AN does not. This persistent connection is not seen during the unexpected receipt of reward. This study has novel results that suggest a generalization of reward system responsiveness in an underweight context. The elevated region-specific sensitivity and the differences in effective connectivity remit with weight restoration but only for receipt and not omission of the salient stimulus. In summary, heightened sensitivity to punishment along with the abnormal processing of unexpected omission of monetary reward may present an obstacle for adolescents undergoing treatment for AN, even after weight restoration.

20) Effect of monoacylglycerol lipase (MAGL) inhibition on aggression in female rats after postweaning social isolation

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Early life adversity, including during adolescence, can lead to alterations in normal brain development and can increase susceptibility to behavioral disorders in adulthood. Post-weaning social isolation (PSI) is a model of early life adversity in social animals, and previous work has shown that after PSI, female rats display increased aggression during a social encounter with a novel conspecific. The endocannabinoid system, which includes 2-arachidonoylglycerol (2-AG), is known to regulate central pathways that are important for emotional regulation. The novel drug MJN110 increases the concentration of 2-AG by inhibiting MAGL, the enzyme responsible for its breakdown. The present study examined the impact of MJN110 on PSI-induced aggression in female rats. Female rats were either group housed (GP) or isolation housed (PSI) for 3 weeks between postnatal days 21 and 42. MJN110 (1 or 5 mg/kg IP) or vehicle was administered 2 hr prior to a single social encounter. Social behaviors and total social interactions were assessed. PSI females displayed more aggressive grooming and more social interaction than GRP females. Both doses of MJN110 decreased aggressive grooming regardless of housing conditions but did not significantly decrease overall social interaction. Thus, 2-AG may selectively modulate aggressive behavior in female rats.

Keywords: Behavior; Endocannabinoids; 2-AG
21) The effect of voluntary exercise during consolidation of auditory fear extinction on renewal and spontaneous recovery of fear
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Fear extinction learning forms the basis of exposure therapy for anxiety and trauma related disorders. Unlike fear memories, fear extinction memories are labile and susceptible to relapse phenomenon such as fear renewal or spontaneous recovery. Fear renewal is characterized by the return of conditioned fear response in contexts outside of the extinction context, even following successful extinction, while spontaneous recovery is the return of fear over time. Previous work suggests that acute exercise, either prior to or following, auditory fear extinction can enhance later extinction memory. It is unknown, however, whether acute exercise can enhance the consolidation of auditory fear extinction memory, and whether fear extinction memory augmented by acute exercise is resistant to different forms of fear relapse. To address this, male Long Evans rats were conditioned to fear an auditory cue followed by two days of fear extinction. Immediately following fear extinction training, half of the rats were placed into a mobile voluntary running wheel (run) for 2 hours, while the other half were placed into an identical locked wheel (locked). Rats in the run group were allowed to run during the consolidation phase of fear extinction. Run rats exhibited enhanced fear extinction memory. To test for fear renewal, a cohort of rats were exposed to the auditory cue in a novel context the day following the last fear extinction trial. Run rats exhibited lower levels of fear than locked rats, suggesting that exercise immediately following fear extinction blocks fear renewal. To test for spontaneous recovery, a different cohort of rats were subjected to the auditory cue in the extinction context one week following fear extinction. Exposure to the auditory cue evoked less fear in the run rats than the locked rats, suggesting that exercise immediately following fear extinction reduces spontaneous recovery as well. Together, these data suggest that acute exercise during the consolidation period of fear extinction promotes enhanced fear extinction memory resistant to multiple types of relapse. Keywords: Fear extinction, exercise, fear renewal, spontaneous recovery

22) Differences in Frontal, Basal Ganglia, and Limbic Activation during a Word-Emotional Face Stroop Task in Monozygotic Twin Pairs Discordant for Severe Stressful Life Events
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Monozygotic twin pairs provide a valuable opportunity to control for genetic and shared environmental influences while studying nonshared environmental influences. In this study we selected ten young adult twin pairs who were discordant in exposure to severe stressful life events during development. Functional magnetic resonance imaging was used to assess brain activation during performance of a word-emotional face Stroop task. Twins who experienced higher levels of stress during development exhibited greater activation in the ventrolateral prefrontal cortex, basal ganglia, and limbic regions including the amygdala and hippocampus compared to their control co-twin without heightened stress during development, across all conditions compared to fixation. The control co-twins, in contrast, showed greater frontoparietal activation for the contrast of incongruent compared to congruent blocks. Overall, the control co-twins showed the more typical pattern of frontoparietal activation while their co-twins with higher stress showed greater activity in limbic and medial regions less commonly activated during Stroop tasks. This study provides a powerful glimpse at the effects of stress during development while accounting for shared genetic and environmental influences such as socioeconomic status, prenatal environment, and schooling. Keywords: Stress, Executive Function, Behavioral Genetics

23) Test-Retest Reliability of N1 and N2 in Adults During a Flanker Task
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Establishing biomarkers in order to understand dysfunction in human development has become a recent interest in research. In order to develop these biomarkers, event-related potentials (ERPs) derived from electroencephalography (EEG) are often used. ERPs are comprised of positive and negative deflections in response to a stimulus. Two such ERP components are N1 and N2 which relate to selective attention and executive discrimination, respectively. In order to develop viable biomarkers, the ERP components must be reliable and stable over time. This study focuses on establishing test-retest reliability of N1 and N2 in adults over a short period of time. We recorded EEG data from 32 adults (19-29 yrs, M = 23.28, SD = 2.31) during two sessions scheduled one-to-two
weeks apart. Participants performed a visual 5-character H/S Flanker task and were instructed to press a button based on the center letter. Reliability in correctly-performed trials was assessed using Pearson correlations on both N1 amplitudes (measured from 70 milliseconds to 110 milliseconds) and N2 amplitudes (measured from 190 milliseconds to 280 milliseconds) measured peak-to-peak and baseline-to-peak. Amplitudes were measured at two central-frontal midline sites, Cz and FCz. Test-retest reliability was found to be greatest for baseline-to-peak measures of both components. The N1 was most reliable measured at Cz, r(29) = .86, p < .0005, and N2 was similarly reliable when measured at Cz, r(30) = .92, p < .0005, as well as at FCz, r(31) = .92, p < .0005. Previous research has also found baseline-to-peak measurements to be reliable, but over a longer period of time, e.g., 1 month or longer. This study found that N1 and N2 baseline-to-peak measures are reliable over a short period of time, suggesting that N1 and N2 may be used for reliable biomarker development in clinical settings and future research. **Keywords:** event-related potentials, test-retest reliability, electroencephalography, biomarker

**24) Test-Retest Reliability of the P300 in Adults during a Flanker Task**

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The P300 component of an event-related potential (ERP) is associated with memory updating and is produced in response to a given stimulus. In order to use information about the P300 clinically as a biomarker for neurological impairment, reliability needs to be established. However, there is a lack of substantial research concerning the test-retest reliability of the P300 elicited during common paradigms like the classic Flanker Task. This study examined the test-retest reliability of adults’ P300 measurements at Cz and Pz over a short period of time. Electroencephalography (EEG) data were collected from 32 adults (19-29 yrs, M=23.28, SD= 2.31) while they performed a 5 character H/S Flanker task during two sessions, one-to-two weeks apart. Reliability was assessed for both baseline-to-peak and peak-to-peak amplitude measures using Pearson correlations and a Spearman correlation (only for peak-to-peak at Pz). The results indicated a high test-retest reliability for baseline-to-peak measurements at Cz, r(31) = .89, p < .0005, and Pz, r(31) = .90, p < .0005 and a lower test-retest reliability for peak-to-peak measurements at Cz, r(30) = .85 p < .0005 and Pz, r(31) = .85 p < .0005, rs(31) = .68 p < .0005. The results suggest baseline-to-peak measurements at Cz and Pz were reliable and can be applied to clinical research. Peak-to-peak measurements at Cz and Pz were not as reliable and are not as desirable for clinical research due to higher levels of variability. The reliability of the baseline-to-peak measurements at Cz and Pz indicate that the P300 has little fluctuation in mature, adult brains. **Keywords:** Event-related potentials, test-retest reliability, P300

**25) Reverse Flow is a Key Epigenetic Regulator of Cardiac Valve Development**

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Congenital heart defects occur when the heart fails to undergo proper morphogenesis. It is estimated that a congenital heart defect occurs in 4 out of every 1000 live births (Pierpont et al., 2007). Blood flow is essential for proper heart development, however the mechanism by which blood effects cardiac morphogenesis has yet to be elucidated. Zebrafish are an ideal model organism for studying heart morphogenesis as their optical clarity coupled with the ease of genetic manipulation make them suited for a plethora of experiments. We are particularly interested in how blood flow forces such as shear stress and reverse flow modulate gene expression. Shear stress is defined as the frictional force exerted on the endothelium by moving blood cells (Gijsen et al, 2013. Reverse flow is defined as the fraction of the blood that flows backwards, from the ventricle to the atrium, in any given cardiac cycle. In 2009, Vermot et al showed a correlation of decreased reverse flow with decreased expression of Kruppel-like transcription factor, klf2a, suggesting a possible mechanistic linkage. To test the hypothesis that klf2a is flow-responsive, we developed genetic models and pharmaceutical approaches to further manipulate the reverse flow in vivo and assess the klf2a transcriptional response. We find that when we knockdown filamin c via morpholino, reverse flow increases by 220%. Concomitantly, we find increased klf2a expression by in situ hybridization and quantitative polymerase chain reaction. Taken together, these results provide strong support for the hypothesis that klf2a is regulated at the transcriptional level by blood flow mediated biomechanical forces, and that blood flow serves as an epigenetic cue to facilitate proper cardiac development.
26) Prenatal administration of glucocorticoids predicts diurnal cortisol regulation during childhood
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Due to the rapid developmental changes that occur during the fetal period, prenatal influences can affect the developing central nervous system with lifelong consequences for physical and mental health. One proposed mechanism by which this occurs is via glucocorticoids, which can pass through the blood-brain barrier and target receptors throughout the central nervous system. Unlike endogenous glucocorticoids, synthetic glucocorticoids readily pass through the placental barrier to reach the developing fetus. The synthetic glucocorticoid, betamethasone, is routinely given prenatally to fetuses at risk for being born preterm. Over 25% of the fetuses treated with betamethasone will be born at term due to an inability to predict who will deliver preterm. Few studies have examined the lasting consequences of prenatal treatment of betamethasone on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Altering the development of the HPA axis is one pathway by which glucocorticoid exposure is thought to influence later childhood outcomes. The HPA axis releases cortisol in a diurnal rhythm. The purpose of this study is to examine whether prenatal exposure to betamethasone alters the circadian release of cortisol by the HPA axis in children who were born full term. School-aged children prenatally treated with betamethasone (n=18, mean (SD) = 8.0 (1.2) years old) were compared to children not treated with prenatal synthetic glucocorticoids (n=61, mean (SD) = 8.3 (1.4) years old). To measure the circadian release of cortisol, multiple salivary samples were collected on a single day in the child’s home, including: at time of awakening; 30, 45, and 60 minutes after awakening; and in the evening. Comparison children showed a typical diurnal cortisol pattern that peaked in the morning (the cortisol awakening response) and gradually decreased throughout the day. In contrast, children exposed to prenatal betamethasone lacked a cortisol awakening response and had a flatter diurnal slope than the comparison children (p’s <0.01). These data suggest that prenatal exposure to synthetic glucocorticoids disrupts the circadian regulation of the HPA axis among children born at term. Because disrupted circadian regulation of cortisol has been linked to a variety of health problems, future research is needed to determine whether children exposed to prenatal synthetic glucocorticoids are at risk for poor mental and physical health.

27) Characterization of zebrafish models of filamin C related cardiomyopathy
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Dilated cardiomyopathy (DCM) is a group of heart muscle diseases in which the heart chambers become dilated and are unable to maintain effective blood circulation, which leads to arrhythmias and eventually heart failure. Familial DCM is caused by mutations in about 30 known genes, most of which function in the cytoskeleton or sarcomere. However, the genetic basis remains unknown for approximately 80% of DCM cases. In collaboration with Dr. Mestroni and Dr. Taylor at UCD, we recently reported a mutation in the filamin c (FLNC) gene in two cardiomyopathy-afflicted Italian families; this is the first data to suggest that FLNC may be a candidate gene for DCM in humans. FLNC is a member of the filamin family of genes that encode actin binding proteins and it is expressed in cardiac and skeletal muscle. FLNC is proposed to play a role in maintaining the integrity of a sarcomere. There is a gap in our understanding of the link between mutations in FLNC and its potential to contribute to cardiomyopathy, especially the familial type. Therefore, we desire to create a zebrafish model to study how FLNC mutation leads to cardiac phenotypes and the progression of cardiac disease. Zebrafish have two paralogs of FLNC (flnca and flncb). Knockdown zebrafish flnca, or flncb by morpholino led to an enlarged heart, abnormal heart development and low heart rate which cumulated in heart failure. An analysis of embryos doubly knocked down (for both flnca and flncb) did not support a high degree of functional redundancy. We will examine how FLNC mutation affects the formation of sarcomere Z-discs in cardiac muscles versus skeletal muscle by using TEM and IHC methods to visualize myofibril bundles and sarcomeric substructures. This project will contribute to the knowledge of the DCM disease pathway and lay the groundwork for potential therapies. Keywords: Cardiomyopathy (DCM), FLNC
28) Exercise Produces Neuroplastic Changes Within the Hippocampus During Discrete Periods of Development
Autumn L. Ingalls-Williams, Kevin R. O'Connor, Kristina A. Hulen, Agnieszka Mika, Nicole L. Rumian, Courtney Bouchet and Monika Fleshner

