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Programme

1:00-2:30
Introductions

Local Speaker Presentations:

**Dynamics of rapid dopamine signaling during decision making**
Regina Carelli, Ph.D., Department of Psychology, University of North Carolina at Chapel Hill

**Can we discover mechanisms of motor learning by just listening to the cerebellum while it works?**
Stephen Lisberger, Ph.D., Department of Neurobiology, Duke University

**Cognitive modulation of oculomotor activity in the frontal cortex of monkeys performing a simple reaction-time task with reward bias**
Emilio Salinas, Ph.D., Neurobiology and Anatomy, Wake Forest University

2:30-2:45
Coffee Break

2:45-3:45
Keynote Address: **Alcohol and the Brain: From Binding Sites to Gene Expression**
Adron Harris, Ph.D., M. June & J. Virgil Waggoner Chair in Molecular Biology Director of Waggoner Center for Alcohol & Addiction Research, University of Texas at Austin

3:45-4:00
Coffee Break

4:00-5:00
Poster Presentations & Judging
Reception with light refreshments
Award Announcements

Special Thanks

S. Alex Marshall, Program Chair
Chintan Oza, Sponsorship Chair
Shannon Farris, SfN Chapter Representative
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Triangle SfN Executive Committee: Amir Rezvani, Patricia Jensen, and Mamta Behl
Program Committee: Jamie Hanson, John Meitzen, Richard Weinberg, and Ying Liu
Sponsorship Committee: Maile Henson and Maggie Rougier-Chapman
Friends and colleagues,

It is with great pleasure that I welcome you to our first annual Spring Neuroscience Meeting. Less than one year ago, the Triangle Chapter of the Society for Neuroscience was reinstated, right here in the heart of North Carolina. As expected, the reinstatement of Triangle SfN was enthusiastically received by local neuroscientists. The Triangle is home to some of the top universities and private and governmental neuroscience research institutes in the country, and it is my hope that through Triangle SfN, individuals from each of these institutions will be able to form a tight and collaborative network.

From the beginning, my colleagues and I strongly believed that by combining the abundance of resources in our area we could have a significant impact throughout the surrounding community by educating the public, legislators, and local officials about the importance of neuroscience research. Since last summer, we have had several successful events designed to engage and inform the community about current research happening right here in the Triangle; I look forward to many similar events in our future.

As our chapter has grown, promoting and fostering collaborations within and beyond our community of neuroscientists has become one of our top priorities. In order to continue building such relationships, we have invited several internationally known neuroscientists to speak at our first annual Spring Neuroscience Meeting, including three from our own backyard. It is with great honor that we present lectures by Drs. Carelli, Lisberger, Salinas, and Harris this afternoon.

I want to thank you for joining us today, at our first annual Spring Meeting. I strongly believe that with your active participation in events such as this, we can collectively achieve our goals and further an appreciation for the importance of neuroscience research here in North Carolina. We need your help in this beautiful journey.

Amir H. Rezvani, Ph.D.
President
KEYNOTE SPEAKER: Dr. Adron Harris holds the M. June and J. Virgil Waggoner Chair in Molecular Biology and is Director of the Waggoner Center for Alcohol and Addiction Research, University of Texas (Austin), where he directs a research program on the molecular actions of drugs of abuse on brain signaling systems. Prior to his appointment at the University of Texas, he was Professor of Pharmacology, University of Colorado Health Sciences Centre (1988-98) and Director, University of Colorado Alcohol Research Centre (1996-98).

Having trained as a pharmacologist, Dr. Harris directs a multidisciplinary team focused on defining the both acute and long-term actions of alcohol and other drugs. He specializes in the study of the neurochemical basis for genetic differences in drug response, using genetically-modified mice that vary in susceptibility to drug intoxication and dependence. He also investigates of the structure and function of ion channels with emphasis on the molecular mechanisms responsible for alcohol and drug actions and the regulation of brain gene expression by drugs.

He has published 68 peer-reviewed publications in the last 5 years and 423 career total and has almost 16000 non-self citations in total (Web of Science) and an h index of 75. These statistics place his work in the top 1% in the field of Neuroscience and Behaviour, 1997-2007.

DISTINGUISHED SPEAKERS

Regina Carelli
Stephen B. Baxter Distinguished Professor and Associate Chair, Department of Psychology, University of North Carolina-Chapel Hill

Stephen Lisberger
George Barth Geller Professor and Chair, Department of Neurobiology, Investigator, HHMI, Duke University

Emilio Salinas
Associate Professor, Neurobiology & Anatomy, Wake Forest School of Medicine
Abstracts

P34: Supplementary eye field activity during and after self-selection of abstract rules
ZM Abzug and MA Sommer
Duke University, Biomedical Engineering Program

Selection and implementation of abstract rules is a crucial part of human cognition. Previous work has shown that neurons in the frontal cortex of monkeys (Macaca mulatta) including Supplementary Eye Field (SEF) encode instructed rules through firing rate modulations (White & Wise 1999). However there is a dearth of work addressing whether or not neurons in frontal cortex encode self-selected rules or if they differentiate between self-selected and instructed rules (i.e. agency selectivity). We previously designed and validated an oculomotor task compatible with neurophysiological recordings in monkeys constructed to answer this question and showed that one monkey was able to perform the task appropriately (Abzug & Sommer SfN 2013). In the first stage of this two-stage task the monkey was required to saccade to one of two colored peripheral targets. The color of each target was associated with a particular rule and the targets could be either different colors (allowing the monkey to select between different rules) or the same color (forcing the monkey to select an instructed rule). After refixating a central location the monkey then had to correctly apply the selected or instructed rule in a perceptual discrimination between two visual targets in order to receive liquid reward. Many neurons in SEF show complex and variable activity throughout the duration of this task. Consistent with previous reports a subset of neurons distinguish between the two rules in various time epochs during the task. We have also found a sub-population of agency-selective cells that distinguish between self-selected and instructed trials. These cells may help fill an important metacognitive role by distinguishing between rules inherited through cognitive decision-making and rules inherited through simple sensory cueing.

P15: Vulnerability in the PFC: Proteomic analysis in binge-drinking adolescent and adult C57BL/6J mice
AE Agoglia, SE Holstein, and CW Hodge
University of North Carolina Chapel Hill; Bowles Center for Alcohol Studies

Adolescence is an evolutionarily conserved developmental period characterized by increased impulsivity risk-taking and social interaction in rodents and humans. Adolescence may also represent a critical period of vulnerability to alcohol abuse as individuals who initiate alcohol use during adolescence are at significantly greater risk of developing alcoholism as an adult. Adolescents have been shown to respond differently to ethanol than adults in terms of behavior effects on brain structure and neurochemical signaling. However differences between the adolescent and adult response to ethanol at the protein level have received less investigation. Here we performed an unbiased proteomics screen of the adolescent and adult male C57BL/6J mouse prefrontal cortex (PFC) following a two-week history of intermittent voluntary ethanol drinking in the home cage. This approach allowed us to qualitatively compare proteins for alcohol-induced changes in expression between adolescents and adults. The screen revealed 21 unique ethanol-sensitive proteins in the adolescent PFC but only 12 unique ethanol-sensitive proteins in the adult PFC. The identified proteins included critical regulators of calcium signaling cell structure and morphology and receptor signaling. Ingenuity IPA Pathway Analysis revealed unique protein interaction networks in the adolescent and adult samples suggesting that ethanol exposure may influence different cellular processes in the two age groups. Together these findings suggest that adolescence represents a time period in which the PFC is uniquely vulnerable to ethanol-induced protein modification. Adolescents and adults have a qualitatively different protein-level response to voluntary ethanol drinking which may underlie age differences in the cellular circuit and behavioral actions of ethanol.
The function of hippocampal area CA2 in behavior has only recently begun to be assessed. Initial studies suggest that CA2 may function in social processing, as shown by the loss of social recognition memory in both the vasopressin 1B receptor knock-out mice (Wersinger et al., 2002) and in mice with CA2 output silenced by tetanus toxin expressed in CA2 pyramidal cells (Hitti and Siegelbaum, 2014). In addition, CA2 may function in spatial information processing, similar to CA1 pyramidal cells. Support for a role of CA2 in spatial processing comes from a knock-out mouse for regulator of G-protein signaling 14 (RGS14). In these mice, long term potentiation at the Schaffer Collateral-CA2 pyramidal cell synapse, absent from wild-type mice, is revealed. Perhaps as a consequence, these mice also display enhanced performance in the acquisition phase of a spatial memory task (Lee et al., 2010). Based on these findings, we hypothesize that CA2 functions in both social and spatial processing. To test this hypothesis, we have taken two independent approaches of querying CA2 pyramidal cell activity and exposed animals to various stimuli. First, we performed in vivo electrophysiological recordings from individual CA2 pyramidal cells in adult male Sprague-Dawley rats and asked how firing rate is affected by behavioral state as well as by spatial navigation, with or without exposure to social stimulation. Second, we exposed a separate group of male Sprague-Dawley rats to identical stimuli, and assessed brain sections for changes in immediate early gene (IEG) expression 5, 15 and 30 min after the exposure. Together, our data show that CA2 pyramidal neurons respond to spatial exploration and that additional social exposure does not enhance IEG expression or firing rate in CA2. Our data do suggest, however, that CA2 firing rate changes depending upon the behavioral state of the animal in a manner that differs from CA1. These results highlight the role of CA2 in processing spatial information but offer no further insights into its role in processing social information. Therefore, modulation of CA2 activity by social stimuli may be more subtle than a general increase in firing rate or may be affected during a later phase of consolidation or recall.

