Pavlovian Society
Annual Meeting, 2015
Portland Hilton

Sept 17–19, 2015
Portland, OR

Overview

All events will be located in the Skyline Level (23rd Floor)

Thur	6:00–10:00 PM	Opening Reception Hors d'Oeuvres & Cash Bar
Fri	7:30–8:25	Breakfast
	8:25–11:40	Morning Sessions
	12:10–1:50	Lunch (Exec Committee Meeting)
	1:50–5:00	Afternoon Sessions
	5:30–7:30	Posters & Cash Bar
Sat	7:30–8:30	Breakfast
	8:30–Noon	Morning Sessions
	Noon–1:30	Lunch (WIL Luncheon)
	1:30–5:00	Afternoon Sessions
	5:30–7:30	Posters & Cash Bar
	7:30–9:00	Banquet

Program

Friday (Sept 18)

7:30–8:25 Breakfast

8:25–8:30 Welcome: **Matt Lattal** (OHSU)

8:30–10:10 ***Symposium: The Ontogeny of Cognition during Adolescence: Behavior and Neurobiology***
David J. Bucci & Mark E. Stanton, Chairs

- 8:30–8:55 **Mark Stanton** (U Delaware): The ontogeny of learning in context
- 8:55–9:20 **Siobhan Pattwell** (Fred Hutchinson Cancer Research Center): Leveraging Dynamic Changes in Neural Circuitry During Adolescence to Persistently Attenuate Fear Memories
- 9:20–9:45 **Heidi Meyer** (Dartmouth): Behavioral and Neurobiological Substrates of Negative Occasion Setting During Adolescence
- 9:45–10:10 **Jill A. McGaughy** (U New Hampshire): Do changes in Prefrontal Norepinephrine Transporter density across Adolescence influence the Development of Cognitive Control in rats?

10:10–10:30 Coffee Break

10:30–12:10 ***Symposium: Drugs and Conditioning***
Chris Cunningham (OHSU), Chair

- 10:30–10:55 **Barbara Sorg** (Washington State): Role of Perineuronal Nets in Cocaine-Associated Memory
- 10:55–11:20 **Rick Bevins** (U Nebraska): Conditioned Enhancement of the Nicotine Reinforcer
- 11:20–11:45 **Linda Parker** (U Guelph): Endocannabinoid Regulation of Nausea is mediated by 2-Arachidonoylglycerol (2-AG) in the Rat Visceral Insular Cortex
- 11:45–12:10 **Chris Cunningham** (OHSU): Deconstructing the Elements of Context in Pavlovian Drug Conditioning

12:10–1:50 Lunch (on your own) and Executive Committee Meeting

1:50–2:20 **Karyn Frick** (U Wisconsin-Milwaukee) Neural Mechanisms Through Which Progesterone Regulates Hippocampal Memory

2:20–3:10 **Jeansok J. Kim** (U Washington) Place and Time of Fear

3:10–3:30 Coffee Break

3:30–5:00 ***Symposium: Remembering the contributions of Richard F. Thompson***
Participants include:
James McGaugh (UCI), chair
Tracey Shors (Rutgers)
Ted Berger (USC)

Joseph Matarazzo (OHSU)

5:30–7:30 Posters and Cash Bar

Saturday (Sept 19)

8:30–10:30 ***Symposium: Remembering the Contributions of Nicholas Mackintosh***
Peter Balsam (Columbia), Chair
Ralph Miller (SUNY Binghamton): The Construct of Attention and Beyond: Homage to NJ Mackintosh
Gavan P. McNally (UNSW Australia): Selecting Danger Signals: Attention, CS Associability, and Fear Learning.
Guillem Esber (CUNY, Brooklyn College): Reconciling the Effects of Predictiveness and Uncertainty on Attention in Associative Learning
Victoria Chamizo (University of Barcelona): Trying to Understand the Differential Use of Spatial Information in Male and Female Rats of Different Age, Juveniles and Adults.

10:30–10:40 Break

10:40–11:05 **Melissa Sharpe** (Princeton/NIDA): The Prelimbic Cortex Directs Attention Toward Predictive Cues During Pavlovian Conditioning

11:05–11:30 **Melissa Malvaez** (UCLA): Histone Acetylation in the Dorsolateral Striatum Selectively Mediates the Formation of Behavioral Habits

11:30–11:55 **Mark Baxter** (Mt. Sinai): Cognitive and Socioemotional Development After Postnatal Anesthetic Exposure

12:00–1:30 Lunch — Women in Learning satellite meeting at the Picnic House
See last page of Program

1:30–3:10 ***Symposium: Neuroimmune and Neuroendocrine Modulation of Memory***
Natalie Tronson (U Michigan), Chair