Voluntary exercise improves hippocampal-dependent learning and memory through a variety of mechanisms, including increased levels of growth factor proteins such as brain-derived neurotrophic factor (BDNF). BDNF binds to and activates its receptor, tyrosine receptor kinase B (TrkB), and in turn, activates transcription factor cyclic AMP response element binding protein (CREB). CREB then increases transcription for many subsequent plasticity proteins, such as synapsin-I. Exercise has been shown to increase levels of CREB, TrkB, and synapsin-I in a BDNF dependent manner. Prior unpublished observations from our lab have demonstrated that six weeks of voluntary exercise initiated in early life can produce long-lasting increases in hippocampal BDNF mRNA expression, while other work has observed similar persistent adaptations in BDNF that correlate with improvements in hippocampal-dependent learning. In contrast, these exercise-induced enhancements in brain function are transient adults and only last as long as exercise persists. In these studies, rats run for six weeks and, thus, run through several stages of early life development into adulthood. Therefore, the specific period during which the hippocampus is susceptible to long lasting exercise-induced neuroplastic changes is unknown. These robust enhancements could be due to the plastic nature of the brain during the early juvenile period, between weaning and puberty, in which increased plasticity proteins augment the ability of neurons to change in response to external events. On the other hand, they could be due to specific neural changes associated to the rise of androgens during the peripubertal period of adolescence. To explore this, rats began exercising during juvenile (PND 24), peripubertal (PND 40), or adult (PND 70) periods, and ran for one week, which is sufficient to produce increases in BDNF mRNA but brief enough to allow us to isolate and compare these discrete developmental stages. After one week of exercise, half of each cohort was sacrificed while the other half had their wheels locked for two additional weeks in order to measure the long-term effects of exercise. BDNF, CREB, and synapsin-I mRNA levels were measured in the dentate gyrus, CA1, CA2, and CA3 regions of the hippocampus at both of these time points. mRNA levels were processed through radioactive in situ hybridization. Preliminary results indicate that one week of exercise produces increases in BDNF and CREB mRNA levels, but not synapsin mRNA levels. Furthermore, one week of exercise is not sufficient to produce long-lasting increases in these mRNA levels regardless of age. Keywords: Exercise, Hippocampus, BDNF, Development, Neuroplasticity

29) Sensory Gating in Children with High Functioning Autism
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Sensory gating is a neurological filtering mechanism that inhibits brain responses to irrelevant incoming sensory stimuli, thereby preventing sensory overload. Results in previous research regarding gating abilities in children with high functioning autism (HFA) compared to typically developing (TD) peers are mixed. Though most children with autism exhibit difficulty in processing sensory information, the neurophysiological evidence of sensory gating for this population remains unclear. This study compared sensory gating in 23 children with HFA with 23 age- and gender-matched, TD peers. Electroencephalographic data were recorded while participants heard 120 pairs of click stimuli every 8 seconds. The first click in the pair is referred to as the conditioning click, and the second as the test click. The clicks were separated by 500 ms. A 2(Group) x 2(Clicks) ANOVA revealed significant interaction effect, F(44) = 12.98, p = .001, η² = .23, and significant group differences, F(44) = 11.76, p = .001, η² = .21. Post-hoc t-test showed that the peak-to-peak P50 amplitude for TD children was smaller in response to the test click than to the conditioning click, t(22) = 5.63, p < .001; however, there was no difference between clicks for children with HFA, t(22) = -.008, p = .994. Post-hoc t-test showed that the peak-to-peak P50 amplitudes in response to the conditioning click of TD children were significantly larger than those of children with HFA, t(44) = 4.55, p < .001. In contrast, peak-to-peak P50 amplitudes in response to the test click of TD children did not differ significantly from those of children with HFA, t(44) = 1.28, p = .21. Difference scores (test click minus conditioning click) of the peak-to-peak P50 amplitude were assessed using an independent t-test, which showed significantly better gating in TD children than in children with HFA, t(44) = 4.12, p < .001. Analysis of T/C ratios (test click/conditioning click) also suggested impaired gating in children with HFA. An independent t-test comparing T/C ratio scores showed that peak-to-peak P50 amplitudes in response to the conditioning and test clicks were significantly more similar (T/C ratio closer to 1) in children with HFA than in TD children, t(44) = 3.60, p = .001. Contrary to some previous literature, these results show that children with HFA have impaired sensory gating. As such, these findings hold noteworthy implications for the
identification of a specific neural biomarker that could differentiate TD children from children with HFA and help to establish effective treatments and therapies. **Keywords:** EEG, Development, Autism

**30) Developmental trends of performance errors and trial-to-trial variations measured by electroencephalography (EEG)**

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**Background:** Response monitoring refers to an individual’s ability to monitor ongoing behaviors and detect errors. In electroencephalography recordings, performance errors are indicated by an error-related negativity (ERN), a large negative deflection appearing after committing an error. Trial-to-trial variability in ERN latency, namely latency jitter, has been shown to be greater in children than adults, and is a confounding factor when interpreting the developmental trends of the ERN. Our research team has successfully implemented the Woody filter, a signal processing technique, to adjust for latency jitter in ERN and believe that it elucidates the true nature of the ERN from a developmental perspective. However, no studies to date have specifically determined whether the Woody filter enhances signal synchronization and intensity of ERN and how it alters developmental trends of these measurements. **Purpose:** Purposes of this study are to: (1) investigate whether the Woody filter increases signal synchronization and intensity, and (2) examine the developmental trends of signal synchronization and intensity of the ERN before and after applying the Woody filter. **Methods:** ERN data were collected from 240 participants (212 children and adolescents, 7 to 18 years of age, and 28 adults, 19 through 25 years of age) while they performed a two-choice flanker task. Data were processed using the Woody filter, which adjusted for the latency jitter of the ERN. The data before and after the Woody filter adjustment were analyzed using time-frequency analysis, which decomposes ERN signal into (1) phase-locking factor, indicating signal synchronization within the theta band and (2) evoked power, indicating signal intensity. **Results:** Two separate one-way repeated-measure ANOVAs with age as a covariate and pre/post filtering as a within-subject factor showed that regardless of age, the Woody filter significantly enhanced phase-locking factor, $F(1,238)=469.91$, $p< .001$, $η^2 = .66$, and evoked power of ERN, $F(1,238)=439.86$, $p< .001$, $η^2 = .65$. Polynomial regression analysis showed that age significantly predicted phase-locking factor as a quadratic trend before implementing the Woody filter, $F(3,236)=32.92$, $p< .001$, adjusted $R^2 = .21$, but not after. Conversely, age did not significantly predict evoked power of ERN before implementing the Woody filter as a quadratic trend but did after, $F(3,236)=34.68$, $p< .001$, adjusted $R^2 = .31$. **Discussion:** The Woody filter effectively increased signal synchronization of ERN, which validated its effect on adjusting the ERN latency jitter. Before the Woody filter, signal synchronization increased with age, supporting the finding that children demonstrated greater latency jitter in the ERN, thus, justifying the need for Woody filter adjustments. Moreover, the Woody filter adjusted such variations in children making the ERN amplitude similar between children and adults. Evoked power decreased with age after implementing the Woody filter but not before, suggesting that at the same level of signal synchronization, brains of adults are still more efficient when performing this task compared to children. **Implications:** The Woody filter is an effective technique for adjusting the signal synchronization and intensity of ERN. Researchers can apply this technique to more accurately examine the developmental changes of the ERN. **Keywords:** Development, event-related potential (ERP), response monitoring

**31) Cellular prion protein promotes axon targeting during adult neurogenesis**

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The cellular prion protein (PrPC) has been associated with varied biological processes including cell signaling and survival, yet its physiological function(s) remain ambiguous. The goal of this study is to determine the role of PrPC in axon targeting during adult neurogenesis using the murine olfactory system model. Olfactory sensory neurons (OSNs) within the olfactory sensory epithelium (OSE) undergo continual neurogenesis, integration, and turnover throughout adulthood, making it a useful model to study neuronal development. Axons from OSNs that express a given odorant receptor migrate to the olfactory bulb and coalesce onto a specific subset of glomeruli where they must make functional synapses within the existing circuitry. Here we determine the effect of PrPC level on OSN axon targeting during homeostasis and in two injury models: acute injury and prion-induced neurodegeneration. To investigate the role of PrPC in OSN axon targeting, glomerular targeting was quantified in transgenic mice expressing Tau-LacZ under the control of the P2 (Olfr17) odorant receptor promoter. Targeting of P2 OSN axons to glomeruli was quantified in wildtype (WT), PrP knockout (KO), and PrP overexpressing (OE) animals under normal
ABSTRACTS

32) Exercise restricted to early critical developmental periods can produce long lasting protection against the debilitating behavioral consequences of stressor exposure
Nicole L. Rumian1, Aggie Mika1,2, Kristina Hulen1, Donald Borchert1, Monika Fleshner1,2 From the 1Integrative Physiology and 2Center for Neuroscience, University of Colorado Boulder

Voluntary physical exercise can prevent stress-related psychiatric disorders, such as anxiety and depression. Using a rodent model, we have shown that male, Fischer F344 rats given access to voluntary running wheels for 6 weeks display less anxiety and depressive like behaviors (exaggerated freezing and shuttle box escape deficits) after exposure to acute inescapable stress (IS) as compared to sedentary controls. We have further demonstrated that adult rats require 6 weeks of running wheel access in order to achieve these protective behavioral effects, as well as the accompanying adaptive changes in brain circuits, whereas 3 weeks of exercise in adult rats is insufficient. Furthermore, when 6 weeks of exercise is initiated in adulthood, the stress protective effects of exercise are transient and quickly dissipate after exercise cessation. Thus, adults need 6 weeks of exercise to initiate stress protection, but they also need to continue exercising in order to remain protected. Intriguingly, 6 weeks of exercise initiated during the juvenile period, postnatal day 24 (PND24) produces persistent protection against the behavioral consequences of stress, indicating that if an organism begins exercising early in development, the stress protective adaptations can persist throughout life despite cessation of exercise. The ability of exercise in early life to produce long lasting changes likely has to do with the highly plastic nature of the brain throughout sensitive developmental periods. Because rodent development is accelerated in comparison to human development, 6 weeks of exercise initiated during the juvenile period spans through multiple discrete developmental stages, including the juvenile (approx. PND24 to puberty onset around PND 45) and adolescent (approx. PND45 to PND65) periods, as well as some early adulthood. During the juvenile period, the brain is highly plastic, exhibits an overabundance of plasticity related proteins and growth factors, and is characterized by rapid growth and an overproduction of synapses. Adolescence, the beginning of which is characterized by the onset of puberty, is a distinct period of neurobiological maturation; during this time, brain circuits underlying higher order functions, such as emotional regulation, undergo reorganization and pruning into their final adult state. Furthermore, an increase in gonadal steroids occurs at this time, signifying puberty. Because the juvenile and adolescent periods are characterized by such discrete neurobiological changes, it is possible that exercise restricted to either the juvenile or adolescent period could be sufficient for producing the long lasting stress protective behavioral effects. To investigate which of these specific developmental periods is the critical period for exercise to produce long lasting stress protection, we used a shorter exercise timeframe (3 weeks) in order to restrict exercise to the juvenile (PND 24-PND45) or adolescent (PND 42-PND66) periods. Male, Fischer F344 rats were allowed access to a running wheel or remained sedentary for 3 weeks. After 3 weeks, the running wheels were locked and all rats remained sedentary for 15 days, in order examine long lasting stress protection. The rats were then exposed to IS and anxiety and depressive like behaviors were assessed 24h later. Preliminary analyses show that exercise restricted to the juvenile period may be sufficient in producing long lasting exercise induced stress protection. Keywords: early life, exercise, stress protection, long lasting

conditions and after injury. During homeostasis, WT P2 OSN axons target, on average, 5.75 glomeruli per olfactory bulb. Total glomeruli targeted by P2 OSNs lacking PrP was higher than WT, and significantly lower in OE as compared to WT. These data indicate that PrPC plays a role in appropriate growth cone targeting of OSNs. Acute nasotoxic injury was induced by methimazole injection into WT and KO mice and results in synchronized OSN regeneration. Previously published work demonstrated that immediately after injury P2 OSN axons project to many glomeruli but the targeting to original glomeruli is refined by 8 weeks post-methimazole treatment. In this study, after a recovery period up to 20 weeks post-methimazole treatment OSNs axons did not refine targeting to original glomeruli. In addition, there was no difference in glomerular refinement between WT and KO P2 OSN axons. In the second injury model of prion infection, there was a decrease in OSN survival combined with an increase in glomerular targeting by P2 OSN axons as compared to mock. These findings suggest a loss of mature OSNs during prion infection either by premature death or a deficiency in OSN maturation that results in more immature neurons projecting axons incorrectly to the olfactory bulb. Keywords: Neurogenesis, homeostasis, olfactory injury, prion infection, axon targeting

32) Exercise restricted to early critical developmental periods can produce long lasting protection against the debilitating behavioral consequences of stressor exposure
Nicole L. Rumian1, Aggie Mika1,2, Kristina Hulen1, Donald Borchert1, Monika Fleshner1,2 From the 1Integrative Physiology and 2Center for Neuroscience, University of Colorado Boulder