P1: The Neurotoxicity of FireMaster 550® in Zebrafish (Danio rerio): Chronic developmental and acute adolescent exposures
JM Bailey and ED Levin
Duke University, Psychiatry and Behavioral Sciences Department

Firemaster® 550 (FM550) is the second most commonly used FR product in consumer goods and has been detected in household dust samples. However neurobehavioral effects associated with exposure have not been characterized. We describe the effect of developmental exposure in zebrafish larvae, the persisting effects of this exposure on adolescent behavior and the acute effects of exposure during adolescence. Developmental exposure to 0, 0.01, 0.1 or 1.0 mg/L via immersion spanned 0-5 days post fertilization with larval testing on day 6 and adolescent on day 40. Acute adolescent exposure to 0, 1.0 or 3.0 mg/L commenced via immersion for 24 hrs with testing 2 hr or 1 week later. Zebrafish behavior was characterized across several domains including learning social affiliation sensorimotor function predator escape and novel environment exploration. Persisting effects of developmental exposure manifested as a significant (p < 0.01) reduction in social behavior among all exposure groups. Acute adolescent effects were generally widespread when tested 2 hr after exposure including hypoactivity and reduced social behavior (p<0.05). These effects were attenuated at the 1 week testing point. Taken together these data indicate that FR mixtures may cause persisting neurobehavioral alterations to social behavior in the absence of perturbations along other behavioral domains and that developmental exposures more costly to the organism than acute adolescent exposure.
RP Bell\textsuperscript{1}, TJ Ross\textsuperscript{2}, EA Stein\textsuperscript{2}, and SB Daughters\textsuperscript{1}
\textsuperscript{1}University of North Carolina Chapel Hill; \textsuperscript{2}Neuroimaging Research Branch NIDA NIH

Cocaine dependence has been associated with neural activation deficits in executive functioning including inhibitory control reward working memory and attention. These deficits are hypothesized to partially result from neuroadaptations that occur as a result of repeated cocaine use and reflect multiple adverse behavioral manifestations that are associated with cocaine dependence. However while specific neural deficits have been identified in multiple cortical and subcortical regions in cocaine dependent (CD) individuals much less is known about the functional connectivity differences in resting state networks (RSNs). Here we investigated resting state functional connectivity utilizing fMRI in CD individuals (n=25) relative to a non-using control group (n=27). We also investigated if functional connectivity in CD individuals was correlated with scores on the UPPS Impulsive Behavior Scale (UPPS). Utilizing probabilistic independent component analysis we identified 30 separate RSNs. These RSNs were then correlated with intrinsic connectivity networks that have been identified with the executive functions that have been found to be deficient in CD individuals (Smith et al. 2009; Laird et al. 2011). This resulted in six RSNs that are associated with inhibitory control working memory reward and attention. Utilizing dual regression to investigate differences in resting state functional connectivity between groups we found that CD individuals displayed increased resting state functional connectivity bilaterally in the precuneus/posterior cingulate cortex bilaterally in the frontal operculum/posterior insula right lateral occipital cortex right frontal pole right caudate and right frontal orbital cortex relative to the non-using controls in a RSN primarily associated with reward processing. In addition for the CD group within a RSN associated with inhibitory control there was a positive correlation between functional connectivity in the left middle frontal gyrus and UPPS scores on the sensation seeking subscale. These results show that CD individuals display aberrant functional connectivity in RSNs that are associated with executive functions that characterize cocaine dependence. Furthermore functional connectivity in CD individuals was also shown to be associated with impulsivity a construct that is hypothesized to be both a risk factor and primary deficit of cocaine dependence. The investigation of RSNs in CD individuals can provide a comprehensive characterization of cocaine dependence and may be utilized as neural biomarkers in future examinations of this disorder.

P37: A gut-brain neuroepithelial circuit
DV Bohórquez and RA Liddle
Duke University, Medicine-Gastroenterology

The homeostasis of core physiological functions like satiety depends on sensory signals arising from the surface of the gut. Here nutrients and bacteria stimulate epithelial biosensors called enteroendocrine cells. Despite being electrically excitable enteroendocrine cells were thought to communicate with nerves only indirectly through hormones but not through direct cell-nerve contact. The missing link appears to be a neuropod that we recently uncovered in intestinal enteroendocrine cells. Using cell-specific transgenic mice and molecular tools for the study of neural circuits we unveiled a neuroepithelial circuit -- a connection between enteroendocrine cells and neurons innervating the small intestine and colon. We found that enteroendocrine cells have the elements necessary for synaptic transmission including genes encoding for pre- post- and trans-synaptic proteins. This neuroepithelial circuit can be reconstituted in vitro by co-culturing single enteroendocrine cells with sensory neurons. We defined the functional connectivity of this circuit in vivo using a monosynaptic rabies virus. Given the conditions a neurotropic virus delivered into the colon's lumen can infect mucosal nerves through enteroendocrine cells. This neuroepithelial circuit can serve both as a sensory conduit for food and gut microbes to interact with the nervous system and as a portal for viruses to enter the enteric and central nervous systems.
P21: Local drug infusions to the site of voltammetric measurement of dopamine in the nucleus accumbens
EL Brightbill1, TA Shnitko1, LA Sombers4, SM Nicola5 and DL Robinson1,2
1Bowles Center for Alcohol Studies, 2Department of Psychiatry, 3Department of Chemistry, University of North Carolina, Chapel Hill, NC, USA 4Department of Chemistry, North Carolina State University, Raleigh, NC, USA 5Department of Psychiatry and Behavioral Science, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York

The role of the brain dopaminergic (DA) system in mechanisms of reward-based learning and addiction is widely studied with fast-scan cyclic voltammetry (FSCV) in behaving and anesthetized animals. Usually, localized effects of drugs on DA neurotransmission are studied in vitro, and in vivo studies are infrequent due to the complex methodology. In this study, we investigated whether drugs affecting DAergic neurotransmission can be infused at the axonal site of DA neurons using an infusion cannula, while DA release is measured with FSCV. We evaluated effects of the well-characterized DA transporter blocker nomifensine (NOM) and vehicle locally applied to the site of DA recording on the dynamic of evoked DA release and uptake in the nucleus accumbens (NAc) of anesthetized rats.

Male Long-Excative rats were used in this study. DA efflux was measured in the NAc with FSCV, where voltage was applied in a triangular waveform from 0.4 to 1.3 V and back to 0.4 versus Ag/AgCl reference at a scan rate of 400 V/s. DA release was induced by electrical stimulation (24 biphasic, square-wave pulses applied at a frequency of 60 Hz, 300 μA, 2 ms/phase) delivered in the VTA every 5 min. Baseline DA signals were recorded during 15 min followed by NOM infusion. NOM was dissolved in saline and infused to the NAc within 100±75 μm from the carbon microelectrode at a concentration of 20 μM at rate of .25 μL/min for 2 min. Post-infusion evoked DA signals were recorded during a 1-hr period. Effect of the drug on [DA]p, and Km were evaluated using the Michaelis-Menten kinetic method and compared to vehicle.

Preliminary results obtained in 2 experimental and 1 control rats show that local infusion of NOM increases [DA]max.

Baseline [DA]max in the three rats was 72±8 nM. [DA]max increased by an average of 244% in NOM animals (n=2) vs 114% for the control (n=1) as measured 4min after the initiation of infusion. In the experimental animals, [DA]max continued to increase to a maximum of 448% above baseline 14 min after infusion start before slowly decreasing. [DA]max still remained at 205% of baseline 1 hour after infusion start. Analysis with Michaelis-Menten kinetics revealed NOM similarly resulted in an increase in [DA]p and Km relative to baseline and vehicle. NOM enhanced [DA]p and Km by 237% and 145% to baseline, respectively.

Overall, the results correspond to previous voltammetric studies with systemic NOM injections, where the drug increased [DA]p and affinity of dopamine transporter. It indicates proper infusion of the drug to the site of DA measurement in the NAc, and reveals a great application of this technique for in vivo analysis of the mechanisms of dopaminergic neurotransmission in the brain with FSCV.

P12: Voluntary binge-like ethanol intake site-specifically increases c-Fos immunoexpression in C57BL/6J.
N.W. Burnham and T.E. Thiele
University of North Carolina Chapel Hill, Behavioral Neuroscience Department

Recent literature implicates a role for norepinephrine (NE) in modulating drug and alcohol use in dependent animals via α1-, α2-, and/or β2- adrenergic receptors. However, the role of NE in voluntary binge-like ethanol consumption among non-dependent animals is poorly understood. Previous experiments utilized intraperitoneal ethanol injections to achieve binge-equivalent BECs and found elevated c-Fos expression in the A2 region of the nucleus of the solitary tract, a noradrenergic brainstem structure. The current study builds upon this research by assessing c-Fos expression in the A2 and A2 efferent neurons following a single “drinking in the dark” (DID) binge episode in ethanol-naïve animals. To this end, male C57BL/6J mice received unilateral injections of green and red RetroBeads aimed at ipsilateral BNST and VTA, regions reported to be A2 efferents. Following surgery recovery, mice underwent one 4-day DID cycle wherein animals were given two hour access to a 20% ethanol solution beginning three hours into the dark cycle. On the fourth day, or test day, tail blood samples were collected within ten minutes following ethanol bottle removal, and animals were subsequently perfused. Immunohistochemistry was performed to label c-Fos and tyrosine hydroxylase (TH). C-Fos and TH double-labeled neurons in the A2 were counted. A2-efferent counts were based on boundaries established via unilateral AAV8-hSyn-DIO-hM3D(Gq)-mCherry injections into the A2 of male TH-Cre mice. T-tests determined that binge-like drinking significantly increased c-Fos immunoactivity in ventral BNST and A2 regions compared to water controls. Green and red RetroBead labeling was also observed in the A2 region. These data suggest that the A2 region and its efferents are active during binge-like ethanol consumption in animals lacking previous ethanol exposure. Additional studies are required to assess voluntary binge behavior following noradrenaline modulation in non-dependent mice. (Support by NIH grants AA022048, AA013573, & AA015148).
A prominent clinical feature of persistent pain is decreased function of endogenous pain-controlling systems, including the descending inhibitory pain pathway. Fear-conditioned analgesia (FCA) represents the reduction in nociceptive behavior expressed following re-exposure to a context previously paired with an aversive stimulus and is facilitated by brain regions involved in the descending inhibitory pain pathway. Although neuronal populations in these brain regions can be distinguished according to their neuropeptide/neuroenzyme-containing phenotype, there is a paucity of data on the precise role of these phenotypically-distinct neuronal populations in processing pain, conditioned fear, and FCA. Using a novel FCA paradigm, C57BL/6 male mice were exposed to a contextual arena and either received footshocks or were exposed an equivalent amount of time without receiving footshocks (conditioning day); 23.5 hours later (test day), mice received an intraplantar injection of either saline or noxious formalin and then, 30 minutes later, were re-exposed to the contextual arena for behavioral measurements over a 15 minute period. Thus, there were four groups: control (non-fear conditioned, saline), acute pain (non-fear conditioned, formalin), conditioned fear (fear conditioned, saline) and FCA (fear conditioned, formalin). Mice in the conditioned fear group showed a significant increase in freezing behavior compared to the control group. Mice in the acute pain group displayed a significant increase in formalin-induced nociceptive behaviors including licking/biting/shaking of the treated paw compared to the control group on the test day. Furthermore, mice in the FCA group displayed a significant reduction in nociceptive behaviors when re-exposed to the same contextual arena 24 hrs later compared to mice in the acute pain group – confirming the expression of FCA in this murine model. Following behavioral assay, mice were euthanized, perfused, and the brains sliced into three serial sets. Sections were immunostained with a neuronal marker (NeuN), a marker for neuronal activation (c-Fos), and CAMKII/parvalbumin, enkephalin/corticotropin releasing factor, or common glutamatergic/GABAergic markers (vGlut2/GAD65/GAD67). Preliminary analysis indicates that the acute pain and conditioned fear groups displayed an increase in c-Fos/CAMKII co-localization in the basolateral amygdala compared to the control and the FCA groups. This work supports our long-term goal of expanding the current knowledge of the functional circuits underlying FCA and developing novel strategies for the treatment for persistent pain.