1:30–1:55 **Ruth Barrientos** (Colorado): Neuroinflammation in the Normal Aging Hippocampus: Causes, Effects, and Therapeutic Interventions

1:55–2:20 **John F. Guzowski** (UCI): Cytokine modulation of hippocampal circuit activity and memory function

2:20–2:45 **Natalie Tronson** (U Michigan): Persistent Memory Deficits and Histone Modifications after a Systemic Inflammatory Event

2:45–3:10 **Andrey Ryabinin** (OHSU): Mice transfer increased pain sensitivity via olfactory cues

3:10–3:30 Break

3:30–5:10 ***Symposium — Retrieval and Extinction Processes***
Tom Gould (Temple), Chair

3:30–3:55 **Brian Wiltgen** (UC Davis): Retrieving memory with the hippocampus

3:55–4:20 **Martha Escobar** (Oakland U.): Synaptic Plasticity Changes After Retrieval of Contextual Fear Memories

4:20–4:45 **Michael Drew** (U. Texas): Dentate Gyrus Controls Extinction of Contextual Fear Memory

4:45–5:10 **Dayan Knox** (U. Delaware): Examining the effects of stress on fear extinction using the single prolonged stress paradigm

5:30–7:30 Posters and Cash Bar

7:30–9:00 Banquet
Speaker: **Steve Sylvester** (Washington State): ***Oysters, Invaders, Sex and CO2***
Awards

Posters

Generally alphabetical by author except for some moved between days.

Posters whose first author's last name begins with A–H will be presented at Friday's Poster Session.

1 Pina, Melanie M.; Cunningham, Christopher L. (*Oregon Health & Science University*) Ethanol-Seeking Behavior is Expressed Directly through an Extended Amygdala to Midbrain Neural Circuit

2 Abraham, Antony; Land, Benjamin; Soltys, David; Chavkin, Charles (*University of Washington*) Kappa Opioid Receptor Activation Disrupts Behavioral Inhibition in Schedule-Controlled Tasks

3 Adkins, Jordan ; Webster, Natalia; Lynch, Joseph III; Vanderhoof, Tyler, T. Gilman, Lee, Jasnow, Aaron (*Kent State University*) Influence of GABAB1 Receptor Deletion in Corticotropin Releasing Factor Neurons on Fear, Anxiety, and Social Stress

4 Allen, Todd; Miller, Daniel; Williams; David; West, Kaitlyn; Servatius, Richard, (*University of Northern Colorado; Stress and Motivated Behavior Institute; Carthage College*) US Alone Trials Are Disruptive When Pre-Exposed Prior to CS-US training, but not when Interpolated into CS-US Training.

5 Asok, Arun; Draper, Adam; Schulkin, Jay; Rosen, Jeffrey B. (*University of Delaware*) Optogenetically Dis-

secting the Function of Corticotropin-Releasing-Factor Neurons During Contextual Fear Learning

6 Blacktop, Jordan M; Churchill, Lynn; Todd, Ryan P; Slaker, Megan; Sorg, Barbara A. (*Washington State University, Vancouver*) Role of Anterior Dorsal Lateral Hypothalamic Area Perineuronal Nets in the Acquisition of Cocaine-Induced Conditioned Place Preference

7 Calub, Catrina; Furtak, Sharon; Brown, Thomas (*California State University, Sacramento; Yale University*) Autoconditioning Hypothesis for Acquired Fear of Ultrasonic Alarm Cries

8 Campese, D. Vinn; Kim, T. Ian; LeDoux, E. Joseph (*New York University*) Different forms of conditioned motivation depend on different regions in the amygdala

9 Collins, Sean; Chau, Lily; Galvez, Roberto (*University of Illinois Urbana-Champaign*) Associative learning temporarily increases SHANK neocortical expression consistent with anatomical plasticity

10 Czerniawski, Jennifer; Guzowski, John F (*University of California - Irvine*) Minocycline Treatment Blocks Cytokine-Induced Alterations in Hippocampal Circuit Activity and Context Discrimination Memory Impairment.

11 Daniels, W. Carter ; Sanabria, Federico (*Arizona State University*) The Effect of Chronic Nicotine Administration on Sign- and Goal-Tracking: A Pilot Study

12 de Solis, Christopher; Holehonnur, Roopa; Banerjee, Anwesha; Luong, Jonathan, Lella Srihari; Ho, Anthony; Pahlavan, Bahram; Ploski, Jonathan (*University of Texas at Dallas*) Viral Delivery of shRNA to Amygdala Neurons Leads to Neurotoxicity and Deficits in Pavlovian Fear Conditioning

13 Elkin, Magdalyn E.; Freeman, John H. (*University of Iowa*) Ontogenetic Changes In Anterior Cingulate Cortical Activity during Trace Eyeblink Conditioning.