Voluntary physical exercise can prevent stress-related psychiatric disorders, such as anxiety and depression. Using a rodent model, we have shown that male, Fischer F344 rats given access to voluntary running wheels for 6 weeks display less anxiety and depressive like behaviors (exaggerated freezing and shuttle box escape deficits) after exposure to acute inescapable stress (IS) as compared to sedentary controls. We have further demonstrated that adult rats require 6 weeks of running wheel access in order to achieve these protective behavioral effects, as well as the accompanying adaptive changes in brain circuits, whereas 3 weeks of exercise in adult rats is insufficient. Furthermore, when 6 weeks of exercise is initiated in adulthood, the stress protective effects of exercise are transient and quickly dissipate after exercise cessation. Thus, adults need 6 weeks of exercise to initiate stress protection, but they also need to continue exercising in order to remain protected. Intriguingly, 6 weeks of exercise initiated during the juvenile period, postnatal day 24 (PND24) produces persistent protection against the behavioral consequences of stress, indicating that if an organism begins exercising early in development, the stress protective adaptations can persist throughout life despite cessation of exercise. The ability of exercise in early life to produce long lasting changes likely has to do with the highly plastic nature of the brain throughout sensitive developmental periods. Because rodent development is accelerated in comparison to human development, 6 weeks of exercise initiated during the juvenile period spans through multiple discrete developmental stages, including the juvenile (approx. PND24 to puberty onset around PND 45) and adolescent (approx. PND45 to PND65) periods, as well as some early adulthood. During the juvenile period, the brain is highly plastic, exhibits an overabundance of plasticity related proteins and growth factors, and is characterized by rapid growth and an overproduction of synapses. Adolescence, the beginning of which is characterized by the onset of puberty, is a distinct period of neurobiological maturation; during this time, brain circuits underlying higher order functions, such as emotional regulation, undergo reorganization and pruning into their final adult state. Furthermore, an increase in gonadal steroids occurs at this time, signifying puberty. Because the juvenile and adolescent periods are characterized by such discrete neurobiological changes, it is possible that exercise restricted to either the juvenile or adolescent period could be sufficient for producing the long lasting stress protective behavioral effects. To investigate which of these specific developmental periods is the critical period for exercise to produce long lasting stress protection, we used a shorter exercise timeframe (3 weeks) in order to restrict exercise to the juvenile (PND 24-PND45) or adolescent (PND 42-PND66) periods. Male, Fischer F344 rats were allowed access to a running wheel or remained sedentary for 3 weeks. After 3 weeks, the running wheels were locked and all rats remained sedentary for 15 days, in order examine long lasting stress protection. The rats were then exposed to IS and anxiety and depressive like behaviors were assessed 24h later. Preliminary analyses show that exercise restricted to the juvenile period may be sufficient in producing long lasting exercise induced stress protection. Keywords: early life, exercise, stress protection, long lasting
33) Prenatal Origins of Obesity Risk
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OBJECTIVE: Obesity affects nearly 18% of children and adolescents in the United States. Increasing evidence indicates that prenatal maternal stress signals influence fetal growth, child obesity, and metabolic risk. Children exhibiting catch-up growth, a rapid and dramatic increase in body size within the first two years of life, are at risk for developing metabolic disorders and obesity. We characterize trajectories of the stress hormone cortisol across pregnancy and assess the role of cortisol trajectory in prenatal programming of early changes in body size and obesity risk. METHOD: Healthy term-born individuals (n=189; 86 girls, 103 boys) and their mothers were followed from early gestation through 24 months of age. Plasma levels of the stress hormone cortisol were measured at 15, 19, 25, 30, and 37 gestational weeks. Child height and weight were collected at birth, 3, 6, 12 and 24 months and used to calculate standardized body mass indexed (BMI). Maternal cortisol trajectories across gestation were characterized using general growth mixture modeling (GGMM). Associations between prenatal cortisol trajectories and postnatal changes in child standardized BMI were evaluated using hierarchical linear modeling. RESULTS: Three distinct cortisol trajectories across pregnancy were identified: Typical (n=151), Flat (n=14), and Steep (n=24). Women in the flat group exhibited an atypical plasma cortisol profile characterized by chronically elevated cortisol across pregnancy. Infants of these mothers showed an exaggerated increase in standardized BMI from birth through six months, a profile resembling catch-up growth, even after accounting for prenatal and postnatal covariates such as gestational age at birth and infant feeding factors. At 3 months old, these infants were significantly larger than infants exposed to the Typical and Steep cortisol trajectories. At 6 months old, infants exposed to the Flat cortisol trajectory remained larger than infants exposed to the Typical cortisol trajectory. CONCLUSIONS: These findings provide evidence that dysregulated patterns of prenatal maternal cortisol predict growth patterns in infancy, independent of postnatal factors. Our findings also suggest that maternal cortisol exposure during fetal development is involved in prenatal programming of obesity risk. Keywords: Stress, prenatal, HPA, infancy

34) Perinatal Maternal Depressive Symptoms and Infant Stress Response
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Objective: Using a prospective longitudinal design we evaluate the influence of maternal depressive symptoms during pregnancy and postpartum on infant hypothalamic-pituitary-adrenal axis (HPA) regulation. Background: Maternal depression is one of the most common prenatal complications affecting 13%-40% of pregnant women. The extraordinary pace of fetal development renders it particularly susceptible to maternal signals. Existing research, however, has focused primarily on the effects of postpartum depression. Methods: The current study followed 68 mothers and their full term infants between 24 and 34 gestational weeks and at 3 and 6 months postpartum. Maternal depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS) during pregnancy (M=6.5, SD=4.7, range: 0-22) and at 3 months postpartum (M=4.86, SD=3.81, range: 0-16). At 6 months postpartum, the infant cortisol response to the still-face procedure was assessed. Infant salivary cortisol samples were collected at baseline, and at 15 minutes and 30 minutes after the challenge. Results: A repeated-measures ANOVA revealed that infant cortisol increased in response to the still-face procedure, p<0.001. Elevated maternal prenatal and postpartum depressive symptoms were associated with a larger infant cortisol response to the still face procedure (p’s<.05). Both pre- and postnatal maternal depressive symptoms jointly contributed to infant cortisol stress response (p<0.05). Conclusions: The relation between infant HPA-axis functioning and maternal depressive symptoms suggests that cumulative exposure to maternal depression across the pre- and postnatal period influences offspring stress regulation. These findings highlight key areas for future research and emphasize the importance of early-targeted intervention for maternal depression to improve developmental health outcomes in children. Keywords: prenatal depression, postnatal depression, HPA axis, stress, infancy
35) Pharmacologic and genetic blockade of NF-κB is neuroprotective in a mouse model of Parkinson’s Disease
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Parkinson’s disease (PD) is the second most common neurodegenerative disease in the US and is characterized by clinical motor symptoms such as bradykinesia, rigidity, postural instability, and resting tremors. PD specifically targets dopamine-producing (DA) neurons in the basal ganglia. Because motor symptoms present only after a significant portion of DA neurons are lost, by the time of diagnosis, the disease is already quite advanced. The motor dysfunction in the early stages of PD is well controlled by symptomatic therapies but as the disease progresses neuronal cell death is magnified by the activation of microglia and astrocytes through the production of neurotoxic inflammatory mediators. These genes such as TNFa, IL-1B and inducible nitric oxide synthase (iNOS/NOS2), are coordinately regulated in glia by the transcription factor, NF-κB. The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) activates NF-κB and progressively degrades DA neurons in the basal ganglia. We tested the efficacy of genetically and pharmacologically inhibiting NF-κB in protecting dopaminergic neurons in the MPTP model of PD using innovative 3D-stereological counting methods to determine the number of neurons in the substantia nigra pars compacta (SNpc). 1,1-bis(3”-indolyl)-1-(p-chlorophenyl)methane (C-DIM12), a small molecule ligand of the nuclear receptor NR4A2 that suppresses NF-κB, displayed DA neuronal preservation. Transgenic mice with astrocyte-specific gene deletion of IKK2, the upstream activator of NF-κB, protected against MPTP-induced loss of dopaminergic neurons in the SNpc, as well as loss of dopamine terminals in the striatum and the dopamine transport proteins DAT and VMAT2. This data demonstrates that the inflammatory pathway NF-κB mediates neuronal degradation relevant to the progression of PD and that interdicting this pathway in glia may offer neuroprotective benefit.

36) Generation of Canine Neural Progenitor Cells from Induced Pluripotent Stem Cells
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New advances in stem cell technology, including the use of induced pluripotent stem cells (iPSC) to produce neurons and glial cells, offers new hope for patients with neurological disease and injuries. Pet dogs with spinal cord injuries offer an important spontaneous animal model for evaluating new approaches to stem cell therapy in a realistic clinical setting. Therefore, we conducted studies to identify optimal conditions for generating neural progenitor cells (NPC) from canine induced pluripotent stem cells (iPSC), in preparation for clinical trials in dogs with chronic spinal cord injuries. iPSC were generated from canine dermal fibroblasts and characterized based on phenotype, gene expression, embryoid body growth, and teratoma formation. Canine iPSC were differentiated into NPC by culture in non-adherent conditions in serum free medium supplemented with growth factors bFGF and EGF. The canine neural spheres express neural progenitor markers nestin, tubulin and vimentin, low expression of neuronal differentiation markers MAP2a and GFAP, and also express pluripotency markers Oct3/4 and Nanog. Canine iPSC could be readily induced to differentiate into neural progenitor spheres (NPS) and maintained this phenotype over several passages. The resulting NPS did not form teratomas in NOD/SCID mice. NPS could be induced to terminally differentiated neural cells, including glial cells and mature neurons, thus confirming their neural progenitor properties. Thus, we confirm that canine iPSC can be readily induced to form NPS by altering cell culture conditions, cell substrate, and by the addition of specific growth factors. These studies provide evidence that iPSC technology can be used to generate NPC in sufficient numbers and purity for use in neural regeneration studies in dogs with spinal injuries.

Keywords: Induced pluripotent stem cells, spinal cord injury
37) Increased mortality of Huntington’s disease mice with Toxoplasma gondii infection: a possible role of elevated Indoleamine-2,3-dioxygenase

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Huntington’s disease (HD) is a progressive neurodegenerative disease caused by a glutamine repeat expansion in the huntingtin gene. Upregulation of the kynurenine pathway of tryptophan metabolism is a feature of HD and is thought to drive disease progression. Indoleamine-2,3-dioxygenase (IDO) catalyzes the oxidation of tryptophan to kynurenine, the first and rate-limiting step in this pathway. Toxoplasma gondii (T. gondii), a common CNS pathogen, requires IDO for immune control of infection thus providing a potential biochemical synergy between a neuroinvasive pathogen and neurodegenerative disease. We hypothesize that an interaction exists between these two diseases via an IDO dependent mechanism. We demonstrate that T. gondii infection results in significantly earlier death of HD mice compared with wild-type infected and non-infected HD mice. We also measured increased cerebral cortical IDO activity in infected HD mice compared to infected WT animals. Overall, our findings demonstrate a novel interaction between murine HD and T. gondii, a prevalent, neuroinvasive protozoan. Our data is highly relevant to understanding human HD and how neuroinflammation and neurotropic pathogens may act as an environmental influence on progression of neurodegenerative diseases. **Keywords:** Neuroinflammation, Huntington's disease, Neurological infection, HPLC-MS/MS

38) Creation of a mutant cofilin conditional knock-in mouse to examine the role of cofilin-actin rods in neurodegenerative disorders

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Bundles of cofilin-actin filaments (rods) form in neurons in response to many stresses, such as ischemic injury and exposure to soluble forms of amyloid beta. Rods are found in Alzheimer disease (AD) brain in humans and in AD mouse models. Rods can disrupt neuronal function, and may represent a common mechanism leading to synapse dysfunction in neurodegenerative disorders. To directly test hypotheses about the role of rods in disease, an experimental system in which rod formation can be prevented is required. The goals of this study are to identify a non-rod-forming cofilin and express it in place of wild type cofilin to make a “rod-less” transgenic mouse. A mutation (K22Q) to cofilin’s non-actin-binding interface was identified which resulted in profoundly impaired rod formation while maintaining sufficient cofilin activity to rescue normal cellular functions. Knockdown and rescue experiments demonstrated that cofilin K22Q could replace WT cofilin in rescuing F-actin aggregation, membrane blebbing, and Golgi fragmentation that occurs with cofilin siRNA treatment of HeLa cells. K22Q also performed as well as WT in an assay of neurite outgrowth in cultured neurons. Despite this, concerns lingered about the mutant’s ability to fully replace WT in all developmental stages, so a strategy for conditional knock-in was designed. An inverted duplication of exon 2 (E2) was flanked by the FLEX switch, so cre expression results in an irreversible inversion, switching gene expression from WT to K22Q. This will allow spatial and temporal control of K22Q expression, dependent on cre. But due to the inability of E. coli to replicate DNA with large hairpins, the targeting plasmid could not be assembled by standard cloning methods. To circumvent this problem, a K22Q E2 was created with extensive mismatches to disrupt hairpin formation, allowing successful creation of the E2 inversion. Cre-induced flipping from WT to K22Q was confirmed at the DNA and protein level in cultured cells. Using this targeting plasmid, 384 clones of C57Bl6/129 hybrid ES cells were generated. These were screened for correct integration of the transgene, and two clones were identified. Removal of the neomycin-resistance cassette was successful in only one clone, which was injected into C57/Bl6 blastocysts. In 12 attempted implantations, only 12 pups were born, and of these only one was chimeric. This male was bred, and germline transmission was obtained (4 out of 10 pups transgene positive). Planned experiments include using primary neurons and brain slices to characterize the rod response and synapse function before and after cre expression. This mouse line represents a unique opportunity to directly test the roles of cofilin-actin rods in synapse dysfunction in neurodegenerative disorders.

39) Scavenging of reactive aldehydes prevents cognitive dysfunction associated with epileptogenesis

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Cognitive dysfunction is a major comorbidity of the epilepsies. However, treatments targeting seizure-associated cognitive dysfunction, particularly deficits in learning and memory are not available. Oxidative stress has been...
40) Nurr1 activation prevents neurotoxic injury in the MPTP model of Parkinson’s Disease

**Sean Hammond**, Katriana Popichak, Pranav Damale, Evan Richman, Lindsay Hunt, Stephen Safe, and Ronald Tjalkens. From the: 1Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO, 2Center for Environmental Medicine, Colorado State University, Fort Collins, CO, 3Institute for Biosciences and Technology, Texas A&M University Health Sciences Center, Houston, TX.

Parkinson’s disease (PD) is characterized by the degeneration of dopaminergic neurons of the ventral midbrain, associated with inflammatory activation of glial cells. The orphan nuclear receptor Nurr1 (NR4A2) suppresses inflammatory gene expression in glial cells and also positively regulates genes associated with the production/release of dopamine (DA) in neurons. Despite these known functions of Nurr1, an endogenous ligand has yet to be discovered. We previously reported that the phytochemical-based compound, 1,1-bis(3′-indolyl)-1-(p-chlorophenyl) methane (C-DIM12), activates Nurr1 in neural cells, suppresses inflammatory gene expression in primary astrocytes and induces a dopaminergic phenotype in neuronal cultures. In current studies, an in vivo approach was undertaken to examine the capacity of C-DIM12 to protect against loss of dopaminergic neurons induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mice were treated (+/-) MPTP (20 mg/Kg) every four days and (+/-) C-DIM12 (25 mg/Kg) every day for 2-weeks. Motor function was monitored during the course of treatment by open field activity and gait analysis. Preliminary data from this study revealed a significant preservation of TH-positive neurons in the substantia nigra (SN), as determined by design-based (3D) stereological methods, as well as preservation of TH protein levels in the striatum, as determined by immunoblotting. C-DIM12 treatment also inhibited the mRNA expression of multiple neuroinflammatory genes in qPCR array studies. To further examine the cell-specific role of Nurr1 in glial cells and DA neurons in the SN, we propose to use adeno-associated viral (AAV) vectors to selectively overexpress Nurr1 in GFAP (+) astrocytes and DA (+) neurons following intracerebroventricular injections in neonates. Optimization of multiple AAV serotypes was validated by whole-brain image montaging and CLARITY tissue transmutation to efficiently infect the cell types of interest. These data suggest that Nurr1 is a direct regulator of dopaminergic function in glial cells as well as neurons and that this receptor may be a viable target for selected small molecule therapeutics. This work was supported by a grant from the Michael J. Fox Foundation (RBT) and by NIH-ES021656 (RBT). **Keywords:** Parkinson’s disease, neurodegeneration, neuroinflammation
41) The Return-to-Play (RTP) recovery time for female concussion sufferers at a military academy

INTRODUCTION Concussions have become a topic of popular interest as well as an important public health issue over the past decade. There is continued interest and debate regarding the potential differences in recovery from concussion in females and non-elite athletes. While women’s interest in sport and integration into the military have both steadily increased, the amount of research dedicated to female concussion and recovery time still lags behind that of men. In particular, the heavy focus on contact sports and the military, both heavily male endeavors, continues to influence the proportion of research addressing concussions in female athletes. Some studies have shown greater susceptibility to concussion in women or more severe immediate post-impact symptoms, but data on the full time course of recovery is lacking. Much of the concussion literature comes from populations of athletes with focus on elite athletes in collision sports. Less is understood regarding the recovery times and sequelae of mild traumatic brain injury in non-athletes. The purpose of this study was to compare the recovery rates for concussion in females versus males and intercollegiate and non-intercollegiate athletes cared for in a single concussion clinic. The primary outcome measures included: time until symptom free, time for neurocognitive testing to normalize, and time to return to sport or full duty. Per our clinic protocol, patients must be symptom-free, have a normal physical exam, and exhibit a normal computerized neurocognitive assessment (i.e. ImPACT), then complete a graduated return-to-play protocol as advocated by the Zurich consensus statement (2012). METHODS We performed a retrospective review of prospectively collected data from a dedicated concussion clinic at a service academy. The data collected included: mechanism of injury, history of prior concussions, demographic data (sex, age, intercollegiate status), time until symptom free, time to return-to-play, time until ImPACT normalized, and number of clinic visits. These data were analyzed using analysis of variance (ANOVA). For all comparisons alpha was set at p < .05. Subjects were organized into groups and data was analyzed twice based on male versus female as well as intercollegiate versus non-intercollegiate athlete status. Exclusion criteria included insufficient data, moderate or severe TBI, or those with post-concussion syndromes with symptoms lasting greater than three standard deviations above the mean.