P41: Relationship of activation of phenotypically-distinct neuronal populations with acute pain, conditioned fear, and fear-conditioned analgesia

RK Butler1,2, S Ehling2,3, AE Thomson1, S Wall1, V Zaric1, B Case1, D Knazovicky1, ME Gruen1, W Bäumer2,3, RM Rodríguez4,5, VM Pogorelov4, DK Aryal4, WC Wetsel4,5, B Duncan, and X Lascelles1,2
1Comparative Pain Research Laboratory, 2Center for Comparative Medicine and Translational Research, Department of Clinical Sciences, 3Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, North Carolina. 4Department of Psychiatry and Behavioral Sciences, 5Mouse Behavioral and Neuroendocrine Analysis Core Facility, Duke University Medical Center, Durham, North Carolina.

P26: Sex differences in mEPSC frequency occurs pre-puberty in medium spiny neurons in the nucleus accumbens core

J Cao, CA Hauser, DM Dorris, and JE Meitzen
North Carolina State University, Department of Biological Sciences

It is well established that the neuroendocrine pathways that regulate reproductive behaviors are sexually dimorphic and profoundly influenced by sex hormone exposure. There is growing evidence that sex differences can exist in other brain areas as well. One of these areas is the striatum, including the dorsal striatum (DS) and nucleus accumbens core and shell. Sex differences and steroid sex hormone sensitivity is found in striatal-mediated behavior and pathologies. The mechanism underlying these sex differences is an area of active research, and one current hypothesis is that the excitatory synaptic inputs onto the output neurons of the striatum, the medium spiny neurons, differs by sex. Here we test this hypothesis by recording miniature excitatory post-synaptic currents (mEPSCs) in pre-pubertal (P16-P23) male and female medium spiny neurons in the nucleus accumbens core. The nucleus accumbens core receives excitatory input from the prefrontal cortex (PFC), hippocampus, amygdala and thalamic nuclei. The nucleus accumbens core plays a central role in motivation and reward behaviors, and addiction pathologies, many of which show sex differences in phenotype. We find that mEPSC frequency is higher in female than in male medium spiny neurons. No sex difference was found in mEPSC amplitude or time of decay. These results suggest that: (1) excitatory synapse number per neuron and/or presynaptic release probability is increased in female nucleus accumbens core, and that (2) sex differences in excitatory synaptic input are present pre-puberty. This data supports the hypothesis that sex differences in excitatory synaptic input may be a potential mechanism by which sex differences in striatal behaviors and pathologies are generated. More broadly, these studies indicate that sex differences in neuron excitatory synaptic properties can exist in brain regions not dedicated to reproductive behavior and in the age-range commonly used for neuronal recordings.
Perineuronal nets (PNNs) refer to a specialized form of extracellular matrix that envelopes specific neuron types in the brain and spinal cord. The matrix, which predominantly concentrates around the soma and proximal dendrites of inhibitory interneurons, has been functionally implicated in inhibiting structural and synaptic plasticity during early adulthood. The lattice-like structure of PNNs is disrupted in several neurological disorders, including schizophrenia and epilepsy, likely by endogenous enzymes. We have identified dense staining for PNNs in mouse hippocampal area CA2 that is distinct from typical PNN expression in that it is associated with excitatory pyramidal cells. CA2 pyramidal neurons are unusual among hippocampal neurons because they are resistant to both the induction of synaptic plasticity and to damage from seizures. In this study, I tested whether PNNs in CA2 are regulated by experience and whether they inhibit synaptic plasticity in CA2. I found that staining for PNNs increases during early postnatal development and appears to be deposited along the membrane of excitatory CA2 cell somata and dendritic spines. Early-life manipulation of experience using environmental enrichment resulted in increased staining for PNNs in adult CA2 compared to control. Furthermore, in preliminary experiments, I found that enzymatic degradation of PNNs in acute hippocampal slices caused enhanced plasticity in normally plasticity-resistant CA2 neurons. Finally, I investigated PNNs in a mouse model of the neurodevelopmental disorder Rett Syndrome. I found that PNNs are strongly upregulated in CA2 of MeCP2-null mice. Together these data provide insight into a previously unknown role for PNNs in restricting plasticity in a population of excitatory neurons. Moreover, they may reveal critical mechanisms underlying alterations of hippocampal plasticity in PNN-associated neurological disorders such as Rett Syndrome and epilepsy.

P20: Rapid and Selective Detection of Met-Enkephalin in Live Tissue using Fast-Scan Cyclic Voltammetry
L Dunaway, Andreas Schmidt, James Roberts, Gregory McCarty and Leslie Sombers
NC State University, Chemistry Department

Met-Enkephalin (M-ENK), an opioid peptide in the brain, has been implicated as an important component in the development of learned-behaviors and motivation. Chromaffin cells, found in the adrenal medulla, are also known to secrete M-ENK along with various other neuropeptides. To date, the analysis of M-ENK has been limited to chromatographic approaches and various static assays, such as immunoassay. These techniques offer excellent chemical selectivity and sensitivity, but are limited in their ability to detect rapid molecular fluctuations. We have developed and optimized a voltammetric method that has the necessary chemical selectivity, sensitivity, and reproducibility to obtain dynamic information for M-ENK using fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes. This approach enables M-ENK to be distinguished from other endogenous enkephalins and opioid peptides. We have used electrical stimulation of rat adrenal tissue to elicit peptide release, and monitored the molecular dynamics with FSCV. Additional work utilizes optogenetic stimulation to excite enkephalinergic neurons and monitor their release in the ventral tegmental area. This work reports the first known instance of sub-second detection of endogenous M-ENK in living tissue, and lays the foundation for future in-depth studies using this real-time approach.
Manipulation of CRF signaling using DREADDs reduces binge-like ethanol consumption of CRF-Cre transgenic mice
SE Ebert, SA Marshall, and TE Thiele
University of North Carolina-Chapel Hill, Department of Psychology and Bowles Center for Alcohol Studies

Binge-like ethanol consumption is characterized by the rapid achievement of blood ethanol concentrations greater than 80mg/dL and is associated with an increased risk of ethanol dependence. Recent evidence has shown that an overlap may exist between systems involved in ethanol dependence and those involved in excessive ethanol consumption. Our laboratory has previously shown that antagonism of corticotropin-releasing factor (CRF) receptors within the central amygdala (CEA) reduces binge-like ethanol consumption. The current study expounds these findings by measuring ethanol consumption following the manipulation of CRF signaling using Cre-induced DREADD technology. This technology allows selective activation or inhibition of specific Cre-positive neuronal populations. AAV8-hSyn-DIO-hM4D(Gi) or a control virus were bilaterally infused in the CEA of CRF-Cre transgenic mice. Ethanol and sucrose were administered using a binge-like model of consumption known as the drinking in the dark paradigm (DID). The DID model is a 4-day procedure in which mice are given access to a 20% (v/v) ethanol or 3% (w/v) sucrose solution three hours into the dark cycle. On the first three days, ethanol or sucrose solution was available for two hours but on the 4th day, known as the test day, mice were given 4 hour access. This procedure was repeated four times. On the first two test days, mice received clozapine-N-oxide (CNO) to inhibit CRF-neurons and ethanol consumption was measured. To determine if the virus had an effect independent of CNO, mice received a saline injection prior to access to the ethanol solution on the third test day. Finally on the test day of the fourth cycle, mice were given access to sucrose solution and CNO was administered to determine if manipulating CRF signaling had an effect on general rewarding solutions. T-tests were used to determine if CRF inhibition within the CEA had a significant effect on ethanol or sucrose consumption. Gi-DREADD activation in the CeA resulted in reduced ethanol consumption ([Test 1: Gi = 2.875 ± 0.3775; control = 5.036 ± 0.3765]; [Test 2: Gi = 3.384 ± 0.2911; control = 5.009 ± 0.4624]). However when mice were injected with saline, no significant difference in consumption was observed. Moreover, no significant effect of inhibition of CRF signaling on sucrose consumption was observed. These data suggest that inhibition of the CRF signaling pathway in the CEA specifically reduces binge-like ethanol consumption and remains a viable target for manipulating binge-like ethanol consumption. (Support by NIH grants AA022048, AA013573, AA015148, AA011605 & NIGMS GM000678).