14 Farley, Sean; Freeman, John (*The University of Iowa*) Cerebellar Feedback is Necessary for Learning Related Activity in the Medial Auditory Thalamic Nuclei During Eyeblink Conditioning

15 Furtak, Sharon; Calub, Catrina; Potter, Nicole (*California State University, Sacramento*) Perirhinal Cortex Involvement in Fear Extinction to a Discontinuous Visual Stimulus

16 Gahtan, Ethan; Bishop, Benjamin (*Humboldrt State University*) Light-Evoked Diving Reflex In Zebrafish Larvae

17 Garr, Eric; Delamater, Andrew R. (*Graduate Center of the City University of New York; Brooklyn College*) Action Sequences are Sensitive to Reward Devaluation

18 Gonzalez, Sarah T.; Fanselow, Michael S. (*University of California, Los Angeles*) The Neural Mechanisms of Safety Learning in Mice

19 Goode, Travis; Jin, Jingji; Holehonnur, Roopashri; Ploski, Jonathan; Maren, Stephen (*Texas A&M University*) Combinatorial DREADD Silencing of Ventral Hippocampal Neurons Projecting to Infralimbic Cortex Prevents Fear Renewal

20 Goosens, Ki; Barbara Gisabella, Junmei Yao (*Massachusetts Institute of Technology*) Enhanced Growth Hormone in Amygdala Neurons Dysregulates Associative Fear Memory Allocation

21 Gould, Thomas (*Temple University*) Adolescent Nicotine Exposure Alters Adult Contextual Fear Conditioning

22 Gray, Michael S.; Lynch III, Joseph F; Winiecki, Patricki A.; Jasnow, Aaron M. (*Kent State University*) Presynaptic GABAB(1a) Receptors Preserve Context Memory During Consolidation

23 Hafenbreidel, Madalyn; Rafa Todd, Carolynn; Smies, Chad. W.; Twining, Robert C.; Mueller, Devin (*University of Wisconsin, Milwaukee*) Blocking Infralimbic Basic Fibroblast Growth Factor (bFGF or FGF2) Facilitates Extinction of Drug Seeking

24 Hanson, Erica E., Mitchell, Suzanne H. (*Oregon Health & Science University*) Fos expression after exposure to an effort discounting procedure

25 Heroux, Nicholas; Robinson-Drummer, Patrese A.; Rosen, Jeffrey B.; Stanton, Mark E. (*University of Delaware*) Role of NMDA Receptors in the Acquisition and Retention of the CPFE in Adolescent Rats

26 Hersman, Sarah; Hoffman, Annie; Hodgins, Lili; Shieh, Shannon; Lam, Jamie; Fanselow, Michael S (*UCLA*) Dissociation of Cholinergic Modulation in Dorsal Hippocampus and Basolateral Amygdala on Stress-enhanced Fear Learning

27 Huckleberry, Kylie A.; Ferguson, Laura B.; Drew, Michael R. (*University of Texas at Austin*) Behavioral Mechanisms of Context Fear Generalization

28 Huda, K. Rebecca; Latsko, Maeson S; Gilman, Lee T; Jasnow, Aaron M. (*Kent State University*) Resistance to Social Defeat Stress is Characterized by Poor Behavioral Inhibition; Assessment in Passive Avoidance

29 Hui, May; Zelikowsky, Moriel; Anderson, David (*California Institute of Technology*) Tac2 mediates chronic stress in mice

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The following posters (first author's last name begins with J–W) will be presented at Saturday's Poster Session.

1 Alfiler, Lauren K. & Pribic, Micaela R.; Rose, Jacqueline K. (*Western Washington University*) Deletion of CaMKII$_\gamma$ in C. elegans Results in an Associative Conditioning Deficit Without Affecting Mobility, Defecation or Fecundity.

2 Johnson, Brandon. A.; Wilson, W. Jeffrey (*Albion College*) Light-induced Attenuation of Response to Light in the Earthworm Lumbricus terrestris

3 Kennedy, Bruce C; Kohli, Maulika; Maertens,

Jamie; Marell, Paulina; Gewirtz, Jonathan C (*University of Minnesota*) Conditioned Object Preference: A Novel Measure of Drug-Seeking in Rodents

4 Kim, Earnest; Lattal, Matthew K. (*Oregon Health & Science University*) Amygdala Modulation of Cocaine-seeking Behavior

5 Knox, Dayan; Stanfield, Briana R; Staib, Jennifer; Keller, Samantha M; DePietro, Thomas. (*University of Delaware*) Using Measures of c-Jun Activity to Determine Mnemonic Processes Through which SPS Exposure Disrupts Extinction Retention