RESULTS A total of 307 subjects were identified of which 245 met the inclusion criteria. One of these did not include intercollegiate athlete status and was excluded in the second analysis. SEX Women comprised 23% of the cadet population and accounted for 64 of 245 (26.1%) of these concussions. All concussions were regressed on full return-to-play time with sex entered as a between-subjects factor. Women took significantly more time (mean = 42.3 days ± 3.9 SEM) to complete return-to-play than men (mean = 28.4 days ± 1.8 SEM) p < .001. IC STATUS A second analysis was performed comparing intercollegiate athletes (N = 85) and non-intercollegiate athletes (N = 159). This comparison demonstrated a statistically significant (p = .029) decrease in mean return-to-play time for intercollegiate athletes (25.91 ± 2.26 SEM days) when compared to non-intercollegiate athletes (35.42 ± 2.55 SEM days).

DISCUSSIONS and CONCLUSION Other studies have shown sex-based differences in concussion susceptibility, but this study is the first to comprehensively track and compare male and female concussions and demonstrate a lengthier return-to-play for women. Further, our data show shorter return-to-play time for elite athletes when compared with non-elite athletes despite similar post-concussive care. Keywords: concussion, mTBI

42) Activation of the nuclear receptor NUR77 by a novel diindolylmethane analog suppresses inflammatory gene expression in primary astrocytes
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Inflammatory activation of glial cells promotes loss of dopaminergic neurons in Parkinson’s disease (PD). The transcription factor, Nuclear Factor-kappa B (NFkB), regulates the expression of multiple neuroinflammatory genes associated in activated glial cells including inducible nitric oxide synthase (iNos), Tnfα and Il1β. These observations suggest that inhibition of NFkB in glial cells could be a promising therapeutic target for the prevention of neuroinflammatory injury. Nuclear orphan receptors in the NR4A family, including NR4A1 (Nur77) and NR4A2 (Nurr1), can inhibit the inflammatory effects of NFkB but there are no approved drugs that target these receptors. We postulated that a novel ligand of Nur77, 1,1bis (3′indolyl)1(pmethoxyphenyl) methane (CDIM5), would suppress NFkB-dependent inflammatory gene expression in astrocytes induced by 1methyl4 phenyl1, 2, 3, 6-tetrahydropyridine (MPTP) and the inflammatory cytokines IFNγ and TNFα. CDIM5 increased expression of Nur77 mRNA and suppressed expression of neuroinflammatory genes. CDIM5 inhibited the expression of multiple NFkB-regulated inflammatory and apoptotic genes in qPCR array studies but did not prevent p65 translocation to the
nucleus, suggesting a nuclear specific mechanism of inhibition. For the first time, we show that MPTP shuttles Nur77 from the nucleus of astrocytes to the cytoplasm, while C-DIM5 keeps Nur77 sequestered in the nucleus. These data demonstrate that CDIM5 prevents the production of neurotoxic inflammatory mediators in glial cells through inhibition of NFκB at a nuclear level, suggesting that this series could be a useful modality in preventing neuroinflammation.

43) Extracellular matrix genes are involved in prion susceptibility
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Transmissible spongiform encephalopathies (TSEs), including Bovine Spongiform encephalopathy (BSE) in cattle, Chronic Wasting Disease (CWD) in Cervids, and Creutzfeldt-Jakob Disease (CJD) in humans, caused by prions. While clinical signs vary between hosts, all types of prion diseases are associated with a slow build up of a malformed configuration of the normal, cellular prion protein (PrPC), referred to as PrPSc. The disease is self-propagating in that PrPSc induces conformational conversion of PrPC to additional PrPSc. It is not known what other cellular factors, other then PrPC itself, allow this propagation to occur. To address this, we compared the transcriptomes using RNA sequencing (RNAseq) of cloned cell lines that are either susceptible (S) or resistant (R) to prion infection to identify gene products that are differentially regulated. We find that the expression of two genes involved in extracellular matrix (ECM) integrity, fibronectin (Fn1) and integrin alpha-5 (Itga-5) are differentially regulated in S and R cells. Our results are in accordance with recent studies in mouse neuroblastoma cells (Marbiah et al., 2014). Both genes are down regulated in S compared to R cells. To validate the differential expression changes seen in RNAseq, Fn1 and Itga-5 expression levels were quantified using real-time PCR and western blots. We confirmed the differential expression profile of Fn1 and Itga-5. We also assessed if retinoic acid (RA) and the subsequent cellular differentiation would cause R cells to become S as seen in the Marbiah et al. study. Using the cervid prion cell assay (CPCA) we assessed prion propagation and found no change when comparing infected plus DMSO treatment to infected plus RA treatment. This finding indicates that, unlike mouse neuroblastoma cells, our R cells are not sensitive to RA treatment. This difference could be due to the different types of cells used in the experiments. As we detect changes in Fn1 in our S and R cells we will take these experiments a step further and use CRISPR/Cas9 genome editing to determine the effect of the removal of Fn1 in R and S cells. Marbiah, M. M., Harvey, A., West, B. T., Louzolo, A., Banerjee, P., Alden, J., (2014). The EMBO Journal, 33(14), 1527–1547. Keywords: prion, RNA sequencing, extracellular matrix

44) Using a novel ELISA to detect prions in infected cell cultures
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Transmissible spongiform encephalopathies (TSE) are fatal, transmissible neurodegenerative diseases caused by prions. Prions consist of a pathogenic form of the prion protein (PrPSc), which is produced by conformational conversion of its cellular counterpart (PrPC). There are only a few diagnostic tools at our disposal to determine prion pathogenicity. The purpose of this study is to develop tools to analyze prion propagation in cell by enzyme-linked immunosorbent assays (ELISA). Such a tool would be very useful in a laboratory or clinical setting. We performed an ELISA using two prion protein-specific antibodies, referred to as PRC5 and PRC7. The ELISA is referred to as the 7-5 ELISA. We used cell extracts derived from prion infected cells expressing mouse PrP, mutated variants thereof, as well as cells expressing deer and elk prion protein. The 7-5 ELISA was able to detect infection-specific responses from our cell models, which was more prevalent especially in our mutated mouse cells. This was determined to be a result of an accumulation of underglycosylated PrP during the prion infection, which was identified by the antibodies used in the ELISA. This method has been found to be useful in diagnosing prion infections and can be a reliable tool for prion drug screening. Keywords: prions, neurodegeneration, diagnostics

45) Adaptation of mouse prions in gene targeted mice
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Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy (TSE) of deer, elk, and moose. The disease causes weight loss and pronounced behavioral changes in infected animals and inevitably leads to death. As with other TSEs, the infectious agent is the pathogenic misfolded isoform (PrPSc) of the normal host cellular prion
Characterization of moose CWD in transgenic and gene-targeted mice expressing elk or deer prion proteins

James E. DiLisio, Jeffrey R. Christiansen, Julie A. Moreno, Seahun Kim, Glenn C. Telling. From the Prion Research Center (PRC) & the Department of Microbiology, Immunology & Pathology, Colorado State University, Fort Collins, CO 80525

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) found in North American cervid populations of deer, elk, and moose. With only two natural cases of moose CWD found and a single experimental inoculation of moose, the pathogenesis and strain capacity of CWD has yet to be characterized for this species. Transgenic (Tg) mouse models expressing high levels of deer and elk prion protein (PrP) have been previously characterized(referred to as TgE and TgD). However the use of gene targeted (Gt) mouse models expressing PrP at endogenous levels increase the biological relevance of this model allowing for more accurate characterization of CWD. Elk and deer PrP primary structures differ by a single amino acid residue at 226 that affects characteristics of CWD including incubation time, infectious prion protein (PrPSc) deposition patterns, and the misfolded protein’s relative conformational stability after denaturation. The mouse PrPC primary sequence is identical to that of CWD. In order to test this, we used Tg and Gt mice inoculated intracerebrally (ic) with infected brain homogenate from the Wyoming experimental moose CWD isolate and natural moose CWD isolates from Alberta. We also intraperitoneally (ip) inoculated Gt mice to assess peripheral transmission and to determine the influence of inoculation route on incubation time in Gt mice elk PrP (GtE) and Gt mice expressing deer PrP (GtD). The incubation times of prions from the Wyoming and Alberta CWD-infected moose were shorter in TgE than in TgD. While the incubation times of both CWD preparations were shorter in GtD and GtE mice than in the corresponding Tg mice, both isolates produced incubation times that were more rapid in GtE than GtD. On second-passage the incubation time of moose CWD prions was reduced by ~50% in TgE, while incubation times remained unchanged in TgD. While the incubation time of GtE challenged ip with moose CWD prions was the same as ic inoculated GtE, remarkably there was an ~30% reduction in the time to disease onset in ip compared to ic inoculated GtD. These findings suggest that moose prions require adaptation to the elk PrPC template during primary passage, however in deer no adaption is necessary as the primary PrPC structure is identical to moose. The remarkable and unexpected response of GtD to different inoculation routes will be a focus of future studies. Keywords: prions, neurodegeneration
47) Monoacylglycerol lipase (MAGL) inhibition differentially alters phosphorylation of mTOR in medial prefrontal cortex neurons and astrocytes in adolescent rats

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The mammalian target of rapamycin (mTOR) is a protein kinase that has been identified in neurons and glial cells and has a role in plasticity through regulation of protein synthesis. Activation of mTOR through phosphorylation (pmTOR) can be mediated through numerous extracellular signals, but it is unknown what the endocannabinoid (eCB) system’s effect on mTOR phosphorylation might be. 2-arachidonoylglycerol (2AG) is one of the primary eCBs present in the brain, and is broken down largely by the enzyme MAGL. The novel compound MJN110 is a potent MAGL inhibitor capable of increasing central 2AG levels. We have observed that MJN110 dose dependently alters social behavior and neuronal activation in the medial prefrontal cortex (mPFC). Here, differing doses of MJN110 were administered systemically to adolescent male rats prior to a single social encounter. pmTOR expression was assessed in neuronal and glial cell types (based on cell morphology) using immunohistochemistry (IHC) in both amygdala and prefrontal cortex. The central (CeN) and basolateral (BLA) amygdalar regions as well as the prelimbic (PL) and infralimbic (IL) regions of the mPFC were analyzed. Similar patterns of pmTOR expression were observed in PL and IL. In vehicle treated rats only, a social encounter increased glial pmTOR expression. A high dose of MJN110 produced a robust decrease in glial pmTOR expression, while neuronal pmTOR expression increased approximately twofold in neurons at this dose. Double label fluorescent IHC revealed that pmTOR was expressed in astrocytes but not microglia. These results suggest that 2AG has opposite and dosedependent effects on mTOR phosphorylation in neurons and astrocytes. Keywords: Endocannabinoid, Glia, Prefrontal Cortex, Amygdala

48) Emerging Roles of Synaptotagmin: Modeling Neurogenic Disease in Drosophila

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Synaptotagmin, a synaptic vesicle protein, is widely known as the fast, synchronous Ca^{2+} sensor that mediates neurotransmitter release. It’s C2B Ca^{2+} binding domain has been extensively analyzed for its essential role in triggering synaptic vesicle fusion in many animal models. Due to its essential nature, many synaptotagmin mutations result in early lethality when expressed in the null background (sytnull/sytnull) in animal models. However, when expressed in a heterozygous background (sytWT/sytnull), some of these same mutations impair synaptic transmission but still support viability. Recently, whole-exome sequencing has identified mutations in synaptotagmin that are associated with human disease. In two families, multigenerational dominant deficits (sytmut/sytWT) have been linked to single adjacent point mutations in synaptotagmin’s C2B domain. These dominant mutations are located in a highly conserved sequence within the Ca^{2+} binding pocket. Patients with either mutation present with symptoms similar to Lambert-Eaton Myasthenic Syndrome (LEMS): including decreased compound muscle action potential amplitude accompanied by synaptic facilitation, as well as muscle wasting and weakness. With a view to identifying the molecular mechanisms underlying the human phenotype, we have generated a homologous point mutation in the C2B domain of Drosophila synaptotagmin. By expressing the mutant transgenic protein in a synaptotagmin heterozygous background (sytWT/sytnull;P[sytmut]/+), we obtained synaptotagmin expression approximately equivalent to that seen in homozygotes (sytWT/sytWT). Thus our expression system should approximate that seen in the human patients. Initial results indicate that we can successfully mimic several of the symptoms seen in the affected family.