Age effects on hippocampal functional connectivity during multifeatural encoding
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During successful episodic memory encoding in young adults (YAs), functional connectivity of the hippocampus decouples from other regions of the Default Mode Network (DMN) to allow for efficient memory formation (Huijbers et al., 2011). The current study tested the hypothesis that older adults (OAs) would show a comparable decoupling of the hippocampus from the DMN during multifeatural source encoding. Functional magnetic resonance imaging was conducted while YAs and OAs intentionally encoded words along with their color and location. Through our experimental design, memory accuracy was deliberately equated between YAs and OAs. Univariate analyses revealed that successful multifeatural encoding, as compared to single item encoding, activated several regions in both age groups, including the left hippocampus. Additionally, while YAs deactivated parietal cortex during multifeatural encoding, OAs deactivated frontal regions. Functional connectivity analyses using left hippocampus as a seed region revealed a set of primarily frontal regions (e.g., superior and middle frontal gyrus and anterior cingulate) that were functionally correlated with the left hippocampus in YAs. In contrast, hippocampal activity among OAs correlated with bilateral superior, inferior, and middle temporal gyrus, as well as left angular and left supramarginal gyrus. Reductions in hippocampal connectivity to DMN regions are consistent with prior studies and have been suggested to support efficient encoding in YAs. Older adults, however, are less able to decouple the DMN even during successful episodic encoding. Given that functional connectivity differences manifested under equivalent behavioral performance, alterations to functional networks appear to precede age-related behavioral changes in source memory encoding.
**P5: A two-layered architecture underlies descending control of limbs in Drosophila**  
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Executing fast and precise movements appropriate to a given context requires the nervous system to integrate multiple streams of sensory information as well as recruit complex multi-jointed limbs in the correct sequence and trajectory. Considerable progress has been made in understanding how a given region of the brain (e.g., motor cortex) controls a single behavior driven by a single stimulus. However, motor control is distributed among many distinct circuits in the brain that work in concert to control behavior. In mammalian systems, the large number of neurons distributed throughout many brain regions presents a major impediment to obtaining an integrated view of motor control. Here we propose to take advantage of Drosophila as a model system. Like humans, Drosophila has also solved the problem of integrating multiple streams of information and controlling multi-jointed limbs, but does so with far fewer neurons, making it possible for us to understand principles of descending motor control at the level of single neurons.

We employ a multi-pronged approach to the study of descending motor control. To understand how sensory information from multiple modalities and instructions related to motor commands are organized in the descending neuron population, we have developed methods to perform in-vivo whole-cell patch clamp recordings in the fly brain while tracking the fly’s leg movements and stimulating the fly with stimuli from multiple modalities. We have also developed methods to genetically activate and inactivate known population of DNs to assess their role in motor control. Finally, to understand the circuit architecture underlying descending control, we will use an ex-vivo preparation to establish connectivity between upstream circuits in the brain, DNs and circuits in the thoracic ganglia.

We hypothesize that descending control in Drosophila is based on a two-layered neural architecture. One set of DNs (driver DNs or DDNs) are strongly-tuned to sensory stimuli, directly contact motor neurons and initiate movement. These DNs also send axon collaterals to activate a second set of DNs (modulatory DNs or MDNs). MDNs are not tuned to sensory stimuli, are activated following movement initiation, and have activity strongly correlated to the speed of movement. Activating MDNs in ex-vivo preparations result in a large increase in the excitability of motor neurons.

**P18: Investigating the role of central interleukin-1 in heroin-conditioned immunomodulation**  
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Heroin use has been shown to suppress several immune parameters that are important to the innate immune response. Previous studies in our laboratory have shown that the effects of heroin on immune function can be conditioned to environmental stimuli. Moreover, we have demonstrated that the nucleus accumbens (NAc) and basolateral amygdala (BLA) are important neural substrates that mediate the expression of heroin-conditioned immunosuppression. Central interleukin-1 has been identified as an important molecule mediating the expression of conditioned responses. Therefore, the present study investigated the role of IL-1 signaling within the BLA and NAc in the expression of heroin-conditioned immunosuppression. On test day, rats were re-exposed to a previously heroin-paired environment followed by systemic lipopolysaccharide treatment to induce an immune response. Bilateral administration of the IL-1 receptor antagonist IL-1Ra in the BLA, but not the NAc, prior to context re-exposure blocked the expression of heroin-conditioned immunosuppression, suggesting IL-1 plays an important role in heroin-conditioned immunomodulation.
P10: Chemogenetic inactivation of the insular cortex selectively increases alcohol self-administration based on alcohol drinking history
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In humans, the insular cortex (IC) is known to be involved in interoceptive states and decision making processes. Additionally, preclinical models have verified the role of the IC in drug-seeking and self-administration of various drugs of abuse. However, a role of the IC in modulating alcohol-related behaviors has not been fully established. Thus, our overall goal was to examine the role of the IC in modulating alcohol self-administration in male Long Evans rats. First, to examine IC neuronal response to alcohol, rats were administered alcohol (0 or 1g/kg, IG; n=6-7) and brain tissue was processed for c-Fos immunoactivity (IR). Alcohol induced an increase in c-Fos IR in the IC, demonstrating a response to alcohol. Next, to validate the use of a chemogenetic approach by which to inactivate the IC, rats were microinjected with inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; hM4D(Gi)). DREADD expression and functional validation were confirmed with IR and electrophysiological recordings. Next, to determine the functional role of the IC in modulating self-administration, rats (n=12) were microinjected with the Gi DREADDs in the IC and trained to operantly self-administer alcohol (15% v/v+2% sucrose w/v). Rats received pretreatment with 1 mg/kg clozapine-N-oxide (CNO; IP), to activate DREADDs 30, 45, or 60 min prior to a self-administration session. An overall escalation in self-administration was observed. Interestingly, when examined based on drinking history, a difference in self-administration following CNO was observed in the 45 min pretreatment group. Low drinkers (<0.5 g/kg) showed escalated self-administration following chemogenetic inactivation of the IC, while no significant change was observed in high drinkers (>0.5 g/kg), demonstrating a potential role for the IC in modulating alcohol SA based on alcohol drinking history.

P42: Sensory neurons directing lung inflammation
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Background. Lung inflammation due to pneumonia can be deadly. The study of inflammation focuses on immune cell (neutrophil) activation since their presence defines inflammation in an innate immune response. However inflammation appears to be orchestrated by sensory neurons of the vagus nerve. The activity of a specific subset of nociceptors or pain sensing neurons which produce TRPA1 is a requirement for an inflammatory response. The mechanism by which nociceptor activity enables immune cell response is unknown. My research seeks to determine changes occurring in the nodose ganglia of the vagus nerve that link immune cells to neurons. I sequenced RNA of the nodose ganglia in healthy versus sick mice and assembled a differential transcriptome in order to identify genes that direct inflammation.

Methods. Female C57Bl/6 mice were exposed intra-nasally to lipopolysaccharide or saline vehicle for 3 consecutive days then euthanized on day 4. The BAL fluid was collected RNA was extracted from the nodose and sequenced using an Illumina HiSeq2500. TopHat and Cufflinks were used to align 50 bp single-end reads and assemble transcripts to determine the fragments per kilobase of exon per million reads mapped (FPKM). Differential expression was based on the following criteria: standard error < 33% of mean one mean FPKM > 1 p-value < 0.05 and a > 2-fold change in expression.

Results. I found significant changes in 34 genes 29 up-regulated and 5 down-regulated. Of these genes 15 are known to mediate immune responses. Notably two known neutrophil marker genes Lrg1 and Lcn2 had a greater than 4-fold increase in the sick mice. The presence of neutrophil markers was confirmed with qPCR. Interestingly the down-regulated gene Postn found to decrease ~2.5-fold is a marker associated with adaptive not innate immune responses. No neuron specific genes were identified in the differential transcriptome.

Conclusions. Lung inflammation due to an innate immune response stimulates the appearance of neutrophils within the nodose ganglia. Their presence allows neurons to interact directly with immune cells. The down-regulation of adaptive immunity markers suggests a differential role of neurons based on the type of immune response innate versus adaptive. These data show that the nodose ganglia enables the direct communication between immune cells and neurons during inflammation. These findings are significant because they present a new venue for neuron-immune cell interactions involved with lung inflammation and provide us with a unique target for future drug development.
P22: The effects of prenatal nicotine exposure on the developing K-complex
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Smoking cigarettes during pregnancy is detrimental to the health of the unborn child yet 15% of expectant mothers smoke. Prenatal nicotine exposure correlates with sensory processing deficits and increased risk of developing disorders of attention (Horst et al. 2012 Heath & Picciotto 2009). The K-complex is produced by the GABAergic networks in the brain and these networks have been implicated in attention. The K-complex can first be seen in infants around 4 months of age (AASM Manual for Scoring Sleep 2007) is crucial for the preservation of sleep (Bastien et al. 2000) is analogous to the orienting response during wakefulness and indicates the degree of sensory processing (Andreassi 2007). In this study brain activity was recorded during a paired-click paradigm (Hunter 2008) to elicit auditory K-complexes during sleep. Thirty-three 4-month-old infants sixteen prenatally exposed to nicotine and seventeen healthy controls completed developmental testing. Then the K-complex task was performed while electroencephalogram (EEG) was recorded during a nap. Stage 2 sleep was identified from the recordings and epochs surrounding the K-complex were analyzed. The amplitude of the K-complex components and delta band (1-4Hz) power in the K-complex was calculated for each infant and compared between groups. The prenatal nicotine exposed group shows a smaller amplitude (p = .036) and less delta power (p = .035) than the control group. This may suggest that infants exposed to nicotine do not appropriately gate out sensory information and fail to proceed into deeper sleep necessary for typical development. It is possible that the K-complex can be used as a biomarker for the development of attentional deficiencies in the future.

P14: Ethanol Withdrawal in Adolescent and Adult Rats
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Background: Adolescents and young adults consume more alcohol than adults, and early initiation of alcohol use is associated with increased risk of alcohol dependence in adulthood. However, the factors that contribute to this increased intake/risk of dependence are not well understood. The immediate and protracted effects of alcohol withdrawal are thought to contribute significantly to consumption of alcohol by alcohol dependent adults. However, there is remarkably little data about either immediate or protracted alcohol withdrawal in adolescents. We evaluated the behavioral and endocrine effects of mild alcohol dependence in adolescent and adult rats.