49) Ca^{2+}-Dependent and –Independent Inhibition of GABA Release onto POMC Neurons by Inhibitory GPCRs

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GABA release onto proopiomelanocortin (POMC) neurons of the arcuate nucleus of the hypothalamus occurs through action potential (AP)-dependent synchronous release, as well as AP-independent spontaneous release. Both AP-dependent and –independent release are robustly inhibited by Ga\textsubscript{i/o}-coupled G-protein coupled receptors (GPCRs) located on GABAergic terminals presynaptic to POMC neurons, but it is currently unknown whether inhibition of both types of release occurs through a common mechanism. GPCR-mediated inhibition of GABA release onto POMC neurons by the mu opioid receptor (MOR) and GABAB receptor (GABABR) was examined under
various conditions that altered Ca\(^{2+}\) influx into presynaptic terminals. Both receptors maintained their ability to inhibit GABA release onto POMC neurons in the absence of external Ca\(^{2+}\), as well as in the presence of unregulated Ca\(^{2+}\) influx into presynaptic terminals. Thus, inhibition of AP-independent GABA release onto POMC neurons occurs through a Ca\(^{2+}\)-independent mechanism, possibly through direct actions at the release machinery. To examine the Ca\(^{2+}\)-dependence of MOR- and GABABR-mediated inhibition of AP-dependent GABA release, Ca\(^{2+}\) was replaced with Sr\(^{2+}\) in the external recording solution. Previous studies have shown that evoking transmitter release with Sr\(^{2+}\) can occlude GPCR-mediated inhibition of release that acts directly on the release machinery. Both MOR- and GABABR-mediated inhibition of evoked release was maintained in the presence of Sr\(^{2+}\), demonstrating that inhibition of evoked release by both the MOR and GABABR occurs through a Ca\(^{2+}\)-dependent mechanism. Further experiments performed in the presence of selective Ca\(^{2+}\) channel blockers demonstrated that evoked GABA release onto POMC neurons was dependent on Ca\(^{2+}\) influx through both P/Q- and N-type voltage dependent Ca\(^{2+}\) channels, and that influx through both type of channel was strongly inhibited by activation of the MOR or GABABR. Together, these data demonstrate that inhibition of AP-dependent and –independent GABA release onto POMC neurons by the MOR and GABABR do not occur through a common mechanism, and AP-dependent and –independent release are inhibited via a Ca\(^{2+}\)-dependent and –independent mechanism, respectively. **Keywords:** Electrophysiology, GPCR, Opioids, Synaptic Release, POMC

**50) New approach to assess the ratio of functional channels in the AIS**
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Voltage-gated sodium channels (Na\(_v\)) have been studied for more than 50 years, and although there is no doubt that Na\(_v\) channels concentrate to the axon initial segment (AIS), there is still a debate regarding what proportion of AIS localized channels are functional. Previous studies that tried to address this question had no means of quantitating the number of channels expressed on the surface, making it difficult to relate channel number to conductance. In our lab, we have developed novel epitope-tagged Na\(_v\)1.6 constructs suitable for live cell imaging (Na\(_v\)1.6-loopBAD) that should allow determination of the percentage of AIS channels that are conducting. Our approach combines the simultaneous imaging of transfected cultured hippocampal neurons via high sensitivity Total Internal Reflection Fluorescence (TIRF) microscopy with whole-cell patch clamp recordings pre- and post- physical axon ablation. Our preliminary results suggest that ~ 40% of the Na\(_v\)1.6 located in the AIS is non-functional, based on the amount of Na\(^+\) current lost when the axon ablation is performed, compared to the proportion of surface-labelled channels observed in the AIS versus soma. The current lost following axon removal wasn’t observed in control experiments where large dendrites were removed. In conclusion, our approach suggests that a significant percentage of the Na\(_v\)1.6 channels located at the AIS are non-conducting, raising the question of how Na\(_v\)1.6 channels located in the AIS are differentially regulated. **Keywords:** Na\(_v\) channels, axon initial segment, TIRF, voltage-clamp

**51) Inhibition of neuronal excitability by post-synaptic mu opioid receptors (MORs) overshadows pre-synaptic disinhibition through a GABAergic synapse**
Philip D. Fox, RL Pennock, ST Hentges. From the Department of Biomedical Sciences, Colorado State University.

Mu opioid receptors (MORs) are widely expressed in neurons of the arcuate nucleus of the hypothalamus (ARC) where they display differential properties whether they are found in axon terminals (pre-synaptic) or the somato-dendritic compartment (post-synaptic). Post-synaptic MORs in proopiomelanocortin (POMC) neurons in the ARC have a lower affinity for opioid agonists than pre-synaptic MORs in upstream neurons, and undergo acute desensitization (~50% in 5 min) which the pre-synaptic MORs resist. Furthermore, the majority of synaptic inputs to POMC neurons are GABAergic such that activation of pre-synaptic MORs could increase POMC excitability through inhibition of GABA release, while post-synaptic MOR activation directly inhibits POMC excitability. Here we used POMC neurons in acute hypothalamic slices expressing the genetically encoded calcium indicator GCAMP6F as a non-invasive reporter of POMC neuron excitability in response to a wide spectrum of MOR agonist concentrations. The action of MOR agonists on POMC neuron excitability was predominated by direct post-synaptic inhibition, even at lower concentrations which favor pre-synaptic inhibition of GABA release. Direct inhibition of ionotropic GABAergic neurotransmission by blocking GABAA receptors with bicuculline caused a modest increase (<50%) in POMC neuron excitability in only a sub-set of neurons (15-20%). Blockade of glutamatergic transmission had no effect on POMC excitability. In conclusion, the predominant effect of MOR agonists on POMC neurons is to silence somatic excitability even at sub-maximal concentrations. GABA inputs onto POMC neurons modulate excitability in
a subset of neurons while glutamatergic inputs are not explicitly required for baseline excitability. **Keywords:** Mu Opioid Receptor, POMC, Gcamp

### 52) Effects of dopaminergic D2 receptor activation on layer I and layer V evoked excitatory synaptic responses in mouse medial prefrontal cortex

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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 inhibitory and excitatory 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 (10Hz-50Hz) in layer V pyramidal neurons in mouse mPFC. 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 had no effect on non-NMDAR mediated EPSPs with layer V or layer I stimulation, while decreasing the amplitude of NMDAR mediated EPSP trains with both layer V and layer I stimulation. These results suggest that D2R activation acts by restricting synaptic plasticity at both layer V and layer I excitatory synapses, stabilizing existing connectivity patterns. Our previous studies demonstrate that D1R and D2R activation have similar effects on layer I excitatory synapses. However, with layer V stimulation, D1R activation enhanced both NMDA and non-NMDA EPSPs, which suggests that when D1R activation predominates, plasticity is promoted; and when D2R activation predominates, plasticity is suppressed. These data provide further insight into mechanisms of dopamine’s bidirectional modulation of executive functions. **Keywords:** NMDA, Working Memory, PFC, Dopamine

### NEUROENDOCRINE

### 53) Early life maternal care programs the neurosteroid/GABAergic system in female offspring: A rodent model of premenstrual dysphoric disorder

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Natural variations in rodent maternal behavior during early life influence affective behavior in adult offspring. Recipients of Low levels of maternal licking and grooming (LG) during the first week of life are more anxious than animals that have received High LG from their mothers. During proestrus, Low LG female offspring also show a greater peak of progesterone, a neurosteroid that exerts anxiolytic effects through the actions of its metabolite, allopregnanolone (THP), at extrasynaptic GABAAR within the limbic system. We hypothesized that the higher peak of progesterone demonstrated by Low LG offspring in proestrus would result in a higher peak of THP in plasma and the brain relative to High LG offspring, as well as an estrous cycle-dependent fluctuation in the THP-responsive GABAAR subunits α4 and δ within the dorsal hippocampus. In Experiment 1, plasma levels of progesterone and THP at proestrus and metestrus in Low and High LG offspring, and GABAAR subunit expression and THP levels were characterized within the dorsal hippocampus. In Experiment 2, levels were assessed at proestrus and metestrus following s.c. treatment with either finasteride (a 5α-reductase inhibitor) or vehicle. Results from Experiment 1 indicate that Low LG offspring have lower levels of THP within the dorsal hippocampus, and that, unlike High LG offspring, plasma and hippocampal THP levels are not correlated with GABAAR expression within this region. In Experiment 2, finasteride only affected plasma THP levels in High LG offspring. In addition, only placebo-treated High LG offspring showed an estrous cycle-dependent plasticity in α4 subunit expression. These data suggest an impaired metabolism of progesterone to THP in adult female recipients of Low LG, and a decrease in the ability of THP to alter GABAAR expression within the dorsal hippocampus of these animals. Our results parallel findings from studies of women with premenstrual dysphoric disorder, a condition which has been associated with an impaired metabolism of progesterone to THP, and the absence of a correlation between THP and GABA levels, suggesting a diminished influence of THP on the GABAergic system.
54) Gonadotropin Releasing Hormone Stimulates Histone Citrullination to Mediate LHβ Expression in Gonadotrope Cells

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Peptidylarginine deiminases (PADs) are a family of Ca2+ dependent enzymes that post-translationally convert positively charged arginine into neutral citrulline residues. Citrullination of arginine residues on histone tails results in chromatin decondensation and changes in gene expression. Previous studies indicate that PAD isoform expression is estrous cycle dependent in uterus and mammary glands suggesting hormones that drive reproduction can potentially regulate PAD expression and activity. Since gonadotropin releasing hormone (GnRH) signaling is critical for maintaining fertility, we were curious if PAD enzymes were involved in anterior pituitary gonadotrope gene regulation. Currently, PAD isoform expression and significance in gonadotropes is unknown. To address this, we first examined expression of the four PAD isoforms (PADs 1-4) in the gonadotrope derived LßT2 cell line. Strikingly, PAD2 mRNA levels were over 600 fold higher compared to the other PADs. To confirm our results in vivo, PAD2 expression was examined by immunohistochemistry in estrous cycle staged mouse pituitaries and showed robust expression during estrus. Next, we tested if the GnRH agonist Buseralin (GnRHa) regulates PAD2 protein expression. LßT2 cells treated with a time course of GnRHa revealed that PAD2 protein levels increased rapidly, showing highest expression at 30 minutes. Following the same experimental paradigm using immunofluorescent confocal microscopy, we observed punctate PAD2 staining in the nucleus of LßT2 cells following 30 minutes of GnRHa treatment. Given the upregulation and accumulation of PAD2 in the nucleus following GnRHa, we hypothesized that the functional consequence of this localization was to facilitate citrullination of histones. To test this, we found that LßT2 cells treated with GnRHa showed an increase in citrullination of histone H3 arginine residues 2, 8, and 17 in a time dependent manner. Consistent with PAD2 localization in the nucleus, GnRH induced histone citrullination was maximal at 30 minutes. In addition, pre-treatment of LßT2 cells with the PAD inhibitor biphenyl-benzimidazole-Cl-amidine (BB-ClA) significantly reduced histone citrullination. At issue is the identity of the genes regulated by PAD2 catalyzed histone citrullination. To identify these genes, LßT2 cells were pre-treated with BB-ClA followed by treatment with vehicle or GnRHa. RNA was purified and subjected to NextGen RNA sequencing. Bioinformatic analysis revealed an anticipated increase in LHβ gene expression following GnRHa treatment, but this increase was blunted in the presence of the PAD2 inhibitor. qPCR analysis of LHβ gene expression in LßT2 cells validated our RNA-seq findings. Taken together, our results suggest that GnRH stimulates PAD2 expression and translocation to the nucleus where it citrullinates histones to alter LHβ gene expression in gonadotropes. Keywords: cell biology, epigenetics

55) Characterization of the gonadotropin-releasing hormone receptor in Aplysia californica

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Gonadotropin-releasing hormone (GnRH) is the most upstream neuroendocrine activator of reproduction in vertebrates. A GnRH-like molecule was previously identified in the mollusk, Aplysia californica, and interestingly, this GnRH (ap-GnRH) does not appear to have a reproductive role. In an earlier study, we cloned the full-length cDNA of a Type II putative ap-GnRH receptor (ap-GnRHR). This receptor contains two potential translation start sites, each accompanied by a Kozak sequence, suggesting the translation of both a long and a short form of the receptor is possible. The putative ap-GnRHR maintains the conserved structural features and motifs of other known Type II GnRH receptors and shares high sequence identity with the octopus GnRHR. The expression of both long and short forms of the putative ap-GnRHR is confined to the central nervous system. The goal of this study is to examine, through a series of functional characterizations, if these two receptor isoforms are authentic ap-GnRHR. The cDNA encoding the long or the short receptor was subcloned into the pAWG vector and transfected into a protostomian cell line, the Drosophila S2 line. Transfected cells were subject to a radioreceptor assay using 125I-labeled ap-GnRH as a radioligand. Further, they were treated with various concentrations of ap-GnRH or a related peptide, Aplysia adipokinetic hormone (ap-AKH), and measured for the accumulation of cAMP and inositol phosphate (IP). Radioreceptor assay revealed that only the long form of the receptor selectively bound to the radioligand, with cold ap-GnRH displacing the bound radioligand at EC50 of 3.54 x 10-8 M. Cells transfected with either form of the receptor did not respond to ap-GnRH or ap-AKH treatment with cAMP accumulation. However, cells transfected
ABSTRACTS

56) Structural and functional disruptions of the suprachiasmatic nucleus in fibroblast growth factor-deficient mice
Annie V. Miller, Scott I. Kavanaugh, Pei-San Tsai. From the Department of Integrative Physiology, University of Colorado Boulder

Fibroblast growth factor (Fgf) 8 and its cognate receptor, Fgfr1, are essential for the development of multiple brain regions. Previous studies from our laboratory showed that reduced Fgf8 signaling led to the malformation of neuroendocrine nuclei that originated within the diencephalon, including the oxytocin system in both the paraventricular (PVN) and supraoptic (SON) nuclei. To further understand the role of Fgf8 in the development of other hypothalamic nuclei, we examined if Fgf8 and Fgfr1 deficiencies also impact the integrity of the suprachiasmatic nuclei (SCN). The SCN are principal regulators of the organism’s circadian rhythm and consist of neurons that produce vasoactive intestinal peptide (VIP) as the main input neurons. The objectives of this study are (1) to examine the number of VIP neurons in the SCN of postnatal day (PN) 0 mice hypomorphic for Fgf8, Fgfr1, or both, and (2) to quantify SCN neuronal activation by cFos immunostaining in adult mice deficient in Fgf8 alone or Fgf8 combined with Fgfr1. Brains were fixed in 4% paraformaldehyde, sectioned in a cryostat, and processed for VIP and cFos immunohistochemistry. The numbers of VIP- and cFos-immunoreactive (ir) neurons were then quantified in the SCN. In general, neonatal mice harboring homozygous deficiencies in Fgf8, Fgfr1, or both combined exhibited the most severe malformation of SCN and very few SCN VIP-ir neurons. Neonatal mice harboring heterozygous deficiencies in Fgf8 alone or Fgf8 combined with Fgfr1 (called DH mice) showed less severe, albeit still significant, reductions in VIP-ir neurons. To determine if these seemingly less severe changes could still disrupt SCN function, adult wildtype, Fgf8 heterozygous and DH mice were examined for SCN cFos activation at three time points: 1 (morning), 6 (afternoon), and 11 (evening) hours after light onset. Although the SCN of WT mice stayed consistently activated at all three time points, a significant change in cFos activation was observed in Fgf8 heterozygous mice between morning and afternoon time points. These data suggest an inherent defect in the morning activation of SCN in heterozygous Fgf8-deficient mice. Overall, our studies provide strong evidence that deficiencies in Fgf8 not only impact the structural integrity of the SCN, the former also impacts the function of the SCN by compromising neuronal activation immediately after the onset of light. Keywords: SCN, Fgf8, Fgfr1, development, cFos, VIP