Methods: Adolescent (PN 28) or adult (PN 70 or older) male and female rats from Charles River Laboratories were used in all experiments. Animals received 5 days of ethanol treatment (1.5 g/kg, 3 injections at 3 hour intervals). Acute withdrawal was assessed 18 hours after the last dose by quantitatively assessing tail rigidity, vocalizations, ventromedial limb flexion, abnormal posture/gait and tremors from 0-2 (0 = no sign, 1 = moderate, 2 = severe). Post withdrawal anxiety was determined 4 days after the final dose via light/dark box testing to assess protracted withdrawal. Anxiety-related measures (latency to emerge into light, percent time in light, rearing) and locomotor measures (total distance traveled, distance in the dark) were measured. Animals were decapitated at the end of the light/dark test for measurement of serum corticosterone to assess stress-induced hormone release. A separate cohort of animals was injected with ethanol and 1 hour after the 1st, 2nd or 3rd injection to measure blood alcohol content (BAC) in an Analox BAC analyzer. Statistics on all results were analyzed by 3 way ANOVA (age x sex x treatment) using NCSS. All experiments were approved by the Duke University IACUC.

Results: Acute withdrawal signs were comparable in adolescents and adults and did not differ by sex. Ethanol withdrawn adults exhibited significant decrease in time spent in light, enhanced latency to enter light and decreased rearing although responses were somewhat milder in females than males. Alcohol-withdrawn adolescent exhibited less rearing, but other behavioral signs (latency to enter light, time in light) did not show significant changes. Overall, the data show that adolescents and adults exhibited comparable acute withdrawal 24 hours after the end of treatment, but that adults experienced more anxiety assessed 4 days later.

Discussion: These results suggest that acute withdrawal from ethanol, which largely reflects CNS hyperexcitability mediated by changes in GABA and glutamate function, is comparable in adolescents and adults after a brief (5 days) ethanol exposure. This finding suggests that the neurochemical adaptation mediating these behaviors may be comparable in adolescents and adults. In contrast, the protracted effects were milder in adolescents and adults. The latter, which are mediated largely by CRF, may reflect lesser adaptation of this neural circuitry in adolescents than adults. Future study of these neurochemical mechanisms in alcohol dependent adolescents may provide insight into age-selective pharmacotherapies for alcohol dependence in adolescents. (Supported by NIAAA 017621)
P13: Membrane properties and GABAergic synaptic transmission in central amygdala neurons in mice lacking BK channel beta-1 subunits: Implication of BK channel in alcoholism
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The central nucleus of the amygdala (CeA) has been implicated in regulating alcohol drinking behavior and alcohol abuse. Alcohol may alter drinking behavior through its action on ion channels of neurons. Large conductance Ca++-activated BK potassium channels are expressed in CeA neurons and regulate neuronal excitability and transmitter release. However, the role of BK channels and their accessory beta subunits in alcoholism remains unclear. Using whole cell patch-clamp recordings in an acute CeA slice preparation from genetically modified mice, we have examined membrane properties and inhibitory synaptic transmission in CeA neurons lacking BK channel beta-1 (BK-?1) subunits. We found no difference in resting membrane potential, time constants or input resistance between neurons from ?1 knock-out (KO) and wild-type (WT) mice. Ethanol (50 mM) had no effect on time constants or action potential threshold in neurons from both KO and WT mice. However, neurons from KO mice fired more action potentials in response to depolarizing current injection than neurons from WT mice. In contrast to neurons from WT mice, action potentials in neurons from KO mice showed a stronger frequency dependent broadening with spike trains at 5, 10, 20 and 20 Hz. However, 50 mM ethanol caused a significant frequency dependent broadening in spike trains at 5, 10, 20 and 20 Hz in neurons from WT mice. Baseline frequency and amplitude of spontaneous postsynaptic inhibitory currents (sIPSCs) mediated by GABA receptors were similar in neurons from KO and WT mice. Ethanol increased the frequency of sIPSCs in both groups without effects on amplitude of sIPSCs. Our preliminary results indicate that functional neuronal BK channels are present in CeA neurons lacking beta-1 auxiliary subunits, although beta-1 subunits may modulate ethanol sensitivity of BK channels.

P39: Mechanosensory neurons contribute to egg-laying induced acetic acid attraction in fruit flies
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Animals often exhibit different behavioral responses to the same sensory stimuli according to their physiological states. Here we show that female flies exhibit attraction to 3% acetic acid prior to egg laying, but usually avoid same stimulus. By genetically manipulating neural activity during behavior, calcium imaging, and analyzing the trajectory of fly locomotion, We identified a group of sensory neurons that sense the distortion of reproductive tract when an egg is passing by. These neurons are essential to initiate the acid attraction induced by egg laying need. Our work uncovers the circuit component represent an important physiological states in fruit flies, and provides a simple model to dissect the neural mechanism that underlies a reproductive need-induced behavioral modification.

P17: K-targeted transgenic mice for preclinical models of drug addiction
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Rodent preclinical models of addiction have been used to determine potential therapeutic approaches to addictive behavior. Transgenic mice afford researchers an opportunity to selectively manipulate signaling in the brain and determine how it affects behavior. Interestingly, there are lines of transgenic mice that allow for cell-specific targeting of cellular processes. Astrocytes are the most abundant neural cell in the brain. Though historically considered a structural support cell, astrocytes have more recently been found to regulate numerous synaptic mechanisms and behavior. In particular, astrocytic processes such as glutamate transport have been shown altered following exposure to drugs of abuse, and restoration of normal activity has returned the animal to non-pathological behavior in some cases. One mechanism by which astrocytes can respond to extracellular stimuli is through the IP3 receptor-dependent Ca2+ pathway. Activation of astrocytes evokes an intracellular flux of Ca2+ that can modulate various pathways within the cell. Among all IP3 receptors in the brain, astrocytes express the type II IP3 receptor (IP3R2), while neurons do not. By genetically deleting IP3R2, we can prevent evoked Ca2+ fluxes in astrocytes without directly altering neuronal Ca2+ signaling. The data presented here represent a first pass analysis of how IP3R2 knockout mice (KO) perform in preclinical models of cocaine addiction, including behavioral sensitization and conditioned place preference.
**P16: Effects of chronic intermittent ethanol exposure and drinking on 3α,5α-THP levels in limbic brain structures following withdrawal in C57BL/6J mice**

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The most widely studied GABAergic neuroactive steroid (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP, allopregnanolone) is altered during ethanol withdrawal in humans, rats and mice. Recently, we observed chronic intermittent ethanol (CIE) exposure decreases 3α,5α-THP levels in the lateral amygdala, ventral tegmental area, medial prefrontal cortex and nucleus accumbens while increasing 3α,5α-THP levels in the CA3 pyramidal cell layer of the hippocampus following 72 hr withdrawal in ethanol-exposed compared to ethanol-naïve mice. Given CIE exposure increases subsequent voluntary ethanol drinking following 72 hr withdrawal, we examined changes in brain regions that showed alterations in 3α,5α-THP immunolabeling in mice with a history of ethanol drinking. Adult male C57BL/6J mice were exposed to the CIE model to produce ethanol dependence. Briefly, after establishing stable baseline drinking using a limited access (2 hr/day) 2-bottle choice (15% ethanol vs. water) paradigm, mice received four cycles of CIE exposure (16 hr/day x 4 days) to ethanol vapor or air in inhalation chambers. Exposure cycles 1-3 were each followed by a week of daily limited access drinking test sessions. All mice were sacrificed and perfused 72 hr after the final (4th) exposure cycle. Free floating brain sections (40 microns; 4-6 sections/region) were immunostained and quantified for 3α,5α-THP labeling for each mouse (n=9/group). CIE-exposed mice showed the expected increase in ethanol drinking across exposure cycles compared to air-exposed mice (p<0.0001). Preliminary results indicate ethanol-exposed mice showed a 23.7±10.8% decrease (p<0.05) in 3α,5α-THP immunolabeling in the CA3 pyramidal cell layer of the hippocampus. There were no significant changes in 3α,5α-THP immunolabeling in the lateral amygdala or in the dorsal or ventral bed nucleus of the stria terminalis. Currently other brain regions that showed reductions in 3α,5α-THP immunolabeling following exposure to four CIE cycles without a history of ethanol drinking are under investigation. These preliminary results suggest that specific adaptations in neuronal 3α,5α-THP levels in discrete limbic subregions may mediate changes in ethanol drinking across repeated CIE exposure. A history of ethanol drinking appears to produce divergent changes in 3α,5α-THP immunolabeling at the time point when elevated drinking is observed, at least in the hippocampus. These differences may have functional consequences that mediate behavioral adaptations to ethanol.

**P11: Components of novelty seeking and the sweet-liking phenotype interact to increase risk of alcohol-related problems in young adults**

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A combination of high novelty seeking (NS) and sweet-liking (SL) are associated with an increased risk of alcohol-related problems in young adults (Kampov-Polevoy et al., 2014). The present analysis reexamined this sample and studied the four components of novelty seeking (exploratory excitability, impulsiveness, extravagance, and disorderliness) with regards to risk for alcohol problems and for interaction with the SL phenotype. The sample consisted of 163 young adults, ages 18 to 26, balanced for gender. Novelty seeking and each NS component were assessed by the Tridimensional Personality Questionnaire (Cloninger, 1987). Binary logistic regression was used to compare the NS components and SL relationship, and the Alcohol Use Disorders Identification Test (AUDIT) and Rutgers Alcohol Problem Index (RAPI) were used as the means of determining history of alcohol-related problems (Saunders et al., 1993; White and Labouvie, 1989). It was found that the NS components demonstrated a similar relationship between SL and alcohol-related problems as denoted by the RAPI. When combined with the SL phenotype, high exploratory excitability had an odds ratio of 5.50 (95% CI: 1.04, 29.11), impulsiveness and extravagance had odds ratios of 5.33 (95% CI: 1.05, 27.25), and disorderliness had an odds ratio of 4.14 (95% CI: 0.84, 20.43). However, the full effect was only seen once all of the components were combined (OR = 19.30, 95% CI: 13.14, 118.63). This suggests that each facet of NS is involved in heightening the risk for alcohol-related problems.
P7: Socially evaluated cold pressor stress renders stimulus-response associations habitual after response devaluation in healthy controls

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Habitual behaviors resist change and theoretically promote compulsive drug use and relapse susceptibility that characterize addiction; stress has been shown to be a predictor of relapse, although the mechanisms by which stress promotes a return to drug use are unknown. A socially evaluated cold pressor test (SECPT) enhances habitual actions, and elevates salivary cortisol/heart rate, but the effect of acute stress on overcoming habitual responses has not been tested empirically. We tested S-R learning and “re-learning” after response devaluation in healthy control participants (n=36) using a conditional S-R paradigm. Participants were shown abstract visual stimuli, and learned, through trial and error, rules associating the stimuli with manual responses (Boettiger & D’Esposito, 2005). Prior to testing, participants learned 2 S-R sets (FAM) to a criterion of ≥90%. After ≥1 night’s sleep, participants returned to show retention of FAM sets and learn 2 new S-R sets (NOV). After 6 blocks, responses for one FAM set and one NOV set were devalued to test the ability to re-learn S-R contingencies. Participants were assigned to one of three SECPT conditions during testing: (1) stress before NOV learning (n=11); (2) stress before re-learning (n=13); (3) no stress control (n=12). Preliminary analyses show no differences in accuracy for FAM and NOV sets prior to devaluation based on stress group (all p’s >0.10). In contrast, stress potentiates habitual responding that is selective to re-learning FAM associations (p<0.001), as demonstrated by increased perseverative responding in the stress groups compared to control. Future work may test the underlying neural substrates of these behavioral differences.

P9: Inhibition of the OX-1 receptor in the ventral tegmental area selectively protects against binge-like ethanol consumption

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Orexin (OX) neurons originating in the hypothalamus are ideally positioned to modulate reward processing as they form connections with several key brain regions of the reward pathway including the ventral tegmental area (VTA). Consistent with these findings a growing number of studies have implicated the OX system in modulating ethanol responding yet its role in binge-like ethanol intake remains relatively unexplored. We have previously demonstrated that the OX system in the lateral but not perifornical area of the hypothalamus is engaged during binge-like intake of both ethanol and saccharose solutions and that peripheral injection of the OX-1 receptor (OX1R) antagonist SB-334867 (SB) blunted ethanol and saccharose drinking while not affecting locomotor activity (Olney et al 2015). Together these data suggest that reward-related OX circuits originating in the lateral hypothalamus participate in the consumption of salient reinforcers regardless of calories that is not secondary to an induction of a hypoactive state. Here we sought to provide a more detailed examination of the OX reward circuitry involved in binge-like ethanol drinking by characterizing the participation of the OX1R and OX-2 receptors (OX2R) within the VTA in binge-like ethanol drinking. The “drinking in the dark” paradigm was used to model binge-like drinking in C57BL/6J mice. In the first experiment we examined binge-like ethanol or saccharose intake following bilateral intra-VTA infusions of 0.0 or 6.0 µg/0.3 µl/side SB. Subsequent experiments examined binge-like ethanol intake following bilateral intra-VTA infusions of 0.0 or 5.0 µg/0.3 µl/side TCS-OX2-29 (TCS) a selective OX2R antagonist. We observed that inhibition of OX1Rs in the VTA via SB blunted ethanol intake during the first hour of testing but did not alter saccharose drinking. Inhibiting the OX2Rs in the VTA via TCS had no significant effect on ethanol consumption during binge testing. Together these data indicate that OX1R but not OX2R signaling in the VTA in part significantly regulates binge-like ethanol drinking behavior. Intriguingly although peripheral SB blunts binge-like saccharose and saccharose drinking intra-VTA infusion of SB did not alter saccharose intake- suggesting that a distinct OX circuit is recruited for ethanol consumption versus a natural reward. (Support by NIH grants AA022048 AA013573 & AA015148).
P3: Interaction between forebrain cholinergic projections and local interneurons in the regulation of adult hippocampal neurogenesis.

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Adult neurogenesis is a robustly documented but poorly understood phenomena that in humans is restricted to the dentate gyrus of the adult hippocampus. While adult neurogenesis recapitulates many of the processes and events of its developmental counterpart, it is uniquely regulated by experience and environmental influence as a function of the activity of long-distance and local neuronal networks—the identity and nature of which are poorly defined. One system strongly linked to both hippocampal function and adult neurogenesis is the forebrain cholinergic system. Cholinergic afferents contribute significant control over the activity of local principal cells and interneurons through both metabotropic and ionotropic receptors. However, the diversity of these afferent inputs (and the cognate heterogeneity of the cholinergic nuclei) obscures the differential role of the cholinergic system, and may partially explain the discrepancy of conclusions in the extant literature. In order to dissect the modulatory influence of differential cholinergic components to the regulation of adult neurogenesis, we are combining electrophysiology, calcium imaging, retrograde trans-synaptic tracing, and in vivo optogenetic manipulations. Here we report that one level of cholinergic regulation of neural stem cell activity may be through the local Parvalbumin-expressing basket cell (PV-cells). GABA release from PV-cells suppresses neural stem cell proliferation, and bath application of acetylcholine suppresses Ca$^{2+}$ in PV-cells. Consistent with a model of cholinergic of PV-cells is our finding that cholinergic terminals are closely associated with PV-cell terminals in the dentate gyrus. Additionally, retrograde monosynaptic tracing utilizing rabies-virus identifies cell bodies in the medial-region of the Diagonal Band of Broca as the primary source of cholinergic inputs to these cells. Direct stimulation of these inputs to the dentate gyrus produces a significant increase in neural stem cell proliferation—consistent with a model of PV-cell inhibition and subsequent release from GABAergic suppression of neural stem cell proliferation. While preliminary, these data identify a specific putative coupling between long-distance neuromodulatory systems and local interneurons that regulates neuronal-activity dependent adult neurogenesis. Ongoing efforts are focused on determining the nature of cholinergic receptor subtypes involved in this process, and the contribution of differential cholinergic activity to both direct and indirect regulation of neural stem cells.

P23: Ventral tegmental area mu opioid receptor modulation of phasic dopamine release in the ventral striatum and effects on reinforcement

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Endogenous opiates play a critical role in reward processing and motivation. Mu opioid receptors (MOR) in the ventral tegmental area (VTA) are of particular interest as this region contains dopamine (DA) neurons projecting to the ventral striatum which are highly implicated in various aspects of reward. However our understanding of the mechanisms by which these MORs underlie VTA function is limited. Both systemic and intra-VTA administration of MOR agonists can produce positive reinforcement and increase DA release in the ventral striatum. By contrast intra-VTA administration of MOR antagonists can elicit place aversion and antagonize increases in striatal DA elicited by intra-VTA agonists. These findings are in accordance with two widely accepted ideas: that robust striatal DA release is sufficient to elicit place preference and that opiates excite VTA DA neurons by disinhibition. However several lines of evidence suggest that more complex mechanisms may be at play. This project monitors the effects of intra-VTA infusion of MOR-specific drugs on sub-second DA release in the nucleus accumbens (NAc) of awake rats using fast-scan cyclic voltammetry and directly correlates these measurements with conditioned place preference experiments. Our data shows that intra-VTA infusion of MOR agonist DAMGO (1.5 µM) significantly increased the frequency and amplitude of DA transients recorded in NAc and elicited conditioned place preference. Interestingly intra-VTA infusion of the MOR antagonist CTOP (0.16 µM) also significantly increased the frequency and amplitude of DA transients recorded in NAc but elicited conditioned place aversion. These results challenge accepted ideas and unequivocally demonstrate that there are complex mechanisms underlying MOR actions in the VTA. Importantly this research will improve our understanding of how this circuitry underlies motivated behavior.
P40: Altered hypothalamic estrogen receptor expression in juvenile and adult animals from NCTR subchronic toxicity evaluation of bisphenol A
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The present studies assessed hypothalamic Esr1 (estrogen receptor alpha) and Esr2 (estrogen receptor beta) expression in juvenile (postnatal day (PND) 21) and young adult (PND 90) Sprague Dawley rats from a NCTR based toxicity study evaluating the effects of BPA (the larger study is described in Delclos et al 2014 Tox Sci). Dams were gavaged daily from gestational day 6 until labor began with vehicle (0.3% carboxymethylcellulose) 2.5 25 260 or 2700 ug BPA/kg by weight/day or 0.5 or 5.0 ug ethinyl estradiol/kg by weight/day. Pups were then gavaged from the day after birth (PND 1) until the day before scheduled sacrifice on PNDs 21 or 90. Brains were collected by NCTR coded (so subsequent work could be done blind to exposure group) and shipped to NCSU for Esr1 and Esr2 expression analysis using in-situ hybridization. Compared to vehicle controls Esr1 expression in the juvenile female rat anteroventral periventricular nucleus (AVPV) of the hypothalamus was significantly decreased in the 25 ug/kg BPA group while 2.5 25 and 260 ug/kg BPA produced significant decreases in Esr2 expression in the adult female rat AVPV and medial preoptic area (MPOA). Decreases in expression in the female AVPV eliminated a sex difference and in the MPOA a sex difference was reduced. These data demonstrate the potential for neural effects at doses below the current lowest observed adverse effect level.

P30: Neuronal activity induces transcription of two sub-classes of immediate early genes
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National Institute of Environmental Health Sciences, Neurobiology

The brain’s ability to learn and form long lasting memories depends on de novo gene transcription in response to environmental stimulation. Following neuronal stimulation, neurons express a select few genes, termed immediate early genes (IEGs), within a short period of time. In cultures of primary cortical rat neurons we have observed that, similar to what is seen in behaving animals, some IEGs, such as activity-regulated cytoskeleton-associated protein (Arc), are up-regulated within 5 minutes of stimulation. We have noted that these rapidly responding genes have RNA polymerase II (Pol II) bound near their transcription start site (TSS) and now define them as Rapid IEGs (Saha et al. 2011). Conversely, we have defined Delayed IEGs such as brain-derived neurotrophic Factor (Bdnf) as those that generally lack a stalled polymerase near their TSS and are expressed with a longer time lag. Here we present evidence that these IEG subclasses can be further differentiated by their induction requirements.