57) Diurnal variations in rhythmic clock gene expression across brain regions important for emotional control of male and female rats
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The molecular clock consists of a counter-regulatory transcription/translation cycle of positive (Bmal1, clock/npas2) and negative (Per1/Per2, Cry1/Cry2) components, whose oscillatory nature consists of a 24-hour period. The molecular clock has been well-characterized in the body’s master clock, the hypothalamic suprachiasmatic nucleus (SCN). Surprisingly, a limited number of studies have examined clock genes of both the positive and negative components in extra-SCN tissue. Furthermore, there has yet to be a direct comparison of basal clock gene expression in extra-SCN brain regions between female and male rodents. This comparison is warranted, as there are sex differences in circadian rhythms, as well as a greater prevalence of mood disorders associated with disruptions in clock gene expression (e.g., depression, anxiety, post-traumatic stress disorder). This study examined in male and female rats basal clock gene mRNA expression (in situ hybridization) of both the positive (Bmal1) and negative (Per1, Per2) components of the molecular clock in brain regions important in emotional regulation (e.g., prefrontal cortex, hippocampus, amygdala), as well as the SCN and the hypothalamic paraventricular nucleus (PVN), the head of the HPA axis. Clock genes were examined at 4-h intervals across a 12:12h light:dark cycle. There was a significant rhythm of Bmal1, Per1, and Per2 mRNA in the SCN, PVN, PFC, subregions of the hippocampus, and the amygdala with a 24-h Period (two-way ANOVA, cosinor analysis, p < 0.05). Importantly, there were three distinct profiles of rhythmic clock gene mRNA acrophase relationship across the brain regions, suggesting diversity amongst molecular clocks. Furthermore, while the clock gene expression profiles were generally similar between males and females, there were a few instances where the robustness of clock gene expression differed between the sexes (e.g., females had less robust Per1 and Per2 mRNA rhythms in the medial PFC, but more robust Bmal1 mRNA rhythms in the CA1 and CA3

Keywords: Cotonotropin-releasing hormone, GnRH, Aplysia californica, Radioreceptor assay

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hippocampal subregions). There was also a general trend for females to be phase-delayed in nearly all brain regions for all clock genes compared to males. Additionally, females with a regular estrous cycle had altered Bmal1 mRNA rhythms in the PFC compared to females that were not cycling. These results indicate that oscillatory clock gene expression is widespread throughout forebrain regions involved in emotional control, and that gonadal hormones may modulate these expression patterns. **Keywords:** in situ hybridization, use of female subjects

58) Characterization of diurnal core clock gene expression in forebrain glucocorticoid receptor knockout mice brains

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Circadian rhythms are maintained through the self-regulatory, oscillatory molecular clock, which includes the core clock genes Per1, Per2, and Bmal1. Disruptions to clock gene expression have been associated with numerous mood and behavior disorders including major depression, anxiety disorders, and bipolar disorders. The molecular clock has been well-characterized in the suprachiasmatic nucleus (SCN), the master clock of the body. Many peripheral tissues and extra-SCN brain regions have also been shown to have circadian rhythms in these core clock genes, but considering that the SCN has few direct projections to these brain and body regions, the question remains how the SCN communicates to extra-SCN molecular clocks. Glucocorticoids (CORT) are a potential candidate by which the SCN signals to other brain and body regions as glucocorticoid receptors (GR) are found ubiquitously throughout the brain and body, with the notable exception of the SCN. Furthermore, CORT is released in a diurnal manner, with peak plasma levels occurring immediately upon the animal's active phase. Interestingly, there is a glucocorticoid response element (GRE) in the promoter region of the Per1 gene, which may be a mechanism by which CORT can induce Per1 mRNA expression, and thereby entrain the molecular clock. We compared clock gene expression in mice that had a conditional forebrain glucocorticoid receptor knockout (FBGRKO) to GR floxed mice to determine the necessity of GRs in diurnal core clock gene expression. FBGRKO (C57BL/6 pure strain of the T29-1 founder line containing Cre+ recombinase transgene) mice have been previously well-characterized to have disruptions in GR expression in the forebrain including the hippocampus, cortex, and nucleus accumbens, while the central nucleus of the amygdala (CEA) had a 50% deletion and the paraventricular nucleus (PVN) was not affected. Mice were sacrificed under basal conditions in the light phase (zeitgeber time (ZT) 1.5) or dark phase (ZT13). In situ hybridization was used to measure mRNA. Our results show there is a time of day difference for Per1, Per2, and Bmal1 clock gene mRNA in the SCN and for Per1 and Bmal1 mRNA in the PVN. The subregions of the prefrontal cortex (anterior cingulate, prelimbic, infralimbic, ventral orbital) and insula show a time of day difference only for Bmal1 mRNA. Only Bmal1 mRNA showed a time of day difference in the subregions of the hippocampus (CA3, supra dentate gyrus, infra dentate gyrus) and amygdala (central, basolateral, medial), while Per1 mRNA was significant only in medial amygdala, and Per2 mRNA only in CA3. CA1 of the hippocampus did not show time of day differences for any clock gene investigated. There were no genotype differences for all brain regions examined. These results are expected in the SCN and PVN, as hypothalamic GRs would not be affected by the FBGRKO. The lack of evident FBGRKO effect in the HPC, AMY, PFC, and insula may be due to the fact that the sacrifice times (1.5 and 13 hours after lights on) are not at the diurnal peaks and troughs of these clock genes and therefore there may not be enough temporal resolution to see slight shifts in the diurnal rhythmic expression. It is also possible that GRs are not necessary for diurnal clock gene expression in these tissues or that CORT modulates the diurnal rhythm of Per1 and Bmal1 in the forebrain regions through a series of non-GR mediated neuronal projections. **Keywords:** In situ hybridization, Cre-LoxP system, clock genes

59) N-methylserotonin from Japanese pepper (Zanthoxylum piperitum), soy isoflavones, black cohosh extract, and combinations thereof regulate skin temperature in a female rat model of menopause-related hot flash

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Oral administration of soy isoflavones or black cohosh extract for the relief of menopause symptoms have some clinical support, but the data are mixed. Soy isoflavones such as genistein can regulate menopause-related hot flashes, but the mechanisms for this activity are not completely understood. Similarly, black cohosh contains N-methylserotonin (NMS) as a minor component, and NMS has selective agonist activity at the 5-HT7 serotonin receptor subtype that is involved in thermoregulation. Soy isoflavones and black cohosh are commonly used as homeopathic approaches to menopause symptoms, but it is unknown whether they work together in additive or
synergistic fashions. We have performed three studies that sought to determine the effects of dietary NMS, or its combination with soy isoflavones and black cohosh, on induced hot flash and measures of mood in female rats. All three studies used ovariectomized (OVX) female rats that were fed diets containing different levels of NMS, soy isoflavone concentrate, or black cohosh extract. OVX animals given estradiol implants served as positive controls. Locomotor activity (open field), anxiety-like behaviors (elevated plus maze; EPM), and depression-like behaviors (forced swim test) were assessed first, and then skin temperature was monitored during experimental hot flashes that were induced with intravenous calcitonin gene-related peptide. None of the dietary manipulations affected OVX-induced weight gain, uterine growth, or mood-related behaviors, but estradiol implants increased the time spent in the center of the open field and on the open arm of the EPM. Furthermore, synthetic NMS, NMS contained in Japanese pepper (Zanthoxylum piperitum; a 25-fold more efficient source of NMS than black cohosh), black cohosh extract, soy isoflavones and estradiol implants all individually blunted the hot flash response. Dietary combinations of Japanese pepper with isoflavones, black cohosh, or isoflavones + black cohosh also reduced the hot flash response, but the pepper alone and the pepper + isoflavones combination were the most effective. These three separate studies provided replication and confirming results that a natural dietary source of NMS was equivalent to synthetic NMS in reducing the hot flash response, that soy isoflavones and black cohosh can have similar, but less robust, effects on hot flash, and that some benefits may be conferred by combinations of these natural products. These in vivo findings also support NMS as a component of black cohosh and Japanese pepper that may reduce menopause-related hot flashes while avoiding the potential side-effects of phytoestrogens or hormone replacement therapy.

60) Control of the hypothalamic pituitary adrenal axis following selective depletion of estrogen receptor beta
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The hypothalamic pituitary adrenal (HPA) axis coordinates responses to stress by recruiting brains regions that relay information regarding a potential threat and prepare the organism for an appropriate response. The main integratory node for the HPA axis is the paraventricular nucleus of the hypothalamus (PVN), where a dense population of estrogen receptor beta (ERβ)-containing neurons resides. Activation of this receptor has been shown to decrease anxiety and HPA axis reactivity. However, the mechanisms and brain regions mediating these outcomes are poorly understood. To determine the role of ERβ in the PVN, we used Cre-loxP technology to delete ERβ in Sim1-Cre-expressing neurons, which are also densely present in the PVN. This deletion, as confirmed by tissue-specific PCR, generated an ERβCKO transgenic mouse line, which displays sex- and stressor-dependent HPA axis reactivity. Two weeks after ovariectomy, female ERβCKO mice responded more pronouncedly to a 30-minute restraint stress (a moderate stressor) when compared to their wild type littermates, by showing higher corticosterone (CORT) levels. However, EPM (a mild processive stressor) had no such effect on CORT levels. Surprisingly, such a genotype effect on HPA reactivity in response to either of the stressors (EPM and restraint) was not observed in the gonadectomized ERβCKO male mice. Treatment with the ERβ selective agonist, R-DPN, decreased HPA axis reactivity in a sex-dependent manner. While R-DPN treatment decreased CORT values following exposure to both stressors in the females, only a robust stressor elicited such a response in males. Interestingly, deletion of ERβ in Sim1-Cre-expressing neurons had no significant effect on anxiety-related behaviors. These findings suggest that ERβ neurons of the PVN are recruited in a sex- and stressor-dependent manner to modulate HPA axis reactivity. These data indicate that males and female mice utilize PVN ERβ neurons in different fashions during a stress response, and that behavioral stressors are modified by extra-PVN ERβ. **Keywords:** Estrogen Receptor Beta, Hypothalamic-pituitary-adrenal axis, Stress Hormones, Cre-loxP technology

61) Colocalization of corticotropin-releasing hormone (crh) with crh transcriptional repressors in the hypothalamus of crh-ires-cre mutant mice
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Crh transcription in the hypothalamic paraventricular nucleus (PVN) has been highlighted as a key target of negative regulation of the hypothalamic-pituitary-adrenal axis (HPA). Yet, the underlying mechanism is not fully understood. Recent in vitro evidence suggests that a repressor complex consisting of glucocorticoid receptor (GR), methylated CpG binding protein 2 (MeCP2), and histone deacetylase 1 (HDAC1) is recruited to the crh promoter and is
associated with increased trimethylation of histone 3-lysine 9 (H3K9me3), a marker of gene repression. However, in vivo support for this observation is necessary to ascertain its physiological relevance. The lack of tools for adequately targeting all CRH neurons in vivo has previously hampered such investigations. Therefore, to facilitate identification of all PVN CRH neurons and provide in vivo support for the role of GR, MeCP2, and H3K9me3 in negative regulation of crh transcription, we utilized mice that express a tdTomato fluorophore in these neurons. These mice were generated by crossing a Crh-IRES-Cre driver line with an Ai14 tdTomato-reporter mouse line (Crh-IRES-Cre;Ai14). Coronal brain sections were immunostained for GR, MeCP2, and H3K9me3, and we determined the percent colocalization of CRH:tdtomato for each. We determined that nearly all (89.1%±3.0%) PVN CRH neurons express GR, over half (55.9%±6.7%) express MeCP2, and some (20.01%±1.5%) express H3K9me3 (all are mean percentages ± SEM) with no differences among rostral, middle and posterior parts of the PVN. Our results support the localization of GR, MeCP2 and H3K9me3 in CRH neurons as players in the negative regulation of CRH in vivo. Keywords: Corticotropin-releasing hormone, transcriptional repressors, hypothalamus, crh-IRES-cre

62) Exercise Counts the Changes in Nutrient Sensing Gene Expression after Caloric Restriction and the Loss of Ovarian Function in Obesity-prone Rats
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Background: Exercise counts the biological drive to regain weight after weight loss, both by reducing the drive to overfeed and by increasing expended energy beyond the cost of the exercise bout. We have hypothesized that exercise alters the nutrient sensing systems in the brain after weight reduction in a manner that would attenuate the drive to overfeed. In the present study, we examined the effect of exercise in female rats after the loss of ovarian function, when the drive to gain weight is known to be exacerbated. We specifically evaluated the gene expression of neural factors involved in glucose sensing and in the regulation of energy balance in the arcuate nucleus (ARC), ventral medial nucleus (VMN), supraoptic nucleus (SON) and amygdala (AMG). Method: Diet induced obese female rats were weight reduced (15% body weight) by restricting caloric intake, with (EX) or without (SED) a daily bout of treadmill exercise (60min/day, 6days/week, 15m/min). The rats were then surgically ovariectomized (OVX), and then allowed ad libitum access for 6 weeks. Food intake, weight gain, and body composition were monitored during this weight gain period. Ad libitum ovariectomized (AL) and sham-operated (SHAM) rats were included in the design as controls. At the time of sacrifice, punch biopsies of ARC, VMN, SON and AMG were obtained and assayed for gene expression (mRNA) by qPCR. Outcomes included mRNA levels of glucokinase (GK), a hallmark of glucose sensing neurons, glucose transporter 2 and 3 (GLUT2, GLUT3), insulin receptor (InsR), oxytocin (OT), OT receptor (OTR), leptin receptor (LepR), and pro-opiomelanocortin (POMC), a precursor polypeptide involved in appetite and metabolic regulation. Results: In the SON, both GLUT3 and InsR mRNAs were significantly lower in AL and SED rats compared to that in SHAM rats (approximately 40% and 60% lower, respectively), but these levels were restored in EX rats. In the AMG, exercise significantly increased OTR gene expression (about two fold). In the VMN, GK and GLUT3 gene expression decreased by approximately 25% and 30%, respectively in AL rats, and these further decreased in SED rats (~ 50% and 45% reduction, respectively), compared to that in SHAM rats. In both cases, regular exercise tended to restore these levels to that found in the SHAM rats. There was no significant change of GK, GLUT3, Glut2 or POMC mRNA in ARC. Conclusion: The loss of ovarian function altered the expression of critical factors involved in glucose sensing in the SON and VMN in a manner consistent with OVX-induced overfeeding. Weight reduction through calorie restriction exacerbated some of these effects. Regular exercise, however, countered these combined effects on nutrient sensing gene expression in the SON, VMN, and AMG, and may elicit appetite suppressing effects by enhancing the capacity for and response to nutrients in circulation. Keywords: brain punches