Previous studies have shown that several (Rapid) IEGs can be induced in vivo with as little as one lap around a track (Miyashita, et al. 2009). Therefore, we tested whether Rapid and Delayed IEGs responded differentially to very brief periods of increased neuronal activity. We find that Rapid, but not Delayed IEGs, are transcribed in response to 2 or 5 minutes of neuronal activity, whereas Delayed IEGs required prolonged periods of activity. Moreover, Rapid, but not Delayed IEGs can be induced with pharmacological stimulation of two upstream activators of the MEK/ERK pathway (phorbol 12-myristate 13-acetate (PMA) and Forskolin, through PKC and PKA respectively) in the absence of neuronal activity. Inhibitors of the MEK/ERK pathway, including PD184352 and 11e, block the induction of both IEG subclasses. These results suggest that Delayed IEGs require signaling factors in addition to the MEK/ERK pathway, but that ERK activity by itself may be sufficient for the Rapid IEG response. In summary, we present further evidence that Rapid IEG induction is mechanistically distinct from Delayed IEG induction in response to neuronal activity.
P28: Prioritizing compounds for targeted developmental neurotoxicity testing through utilization of human induced pluripotent stem cells in a high throughput high content screening assay
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With the recent increase in the prevalence of developmental neurotoxicity (DNT) in children, there has been recognition that rapid, reliable, and efficient screening tools are needed for better identification, prioritization, and evaluation of chemicals with the potential to induce DNT. In an effort to develop and characterize an in vitro model system for DNT screening, we exposed human iPSC-derived neurons to a diverse set of 80 compounds comprising different functional and structural classes. The test set included: (i) known DNT and neurotoxicants, (ii) NTP (National Toxicology Program) chemicals of interest with unknown neurotoxic potential (flame retardants (FRs), polycyclic aromatic hydrocarbons (PAHs)), (iii) known positive and negative compounds for neurite outgrowth, and (iv) test replicates as internal controls. Neurons were treated in duplicate across a 6-point concentration range (~0.3 to 100 μM) in 384-well plates. Using high content imaging, effects on neurite outgrowth parameters (total outgrowth, processes, branching) and cell viability were monitored after 72 h of exposure. Also, mitochondrial membrane potential (MMP) was evaluated at 1 h to assess the potential contribution of MMP to altered neurite outgrowth. The assay-specific noise threshold was calculated based on DMSO control variability and concentration-response profiles were evaluated using a Hill model to derive benchmark concentration (BMC) point-of-departure values. Following assay validation with controls and test replicates, chemicals were ranked by toxicity and selectivity (i.e., effects on neurite outgrowth parameters independent of cytotoxicity). Neurite total outgrowth and branching were the most sensitive endpoints; 16 compounds had an effect on neurite outgrowth independent of cytotoxicity (the pesticide rotenone was the most selective) including 1 FR (triphenyl phosphate) and 3 PAHs (chrysene, dibenz(a,h)anthracene, acenaphthylene) inhibited neurite outgrowth. Of the 80 compounds, 39 (49%) decreased MMP. Nine compounds were active in both assays, which might indicate that alterations in MMP are linked with neurite outgrowth inhibition for these compounds. These studies have important implications for moving the DNT field forward from a more traditional assessment in animals to implementing novel and improved methodologies that will allow for a more rapid screening and prioritization of potential developmental neurotoxicants for further in hazard characterization in vivo.

P43: Effects of perinatal nutritional deficiencies on anxiety- and depressive-like behaviors in mice
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Nutritional deficiencies during the perinatal period have been linked to increased risk for development of psychiatric disorders in the human population and have been modeled in rodents and result in behavioral changes. The mechanism in both humans and animal models is believed to be epigenetic changes that alter gene expression. We designed an experiment to examine the effects of maternal dietary modifications on various genetic backgrounds to study alterations in behavior and gene expression in mice. We utilized a disjoint reciprocal diallel with 16 Collaborative Cross (CC) mouse lines crossed to produce recombinant inbred intercross (RIX) mice. Females from each line are exposed to one of four diets (vitamin D and protein deficient, methyl enriched and standard) prior to mating and throughout gestation and weaning. Adult female offspring are either tested in behavioral assays to measure stress response, anxiety- and depressive-like behaviors or whole brain gene expression analysis using RNA-Seq. Comparison within reciprocals and across CC-RIX lines will reveal genetic, diet, parent-of-origin and diet-specific parent-of-origin effects. We have completed behavioral phenotyping for over 600 CC-RIX offspring and assessed gene expression in 96. We have identified significant strain, parent-of-origin, and diet effects on behavior and are in the process of analyzing the gene expression data and identifying possible candidate genes that interact with diet during development to change behavior in adult offspring for validation and further testing.
Cocaine abuse alters cellular dynamics within several regions of the brain’s reward circuitry, including the ventral tegmental area, nucleus accumbens and prefrontal cortex, among others. Elucidation of these cellular adaptations can help identify pharmacotherapeutic candidates for cocaine addiction. One such adaptation is a cocaine-dependent decrease in glutamate transporter-1 (GLT-1) expression in the nucleus accumbens. GLT-1 is responsible for approximately 90% of glutamate uptake in the brain, and is critical for neuroprotection and fidelity of synaptic processing. Previous studies have reported that compounds which restore expression of GLT-1 can also reduce behavioral measures of drug seeking. Thus, we wished to test the hypothesis that another known regulator of GLT-1, riluzole, might also reduce cocaine seeking. Riluzole is an FDA approved drug for Amyotrophic Lateral Sclerosis (ALS), which decreases neuronal activity by blocking voltage gated sodium channels. In addition, riluzole upregulates expression of GLT-1 in astrocytes in animal models of depression, resulting in anti-depressant behavioral effects.

However, no studies have investigated the effect of riluzole in cocaine addiction. To determine if riluzole has an effect on cocaine seeking, we employed the rat self-administration/extinction/reinstatement model of cocaine abuse. During the extinction phase, rats received chronic intraperitoneal injections of 4 mg/kg or 1mg/kg riluzole, or vehicle. We observed a dose-dependent reduction in cue-primed reinstatement to cocaine. However, riluzole had no effect on cue-primed reinstatement of sucrose seeking. In addition, we recorded intrinsic excitability in prefrontal cortical neurons using whole-cell patch clamp recordings in slices of rats trained to administer cocaine or saline, receiving chronic riluzole or vehicle injections during extinction. Preliminary data from whole cell patch clamp recordings indicate an increase in number of evoked spikes in prefrontal cortex neurons in rats with a cocaine history, which is reversed by administration of riluzole. These results suggest that riluzole restores levels of intrinsic excitability in the prefrontal cortex, which may contribute to its effect on cocaine seeking. These results further support an existing body of literature which indicates GLT-1 regulators as therapeutic candidates for psychostimulant addiction. Riluzole may restore cocaine-induced adaptations within the brain’s reward circuitry, and may thus represent a novel pharmacological treatment for cocaine addicts.
Expression levels of Ubiquitin-protein ligase E3A (UBE3A) must be properly regulated for healthy neurological development. Increased UBE3A gene dosage is associated with autism spectrum disorders (ASDs), and loss of UBE3A expression causes Angelman syndrome (AS). AS is a severe neurodevelopmental disorder characterized by intellectual disability, lack of speech, and sensory processing deficits—domains that are also impacted in ASDs. Since neocortical circuitry is crucial for these functions, a pivotal question is how these circuits are disrupted in AS. A mouse model of AS, which lacks expression of the maternal Ube3a allele, recapitulates many features of AS in humans, including learning impairments. These AS model mice have deficits in synaptic function and experience-dependent plasticity in primary visual cortex. However, it was unknown how circuit-level development might be affected in AS mice, prompting us to examine how functional responses to visual stimuli across multiple cortical areas are disrupted in AS mice. We hypothesized that loss of expression of the maternal Ube3a allele impairs cortical circuit development and functional responses in vivo. Using intrinsic signal optical imaging to measure cortical activation in response to complex visual stimuli, we examined the functional development of primary visual cortex and surrounding higher visual areas (HVAs) in developing (postnatal day 20) and adult (postnatal day 85) AS model mice. Visual responses of developing AS model mice to complex stimuli were indistinguishable from their wildtype littermates. In contrast, adult AS mice exhibited impaired activation of HVAs specifically in response to stimuli with changing speeds, relative to wildtype littermates. These results implicate Ube3a in the development and function of higher order circuits in the cortex. Further dissection of circuit deficits in AS model mice will provide insight into the cellular mechanisms that underlie these impairments, informing our understanding of neocortical circuit development in Angelman syndrome and ASDs.

P6: Genetic modulation of concussion and football exposure effects on BOLD response and functional neural connectivity
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Recent studies have shown a link between concussions (or subconcussive episodes) sustained earlier in life and memory problems, dementia, and Alzheimer’s Disease (AD) later in life. However, little is known about individual differences in the long-term effects of concussion, and specifically whether genetic risk factors for AD, such as the Apolipoprotein-ε4 (APOE-ε4) allele, may better account for some of these effects or interact with concussion and exposure history to influence cognitive functioning years after concussive injury. In the present study, participants between the ages of 50-65 (N=63) were recruited based on concussion history (0-1 or 3+), and football exposure (college or college+NFL). Participants completed two batteries of neurocognitive tasks, and performed an fMRI- adapted N-back task to assess functional connectivity during working memory performance. Results from the functional neuroimaging analyses revealed that the interaction between concussion history and genotype accounted for some differences in parametric modulation of BOLD response within a fronto-parietal working memory network. Additionally, the interaction between APOE-ε4 status and exposure also accounted for some differences in magnitude of functional connectivity strength of this network. These findings suggest that not only do individuals with a history of repeated sport-related concussion who possess the APOE-ε4 allele seem to show a diminished ability to modulate recruitment of task-specific regions per task demands, but that the functional neural connectivity during working memory performance may be altered by the interaction between football exposure and APOE-ε4 status.
What are the behavioral and reproductive effects of SFPs in prairie voles?
A Vogel and L McGraw
North Carolina State University, Department of Biological Sciences

It has long been known that seminal fluid proteins (Sfps) play an integral role in fertilization, yet studies in insects and a handful of recent studies in mammals suggest that these proteins play important roles beyond merely supporting sperm health and longevity. These roles could include inducing ovulation in some species, or support conception and maintenance of pregnancy. Sfps could even impact the health of the offspring, due to maternal factors in utero. To increase our comprehension of the complex role of Sfps in mammalian reproduction, I am using a multidisciplinary approach incorporating behavioral, physiological, neurobiological, and genetic measures in prairie voles, a socially monogamous rodent. First, to understand whether seminal vesicle proteins cause behavioral and physiological changes in females and their offspring, we removed the seminal vesicles from male prairie voles. Females mated to removed, sham, or control males are being used to examine the short- and long-term behavioral and physiological effects of seminal vesicle proteins on the females and their offspring. Second, to understand the neurogenetic consequences of Sfps in the mated female, brains of females mated to males from each treatment group will be examined for Sfp induced changes in neuronal activity and in the brain transcriptome. These studies are the first of their kind and will enhance our knowledge in a largely unexplored field, with implications for human health.