SENSORY AND MOTOR SYSTEMS

63) Non-canonical rubro-cerebellar afferents form both positive and negative feedback loops via diverse postsynaptic targets
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The interposed nucleus (IN) of the cerebellum targets the premotor midbrain structure the red nucleus (RN). Previous retrograde tracing studies suggest that the RN projects back to the cerebellum, preferentially targeting the IN over
the cerebellar cortex. The RN-to-IN afferents represent a break from canonical mossy fiber inputs, which mainly collateralize to innervate both the IN and overlying cortex. Furthermore, reciprocal innervation of the IN and RN indicated by retrograde tracing suggests a positive feedback loop exists between the two structures. To clarify the organization of this circuit, we injected viral-mediated anterograde tracers into transgenic mice to label specific neuronal populations. Following injections into the RN, we observed dense terminals in the IN of the cerebellum with very few mossy fibers in the cortex (n=9 mice). In stark contrast, injections into the pontine nuclei of wild type mice preferentially targeted the cerebellar cortex, producing relatively few boutons in the IN (n=5). Both overall bouton numbers and distributions were distinct between the two injection sites. RN injections labeled 3-fold more boutons to the IN than the pontine injections, but 15-fold fewer mossy fibers to the cortex. Indeed, the mossy fiber:IN bouton ratio was 23.5 for pontine injections and 0.34 for the RN injections, suggesting a glutamatergic phenotype. VGlut2-positive RN terminals formed close contacts with Ntr1-Cre expressing glutamatergic principal output cells (n=3), glycinergic inhibitory interneurons (n=6), and GAD1-Cre (n=4) expressing cells in the IN, indicating heterogeneous connectivity. These results support the view that RN-to-IN afferents preferentially target the cerebellar nuclei over the cerebellar cortex but argue against the view that the RN-to-IN projection solely forms a positive feedback loop, since inhibitory neurons are also targeted. These data support an emerging view that innervation of the cerebellar nuclei may be independent of the innervation of the cerebellar cortex (Luo and Sugihara, 2014) and could therefore reflect the anatomical substrate for a novel computation made by the cerebellum.

64) Adeno-associated Viral Transfection and Optogenetic Manipulation of Auditory Brainstem Regions

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Low-frequency sound localization relies upon the difference in the times at which sound reaches the two ears (interaural time difference; ITD). Depending on the location of the sound source along the azimuth, ITDs are very small for sounds originating from or near the midline, and increase up to several hundred microseconds for sounds originating directly from one of the two sides. Thus, ITDs systematically vary with location along the azimuth, and this cue is extracted and evaluated by the auditory system during the localization process. While the medial superior olive (MSO) is the nucleus performing the actual ITD analysis, both the medial and the lateral nucleus of the trapezoid body (MNTB and LNTB, respectively) project fast and well timed monaural glycinergic inhibition to MSO neurons. Several concepts have been put forward that explain how these inhibitory inputs contribute to ITD processing in mammals. Both the MSO but also the MNTB and LNTB are deep auditory brain stem nuclei, making them difficult targets for optogenetic manipulation. Additionally, the auditory brain stem is heavily myelinated, making it challenging to deliver light to neurons in these nuclei. We have successfully transfected MSO, MNTB and LNTB neurons with inhibitory light-sensitive adeno-associated viruses (both halorhodopsin and ArchT). Once the virus has been expressed in these brain areas, we have shown that we can reduce or eliminate sound-evoked multiunit neural activity with light. Until recently, experimental manipulations that allowed for fast and reversible activation and inactivation of MSO, MNTB, and LNTB, thus preventing direct experimental tests of the function of this circuit were not possible. Successfully transfection and manipulation of this circuit will be able to lead us forward into further investigating the processing of sound and ITDs in the auditory brainstem. Keywords: Optogenetics, auditory system, sound localization, inhibition

65) Effects of Visual Ambiguity on Audiovisual Sentence Perception

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The idea that seeing a speaker’s face increases comprehension is very intuitive, but we still have a poor understanding of the mechanisms responsible for this. The current study manipulates point-light facial display (PLFD) presence (absent, present) and speaker illumination (no light, low light, full light). Previous studies have only investigated the effect of full illumination and no illumination. Regis University undergraduate students participated for class credit or on a volunteer basis to watch 18 videos (6 conditions, 3 videos per condition), where comprehension was measured using speech reception thresholds in noise (SRTN). As expected, main effects of PLFD presence and speaker illumination were very significant (p = .004, p < .001, respectively). However, a decrease in comprehension was seen in the low-light condition when no PLFD was present (d = .22) but an increase was seen when a PLFD was present (d = .32). The difference between PLFD present and PLFD absent in low-light was very
significant (p = .005, d = .73). This raises questions about the effect of low-light conditions on cognitive load and visual attention. Overall, the current study confirms previous findings of the audiovisual gain in PLFDs and fully illuminated speakers, but raises questions about the effectiveness of partial speaker illumination. **Keywords:** audiovisual, speech, sentence, perception, point-light facial display

### 66) Putative pathways underlying female songbird mate choice: An anterograde tract tracing study
**Jeffery L Dunning, Ethan Atwood, Jonathan F. Prather.** From the Program in Neuroscience, University of Wyoming.

Females of many species use male courtship displays as a proxy of male fitness to inform decisions of mate choice. The process of female sexual selection has been studied extensively in songbirds, in which males produce songs for the purpose of attracting female mates. 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 playback of songs through a speaker, even when males are no physically present. It remains unknown, however, how the female brain uses song to influence copulatory behaviors. Studies of female responses to male 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 of the female CM in Bengalese finches (Lonchura striata) using an anterograde neural tracer to determine pathways through which CM may be able to exert its effects on brain regions associated with female copulatory behaviors. Our results demonstrate that CM projects to two regions within the arcopallium, the robust nucleus of the arcopallium (RA) and a region we believe to be the ventral intermediate arcopallium (AIV). These projections may provide CM the ability to indirectly influence brain regions downstream of the arcopallium implicated in female courtship behaviors. In other species, AIV projects to the ascending auditory stream which 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 and sufficient for female call production. Collectively, these data demonstrate putative pathways through which CM may influence both CSDs and calls in response to preferred song. Future studies need to address functionality and sufficiency of these putative pathways emanating from CM in driving female courtship behaviors. **Keywords:** Songbirds, Behavior, Microinjections, Tract tracing, Optogenetics

### 67) An Electrophysiological Study of Auditory-Motor Entrainment
**Jewel E Crasta**, Michael Thaut, William J Gavin, & Patricia L Davies. From the Department of Occupational therapy, Center for Biomedical Research in Music, Human Development and Family Studies, Colorado State University, Fort Collins.

Neurophysiological research has shown that the auditory and motor systems interact during movement to rhythmic auditory stimuli, through the process of entrainment. However, the mechanisms by which auditory entrainment enhances motor performance still remain unclear. This study used electroencephalography to explore the neural mechanisms underlying auditory-motor entrainment. A cross-sectional quasi-experimental quantitative study design with convenience sampling procedures was employed to compare two groups. Twenty-eight young adults were randomly allotted to an auditory group or a motor group. Participants in the auditory group were asked to listen to auditory stimuli presented every 800 milliseconds (ms) (auditory only condition), while participants in the motor group were asked to press a button every 800 ms without any external stimuli (motor only condition). Participants in both these groups were then asked to press a button along with auditory stimuli every 800 ms (combined condition). As expected, synchronization error (response time) was lower for the combined condition compared to the motor only condition, confirming that individuals have better synchronization in the presence of an external auditory stimulus compared to an internal rhythm. Synchronization error for the combined condition was not significantly different for the two groups. Averaged event-related potentials (ERPs) for the auditory only condition displayed the characteristic P1-N1-P2-N2 pattern. This was followed by the presence of alpha waves from 300 ms to 800 ms post-stimulus onset, suggestive of entrainment. Averaged ERPs for the motor only condition displayed the characteristic waveforms representative of motor activity, however, without the presence of alpha waves. Interestingly, in the ERPs for the combined condition, the motor group displayed greater alpha activity than the auditory group, suggesting different neural responses based on prior exposure. These findings were further validated with time-frequency analysis. **Keywords:** auditory, motor, entrainment
auditory signals in therapeutic interventions for individuals with motor disabilities. **Keywords:** Entrainment, EEG, Time-Frequency Analysis

### 68) Somatosensory Stimulation of the Tongue as Sensory Substitution for Audition

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Hearing loss or impairment affects more than 30 million people in the United States (NIDCD) and is associated with communication difficulties that can greatly impact the quality of life for impacted individuals. For less severe cases, hearing aids can be very helpful and for more severe cases, implantable devices have been developed. Cochlear implants stimulate the auditory nerve directly and are one of the most successful neural prostheses to date (NICDC; Moore and Shannon, 2009). Unfortunately, these are only effective if hearing loss is due to peripheral damage, and normal functioning of the remainder of the auditory pathway is required. In addition, they are expensive and require invasive surgery. Brainstem and midbrain implants also have been developed, but due to the complexity of neuronal processing at these levels, and the extensive surgery required for implantation, these devices are much less effective. Thus there is a great need for alternate approaches to help people who have injuries or defects central to the auditory nerve or who are unable to utilize cochlear implants for other reasons (financial, medical). Sensory substitution is a growing field that focuses on using alternate sensory systems to provide information to the nervous system that can no longer be carried by a damaged sensory pathway. This technique has been used for centuries by the visually impaired in the form of reading Braille, where the somatosensory system provides the individual with information normally gathered by the visual system. More recent applications include electrotactile stimulation of the tongue as an alternate pathway for sensory substitution. This method has been used successfully to compensate for vision and balance deficits in human subjects (Vuillerme et al., 2007; 2009; Wildenberg et al., 2013), and is promising because the somatosensory system of the tongue contains a dense nerve supply and the mouth provides a protected, moist environment for electronic stimulation (Kaczmarek, 2011). To date, little work has been done to investigate the utility of using tongue stimulation for auditory substitution and the focus of our work is to develop an advanced electrotactile stimulation device for the tongue that is capable of transmitting auditory information. This will provide a less expensive, more flexible and less invasive option for people who may be considering a cochlear implant, and will provide an option for people who currently can’t benefit from available technologies. The current study focuses on mapping the sensitivity and 2 point discrimination of the tongue surface and investigates whether there are differences in these parameters in tasters relative to non-tasters of Propylthiouracil, a bitter compound associated with increased sensitivity to bitter stimuli and other oral stimuli. Our preliminary evidence suggests that tasters of PROP are more sensitive to electrotactile sensitivity than non-tasters and surprisingly, many people have asymmetry in the ability of their tongue to detect and discriminate electrotactile information. These studies were supported by the State of Colorado (OEDIT award to Sapien, LLC), and by the College of Veterinary Medicine and Biomedical Sciences College Research Council (CRC award to LMS).

### 69) 3D Imaging of Neurons In Vivo Using a Variable Focus Fiber-Coupled Microscope

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We have developed a chronically implantable fiber-coupled microscope (FCM) that incorporates an electrowetting variable focus lens to obtain real-time 3D functional images of neurons in a mouse brain. The device is coupled to a laser-scanning microscope through an optical fiber-bundle for lateral scanning with ~2 µm resolution. Axial scanning is accomplished by altering the focus of the electrowetting lens distal to the fiber-bundle and allows for a Z-scan range of ~200 µm. The FCM is manufactured through a 3D-printing process for rapid assembly, and allows for a variety of dimensions depending on the target brain region. The assembly includes a chronically implanted lightweight (< 5g) objective lens adapter and an easily attachable FCM head for imaging. We have demonstrated the FCM by performing real-time imaging of neurons in a mouse brain. Our FCM shows a robust and customizable implementation of a fast miniature 3D fluorescence imaging system.
70) Shedding light on Type III taste cell function
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Type III taste cells in the mammalian taste bud respond to both sour and salty stimuli. Unlike Type II taste cells, Type III cells form classical synapses with afferent nerve fibers. The transmitter released at this synapse and its cognate receptors remain unclear. Studies of Type III cells in the intact bud are complicated, however, by the widespread effects of acid application (sour stimuli) on taste tissue. To circumvent this issue and isolate Type III cell activity, we have developed a Cre-dependent optogenetic system to modulate Type III cell activity using light as a stimulus. We created a knock-in mouse expressing Cre recombinase in Type III cells by inserting a bicistronic IRES Cre recombinase construct directly following the Polycystic Kidney Disease 2-Like 1 (Pkd2l1) stop codon. As PKD2L1 is expressed exclusively in Type III cells, this Cre construct allows for the expression of Cre-dependent channelrhodopsin-YFP specifically in Type III cells. Initial findings indicate that the expression of our construct is faithful as channelrhodopsin-YFP is expressed in PKD2L1 immunoreactive Type III taste cells, with no expression in Type II taste cells. Light application to the tongue in an anesthetized PKD2L1-Cre, channelrhodopsin mouse produces a chorda tympani nerve response, indicating the functionality of our system. Optogenetic modulation of PKD2L1 positive cells will allow us to examine Type III cell function in intact taste buds without the confounding effects of intracellular acidification.

71) Guts, Bugs and Brains: Interactions Ex Vivo
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Evidence for gut having an impact on central nervous system function and development, as well as immune surveillance and response, suggests a central role for the integrated signaling of commensal bacteria, gut associated lymphoid tissue, and components of the enteric nervous system. To understand these interactions further, we are examining signaling pathways thought to be involved. Receptors in the Toll-like family (TLRs) are expressed by both the neural and immune cells of the gut, and play key roles in bacterial-host interactions. Bacterial – TLR signaling impacts gut function in several inflammatory and infectious diseases in vivo (Frosali et al., J Immunol Res 2015:12, 2015). How TLRs, commensals/bacterial products and neural and immune cells interact has yet to be shown with cellular resolution. To address these issues, we developed an organotypic slice model that maintains mouse intestinal tissue ex vivo. The model maintains structural components of the gut, including the muscle layers, sub-mucosa, and the mucosal layer for up to 6 days ex vivo. Epithelial cells in these slices undergo cell proliferation and migration, based on the incorporation of the thymidine analog 5-Ethynyl-2’-deoxyuridine during DNA synthesis and subsequent changes in the position of labeled cells. Enteric neuronal structure is maintained based on the live visualization of neurons with yellow fluorescent protein driven selectively in neurons under the control of the Thy-1 promoter. Slices undergo segmental contractions at rates within 25% of those reported in vivo. To begin testing the utility of the model, the impact of commensal bacteria on gut contractions was examined. After slicing, penicillin-streptomycin (PS) was used to kill roughly 50% of native bacteria in the slices, and contraction rates were measured compared to non-PS treated slices whose microbiota were kept alive. Slices treated with PS demonstrated 64% lower contraction rates compared to their PS-free counterparts. This experiment demonstrates the efficacy of the model for studying gut-microbiota functional interactions, and agrees with in vivo studies that show dysmotility in the presence of antibiotic treatments (Grasa et al., Microbial Ecology, 70:835-848, 2015). Further research will focus on the secretory outputs of commensal bacteria to determine chemical signaling among gut components. The availability of cell-selective fluorescent transgenic mice provide for direct observation of the effects of chemical signaling in the gut over time. Keywords: Organotypic slice, microbiome, enteric nervous system.

72) Duration Mismatch Negativity in High-Risk Populations for Schizophrenia
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Schizophrenia is an intractable disorder that is categorized by disturbances in sensory information processing and the onset of a psychotic episode. Recent research has targeted clinically high-risk populations and used mismatch negativity (MMN) to provide predictive information regarding the onset of psychosis. Patients with schizophrenia
73) Oliver Sacks' “The Man Who Mistook His Wife For a Hat” – Using Performance to Stage Neurology
Naomi Rusk1, Livia Batista Silveira1, Ali A. Goldfarb2,* Niki Tulk2,*. From the 1Brain and Behavior Clinic, Boulder; 2University of Colorado Boulder, Department of Theatre & Dance; 3Neuroscience Institute, Children's Hospital Colorado. *authors contributed equally to the project.

Neurologist and writer Oliver Sacks used storytelling to share his commitment to humanizing neurological disorders; in turn, storytelling is an inherent part of theatrical processes. In this particular project, Sacks' best-selling book "The Man Who Mistook His Wife For a Hat" was adapted to a performance, looking at on-stage representation of neurological disorders and with a particular focus on aphasia. Ethics of performance, narratives from neurology professionals, and audience participation were addressed in this project. Keywords: theatre; performance; neurology; neurological disorder; applied theatre; Oliver Sacks; translational research; aphasia; diagnosis; patient experience.

74) The effect of nicotine administration and withdrawal on sleep in mice
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Sleep disturbances are a commonly reported symptom during tobacco cessation attempts. They are currently the only symptom of human nicotine withdrawal syndrome that has not been correlated in a rodent model. The current study investigates the effect of nicotine administration and withdrawal on sleep quantity and quality in a forced oral nicotine mouse model. Nine subjects were implanted with EEG and EMG recording devices using standard procedures. After a recovery and acclimation period, data was recorded continuously for a 4-week period, certain days were chosen over each condition for sleep scoring. Mice had ad libitum access to food and a drinking water solution containing .2% saccharin. Baseline sleep and wake data was scored for three consecutive 24 periods, and subsequently averaged. Immediately following baseline, five of the subjects began receiving 200 μg/ml of nicotine for a period of 2 weeks (nicotine group). The control group did not experience any changes. Data for this condition was scored on days 1, 4, 8, 11, and 13. Withdrawal was precipitated spontaneously by excluding the nicotine from the drinking solution; the first two days of withdrawal were scored. Nicotine consumption tended to decrease total sleep. The effect was primarily seen during the lights off period and can mostly be explained by a decrease in time spent in NREM. Additionally, nicotine withdrawal appears to have an effect on the number of stage changes, both the number of awakenings from sleep and the number of total stage changes. The current data suggests of effect of nicotine consumption and withdrawal on the sleep wake cycle. Keywords: Nicotine, Withdrawal, Sleep

75) A Brain Computer Interface for Controlling Mobile Robots
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Brain-Computer Interfaces (BCI) are technologies that enable a user to communicate with a computerized device using only voluntary changes in mental state. Although there are many potential uses for BCI, an important application is the development of assistive technologies for people with motor impairments. For these people, restoring the ability to perform day-to-day functions can be extremely beneficial. For those afflicted with severe...
progressive neurodegenerative diseases, BCI may even become their only means of communication. Current BCI systems are typically designed to perform relatively simple tasks, such as typing a message or moving a mouse cursor. We believe, however, that a long-term goal for BCI should be to control more sophisticated devices that restore high-level functions, such as electric wheelchairs, prosthetic limbs or, perhaps, robotic exoskeletons. As a first-step toward these goals we have developed a novel BCI system that allows users to control mobile robots using simple, goal-directed instructions. This BCI system is built on top of The Colorado Electroencephalography and Brain-Computer Interfaces Laboratory (CEBL) software platform. Electroencephalography (EEG) is used to monitor the user’s brain activity while they attend to an item on a circular pie-menu that lists all possible instructions that may be sent to the robot. Each section of the pie-menu is then enlarged in a random order. When the section that the user is attending to is enlarged, a P300 event-related potential (ERP) is elicited in the user’s EEG signal and Linear Discriminant Analysis (LDA) is used to identify the corresponding pattern. The appropriate instruction is then sent to the robot over a wireless connection. So far, we have tested this interface with two different robotics platforms: ER1 and Baxter. The ER1 configuration consists of a laptop and digital camera placed on a cart with wheels. The ER1 robot accepts one of four commands: move forward, move backward, turn left and turn right. We have performed several real-time demonstrations showing that a user can reliably drive the ER1 robot in real-time; however, it is important to note that only a few instructions can typically be sent per minute. The Baxter robotics platform consists of a versatile human-like robot with arms, hands and cameras. Since we do not currently have access to a Baxter robot, our initial trials were performed using the VREP simulator, which presents a 3D virtual Baxter on a separate computer. We have demonstrated that a user can reliably raise and lower both arms of the Baxter robot in real-time. In our future work, we plan to implement more useful functionality for the Baxter robot, such helping a user to move an item, read a book or eat. Keywords: Brain-Computer Interfaces (BCI), Electroencephalography (EEG), Event-Related Potentials (ERP), P300, Robotics, Assistive Technology

76) Convolutional Networks for EEG Signal Classification in Non-Invasive Brain-Computer Interfaces
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Non-Invasive Brain-Computer Interfaces (BCI) are systems that seek to establish a direct channel of communication between the human brain and a computerized device. Typically, BCI are constructed by combining a communication protocol with Electroencephalography (EEG) for monitoring brain activity and machine learning algorithms for classification of the user’s mental state. In recent years, a number of BCI prototypes with potential applications in assistive technology have been successfully demonstrated. However, the speed and reliability of current BCI systems is less than ideal and a sophisticated manual process is often required for selecting features and filter parameters. Convolutional Neural Networks (CNN) are a type of artificial neural network that have recently gained considerable traction in the machine learning community, particularly in the field of computer vision. CNN are deep networks with several layers that act as non-linear convolutional filters followed by downsampling. The output of the final convolutional layer is then passed to a fully connected network that identifies which category of mental activity the EEG signal is most similar to. Parallels have been drawn between convolutional layers and the local receptive fields observed in biological neural networks. We believe that CNN may outperform other methods for EEG signal classification for several reasons. First, CNN typically have a degree of shift-invariance, meaning that they are able to identify events that occur in the EEG signal regardless of the time at which the event occurred. Second, convolutional layers may be viewed as non-linear finite impulse response decimation filters. In other words, a CNN may be able to automatically learn to filter EEG signals and to select appropriate features without manual intervention or the introduction of prior assumptions. Preliminary results suggest that CNN yield a mean performance improvement of about 2% AUC across 16 subjects for P300-based BCI when compared to Linear Discriminant Analysis. Experiments related to mental-task BCI are ongoing. Currently, training performance is near 100%, suggesting that regularization may be necessary in order for CNN to work effectively with the small amount of data that can be collected during a BCI calibration phase. Additionally, determining the optimal network layout, i.e., width and number of layers and neurons, is a challenge that remains to be fully addressed. Keywords: Brain-Computer Interfaces (BCI), Electroencephalography (EEG), Artificial Neural Networks (ANN), Convolutional Neural Networks (CNN)
77) Perivascular stromal cells and CNS injury
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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 in less than 3 hours following onset of symptoms. The development of novel therapies with broader application times that treat acute and chronic stages of stroke is an active area of pre-clinical research. Neuroprotective agents are the primary focus of new stroke therapies for the acute phase, however no therapies exist for the chronic, regenerative stage following stroke. CNS regeneration following injury is impaired by fibrosis. Perivascular stromal cells (PSC) are a major component of the fibrotic scar following brain injury like stroke. Prior to injury, PSCs surround large diameter blood vessels and occupy the same perivascular niche as vascular smooth muscle cells and pericytes. PSCs uniquely express the RA synthesis enzymes Raldh1 and 2 and collagen 1a1 (Col1a1). We find PSCs along with activated inflammatory cells synthesize RA in the stroke lesion and activate signaling in adjacent neurons and astrocytes. This sets up the possibility that PSCs have a role beyond fibrosis and may be important for RA-mediated regeneration following injury. The signal(s) in the injury niche that activates PSCs following injury has not been identified and no signaling pathways that underlie their fibrotic activity have been described. We are currently utilizing Col1a1-GFP mice to identify the cellular source of activation signals in the ischemic core and an in vivo model of stroke injury to obtain “activated” PSCs for unbiased gene profiling experiments.

78) Influence of Bifidobacterium infantis on the Development of Anxiety, Depression, and Stress in Adolescent Rats
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The importance of commensal gut bacteria in shaping nervous system structure and function is becoming increasingly evident. For example, oral supplementation of the beneficial bacterium Bifidobacterium infantis to rodents can alleviate depressive behaviors resulting from maternal separation. However, it is unknown whether these beneficial effects of gut bacteria on the brain are mediated through immune, hormonal, or neural (i.e., through the vagus nerve) pathways. The purpose of the current study was to elucidate the effects of the probiotic Bifidobacterium infantis on the development of anxiety, depression, and stress in healthy adolescent rats. Furthermore, we explored the unique contribution of the vagus nerve to such effects. It was hypothesized that supplementation with B. infantis would decrease anxious and depressive behaviors, as well as the HPA-axis response to stress, in a vagally-dependent manner. As weanlings, rats were subjected to either a transection of the vagus nerve or a sham surgery; they were then supplemented daily with B. infantis or vehicle for 14 days; finally, they underwent a series of behavioral and chemical tests. B. infantis treatment increased the number of arm crosses and time spent in open arms in the elevated plus maze (EPM), a model for anxious behavior in all female rats, but in males, only rats with an intact vagus nerve benefited. There was no significant alteration in behavior in the forced swim test (FST)–a depression model–or levels of serum corticosterone as a result of probiotic supplementation. These results suggest that the vagus nerve plays a necessary role in anxiolytic communication between gut B. infantis and the CNS in male rats, but that other pathways, immune or otherwise, are sufficient to communicate the beneficial effects of B. infantis in females. More research is warranted to further disentangle the pathways utilized by commensal gut bacteria in producing behavioral and neurochemical effects, while taking into consideration the therapeutic benefits that result from dietary probiotic supplementation. Keywords: Microbiome, Development, Anxiety, Depression, Probiotic, Vagotomy

79) Computing individual voltammetric electrode calibrations in vivo using a standardized model
Devan Gomez, Matthew Harrington, Erik Oleson.

Fast-scan cyclic voltammetry (FSCV) is a powerful electrochemical method used to detect sub-second changes in neurochemical concentration. This technique enables researchers to assess information regarding transient release of oxidizable neurochemical messengers, including their concentration in vivo. A significant unresolved issue with the use of this technique, however, concerns how to uniformly perform post-calibration assessments of the working electrodes used for individual measurements. Researchers using FSCV lack a standardized protocol allowing for convenient in vitro post-calibration, a process that is oftentimes impractical, if not impossible to perform.
Furthermore, criticisms regarding face-validity surround the issue of generalized calibration values, as significant electrode-to-electrode variability exists. Here we implemented a mathematical model specific to the materials and equipment used within our lab to perform calibration in vivo, thereby expediting a valuable yet otherwise lengthy process. By replicating a now validated study, we obtained a set of empirical values using multiple linear regression analysis, which enables the use of background current produced by our equipment to solve for concentration in individual electrodes. This method provides a fast and practical means of computing electrode-specific changes in neurochemical concentration and addresses an issue of validity regarding FSCV.

80) Promoting child brain development and health through classroom neuroscience education

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The University of Colorado (CU) Boulder’s Intermountain Neuroimaging Consortium (INC) is at the forefront of research on the role of healthy lifestyle choices such as good sleep habits and exercise in the prevention and treatment of brain-related illnesses and disorders. Given the importance of this research for our community’s health and well-being, the overarching goal of the INC’s outreach efforts is to bring CU research directly to the community through targeted neuroscience classes and local community lessons. In this program INC faculty, staff and a graduate student trained 14 CU undergraduates from neuroscience and related majors in classroom management and cognitive development. The undergraduate students then designed and delivered targeted lessons to local elementary classrooms. Lessons focused on how information is transmitted to and from the brain, as well as ways that children can enhance their brain’s development and function through healthy lifestyle choices (i.e., sleep habits, exercise, nutrition, stress management, abstaining from drug use and brain injury prevention). Lessons were age-appropriate in content and activities. The undergraduate teaching team reached over 800 elementary students including many Title I schools, over 60 K-12 educators, and approximately 50 members of the public in the 2014-2015 academic year. The inclusion of undergraduates enhanced their educational experience and afforded a much broader outreach effort, expanding the reach of the program to more students, educators and community members than in the program’s previous year. Student and teacher responses were overwhelmingly positive. Teachers consistently rated the lessons as having a high degree of applicability and accessibility to their students, and spontaneously incorporated lessons into their own classroom routines. The program is expanding in 2015-2016 to include middle and high school classes, parents and educators. Additionally, we are looking for avenues to directly interact with and receive feedback from students and parents, to expand our marketing strategies to include a larger website and social media presence and to develop more concrete data collection tactics to measure how the lessons are informing kids’ choices around exercise, nutrition, sleep, stress management, and injury prevention. This program gives students the tools to make choices that enhance their brain’s health and development and enables them to incorporate this information into their everyday lives. Keywords: outreach, teaching, k-12, undergraduate, community