Intrinsic Excitability and Excitatory Synaptic Input Do Not Differ by Sex in the Nucleus Accumbens Shell
JA Willett, T Will, CA Hauser, J Cao, DM Dorris, and JE Meitzen
North Carolina State University, Department of Biological Sciences

Sex differences exist in how the brain mediates motivated behavior and reward, both in normal and pathological contexts. For example, women are more susceptible to addiction and advance more rapidly through the stages relative to men. Investigations into the underlying neural mechanisms yield accumulating evidence of sexually different cellular morphology and neuromodulator/hormone action in the striatal brain regions, including the nucleus accumbens shell. It is unknown whether these sex differences influence the electrical properties of neurons in this brain region. This is a critical unaddressed question because the electrical activity of neurons directly underlies behavior, including motivation and reward. Thus, I hypothesize that the electrophysiological properties of medium spiny neurons (MSNs), the output neurons of this brain region, differ by sex. To test this hypothesis, I performed whole-cell patch clamp recordings on 35 female MSNs and 27 male MSNs in acute living brain slices of pre-pubertal rat nucleus accumbens shell. Blind analysis of the electrophysiological properties is ongoing, with a particular emphasis on excitatory synaptic properties and intrinsic excitability, which have been found to differ by sex in other striatal regions. Currently, analysis does not detect a sex difference in either the response of nucleus accumbens shell MSNs to positive or negative current stimuli or in excitatory post-synaptic current properties. Analysis of potential sex differences in action potential characteristics will further inform these results. Overall, given the significant sex differences in addiction and the normal behavioral output of these circuits, understanding the nature of sex differences in the nucleus accumbens shell is an important research goal.
Highly selective and mechanically robust sensors for electrochemical measurements of real-time hydrogen peroxide dynamics in brain tissue
LR Wilson, AC Schmidt, and LA Sombers
NC State University, Chemistry Department

Hydrogen peroxide whose role in the complex environment of the brain is not well understood has been implicated in the slow destruction of dopaminergic neurons in Parkinson's disease. This neurodegenerative disease affects more than a million people in America creating a critical need to identify the mechanisms through which hydrogen peroxide interacts with dopaminergic neurons. Real-time in vivo detection of this analyte has recently been described using fast-scan cyclic voltammetry at carbon-fiber electrodes. However selectively identifying hydrogen peroxide from interferents such as adenosine and pH shifts remains a challenge. Additionally some chemical agents used to pharmacologically verify the presence of hydrogen peroxide production in the brain such as mercaptosuccinic acid also have a similar oxidation peak as that of the target analyte further convoluting the characterization of hydrogen peroxide dynamics in the brain. We have addressed these problems by fabricating a hydrogen peroxide-selective electrode. 1 3-phenylenediamine (mPD) was electrodeposited onto the surface of a carbon-fiber electrode to render it sensitive to hydrogen peroxide fluctuations and pH shifts but not other analytes. Since pH changes generate a well-characterized and distinct voltammogram they can easily be removed from the signal using principal component regression to reveal an electrochemical signal due solely to the oxidation of hydrogen peroxide. This technology was fully characterized on glass-insulated electrodes for acute implantation as well as electrodes designed for chronic implantation. The work presented herein will facilitate the selective detection of hydrogen peroxide opening the door for further elucidation of the neurodegenerative role it plays in PD as well as other neuropathies involving oxidative stress.

Quantitative Stereologic Volume Analysis of the Dorsal Striatum and Nucleus Accumbens Core and Shell in the Brain of Rattus norvegicus.
JE Wong, J Cao, and JE Meitzen
North Carolina State University, Department of Biological Sciences

Sex differences and hemispheric bias are widespread across vertebrate nervous systems. Such differences are sometimes reflected in the neural substrate via neuroanatomical differences in brain region volume. One brain region that displays sex and hemispheric differences in associated behaviors and pathologies is the striatum, including the caudate-putamen (dorsal striatum), nucleus accumbens core and shell. The extent to which these differences can be attributed to alterations in volume is unclear. We thus tested whether the volumes of the caudate-putamen, nucleus accumbens core, and nucleus accumbens shell differed by region, sex, and hemisphere in adult Sprague-Dawley rats. For a positive control, we measured the volume of the sexually dimorphic nucleus of the medial preoptic area (SDN). As expected, SDN volume was larger in males than in females. No sex differences were detected in the volumes of the caudate-putamen, nucleus accumbens core, or shell. Nucleus accumbens core volume was larger in the right than left hemisphere across males and females. These findings complement previous reports of lateralized nucleus accumbens volume in humans, and are the first to show that this may be driven via hemispheric differences in nucleus accumbens core volume. In contrast, striatal sex differences seem to be mediated by factors other than striatal region volume. This conclusion is presented within the context of a detailed review of all known studies addressing sex differences and similarities in striatal neuroanatomy.

Contributions of Interdisciplinary Communications to Neuroscience Research
BIN YIN

Neuroscience is inherently interdisciplinary in its widespread scope of investigation of brain-mind-behavior relationships. However not enough focus has been placed on enhancing interdisciplinary knowledge sharing and cross-field communication skills building that would enable effective thinking style and collaborations across disciplines. We have established an interdisciplinary knowledge-sharing platform (named Triangle SmartTalk) among students and scholars at Duke UNC and NCSU and have successfully held 18 knowledge-sharing SmartTalks and involved more than 400 members. Here we demonstrate how our interdisciplinary SmartTalks could benefit future neuroscience research.
P24: Involvement of glutamatergic and cholinergic modulations of mesolimbic dopamine neurotransmission in a visual stimulus-reinforced instrumental behavior
X Xie, M Spanos J Gras-Najjar, SC Hughes, and LA Sombers
NC State University, Chemistry Department

The primary reinforcing effects of sensory stimuli (e.g. visual stimuli) play a critical role in self-administration of addictive drugs such as nicotine and methamphetamine. However the neural mechanisms underlying this phenomenon are still not clear. To this end we trained male Sprague Dawley rats (n=7) to perform a visual stimulus-induced instrumental task during which pressing one active lever resulted in activation of the above stimulus light for one second while pressing the second inactive lever had no programmed consequences. After the rats achieved the stable performance (i.e. less than 20% variability in active lever responding) the behavioral effects of microinjections of vehicle (phosphate buffered saline 0.5 µl/hemisphere) or Carbachol (acetylcholinergic receptor agonist 1 mM/0.5 µl/hemisphere) alone or Carbachol mixed with NMDA (NMDA glutamatergic receptor agonist 0.3 mM/0.5 µl/hemisphere) or AP5 (NMDA glutamatergic receptor antagonist 10 mM/0.5 µl/hemisphere) into the ventral tegmental area (VTA) were evaluated using a within-subject testing design. The effects of these intra-VTA pharmacological manipulations on dopamine transients in the nucleus accumbens shell (NAc shell) subregion in a separate group of freely moving rats (n=7) were also investigated using Fast-scan cyclic voltammetry (FSCV). Intra-VTA infusions of Carbachol potentiated active lever responding without altering inactive lever responding as compared to vehicle during the behavioral test. Furthermore intra-VTA co-infusions of Carbachol with AP5 but not NMDA attenuated active lever responding without altering inactive lever responding as compared to vehicle during the behavioral test. Finally intra-VTA administrations of Carbachol enhanced the magnitude and frequency of dopamine transients in the NAc shell and such effects were abolished by co-administrations of Carbachol with AP5 but restored by administrations of Carbachol with NMDA. Together these results suggested that cholinergic receptors and NMDA receptors play a synergistic role in modulation of VTA-NAc shell dopaminergic output which is likely critical for motivation for a visual stimulus.

P44: Spinal dopamine / morphine interactions in an animal model of Restless Legs Syndrome (RLS)
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Restless Legs Syndrome (RLS) involves abnormal limb sensations that diminish with motor activity, worsen at rest, and can severely disrupt sleep. Primary treatment is directed at CNS dopaminergic systems, particularly activation of D2-like (D2, D3 D4) receptors, however long-term therapy can lead to augmentation, a switch of initially beneficial therapeutic actions into adverse effects and a subsequent worsening of symptoms that will require opioid treatment. Our lab recently reported that a dysfunction of the D3 receptor (D3R) system was associated with a lack of responsiveness to morphine and an increase in D1 receptor (D1R) protein levels in the spinal cord. Based on these and data from other labs that point to a role of the D1R in controlling locomotor output in other movement disorders, we hypothesized that a modulation of the D1R system will provide a novel means by which to prevent the development of D3R agonist-induced augmentation and lack of responsiveness to morphine.

We tested thermal pain withdrawal latencies over the life span of wild type (WT) and D3R knockout mice (D3KO) with varying dosages and/or combinations of morphine and D1 antagonist. After establishing baseline withdrawal latencies, animals were treated with morphine (2 mg/kg and 5 mg/kg respectively), D1R antagonist (0.1 mg/kg), and morphine-D1 antagonist combinations (2 mg/kg + 0.1 mg/kg; 5 mg/kg + 0.1 mg/kg).

We found that morphine was effective in extending pain withdrawal latencies at both concentrations tested and in both young and old WT animals, but that low morphine had no effect on its own in D3KO at either age. In contrast, high morphine increased latencies in young and old but not middle-aged D3KO albeit to a lesser extent that in WT. Blocking D1Rs did not alter responses in WT, but increased latencies in young D3KO only. Further, the combination treatment of D1R antagonists and low morphine increase latencies in young and old WT, but only in young D3KO. Lastly, the combination treatment of D1R antagonists and high morphine was efficient in WT and young and middle-aged D3KOs. These data suggest that D1-D3 receptor interaction mediate morphine responsiveness, and that blocking D1Rs can restore opioid sensitivity, including in an animal model of RLS with compromised D3R function.
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Spinal dopamine / morphine interactions in an animal model of Restless Legs Syndrome (RLS)
AP Yllanes1, S Samir1, K Brewer2, and S Clemens1

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