6 Koraym, Adam; Gallimore, Darci; Kidd, Tara; Dodd, Maria; Hennessy, Michael B.; Claflin, Dragana I. (*Wright State University*) Effects of Early-Life Stress and Social Buffering on Learning in Juvenile Rats

7 Kwapis, Janine L.; Alaghband, Yasaman; Kramar, Eniko A.; Matheos, Dina P.; Rhee, Diane; Lopez, Alberto, J.; Wood, Marcelo A. (*University of California, Irvine*) HDAC3: An Epigenetic Key to Ameliorating Synaptic Plasticity and Memory Deficits in the Aging Brain

8 Lacagnina, Anthony F; Drew, Michael R (*University of Texas at Austin*) Enhanced Context Fear Memory with Spaced Context Exposures

9 Latsko, Maeson S.; Farnbauch, Laure A.; Jasnow, Aaron M. (*Kent State University*) Enhanced juvenile glucocorticoid response is associated with an adult resistant phenotype following juvenile social defeat

10 Laude, R. Jennifer; Fillmore, T. Mark (*University of Kentucky*) Activities Engaged in while Drinking Alcohol Affect Perceived Intoxication and Liking of Effects

11 Lee, Ji-Hye; Kimm, Sunwhi; Choi, June-Seek (*Korea University*) Effect of Emotional Trauma during Adolescence on subsequent Aversive Memory Formation in rats

12 Lee, Yeon Kyung; Hong, Eun-Hwa; Kim, Sunwhi; Jie, Hyesoo; Choi, June-Seek Choi (*Korea University*) Uncontrollable stress effects on discriminatory avoidance learning

13 Loh, Ryan; Galvez, Roberto (*University of Illinois at Urbana-Champaign*) Neocortical Kappa Inhibition Attenuates Forebrain-Dependent Associative Learning

14 Lynch III, Joseph; Gray, Michael; Ouellette, Emily; Adkins, Jordan; Winiecki, Patrick A.; Riccio, David C.; Jasnow, Aaron M.; (*Kent State University*) Estradiol-induced Fear Generalization is Blocked by Inactivation of NMDA or AMPA Receptors Prior to Retrieval

15 Murawski, Nathen J.; Asok, Arun (*Center for Neuroscience, UC Davis (NJM); Department of Psychology and Brain Sciences, University of Delaware (AA)*) Digital Context Fear Conditioning in Rats: Utilizing LCD-Based Visual Context Manipulations During Conditioning

16 Noble, J. Lindsey; Gonzalez, Ian J; Ngo, Tiffany T, Malhotra, Simran; Meyers, Eric; Bleker, Nathaniel, Carrillo, D'angelique D; Ramananthan, Karthik R; Rennaker, Robert L; Kilgard, Michael P; McIntyre, Christa K* (*The University of Texas at Dallas*) Vagus Nerve Stimulation Enhances Extinction of Conditioned Fear in an Animal Model of PTSD

17 Ouellette, B. Emily; Lynch III, F. Joseph; Riccio, C. David; McEwen, S. Bruce; Jasnow, M. Aaron (*Kent State University*) Corticosterone Attenuates Context Fear Generalization in Male Rats

18 Pennington, Zachary T.; Avershal, Jacob Z.; Anderson, Austin S.; Fanselow, Michael S. (*University of California, Los Angeles*) Broadening the stress enhanced fear learning (SEFL) model: acute shock exposure augments subsequent startle responses and contextual fear of a startle-paired context

19 Pisansky, Marc T.; Gewirtz, Jonathan C. (*University of Minnesota - Twin Cities*) Intranasal oxytocin enhances socially transmitted fear behavior in mice

20 Pizzimenti, Christie; Lattal, K. Matthew (*Oregon Health & Science University*) Context-independent effects of shock on drug-seeking

21 Pochiro, Joseph M; Goodfellow, Molly J; Lee, Michael A; Lindquist, Derick H. (*The Ohio State University*) Trace Fear Conditioning and Hippocampal NR2B-NMDA Receptor Signaling in Adult Rats Administered Ethanol During the Third Trimester-equivalent Period

22 Rafa Todd, Carolynn; Hafenbreidel, Madalyn; Otis, James, M.; Twining, Robert, C.; Mueller, Devin (*University of Wisconsin, Milwaukee*) Infralimbic NR2A-containing NMDA Receptors are Necessary for the Reconsolidation of Cocaine Self-administration Memory

23 Rankin, Catharine H.; Giles, A.C., Ardiel, E. A., Yu, A. (*University of British Columbi*) Response Based Analyses of Behavior Overlook Other Important Behavioral Changes: Integrating Habituation into Ongoing Behavior

24 Reyes, Kyrie-Anne E.; Kudva, Priya S; Todd, Ryan P; Sorg, Barbara A. (*Washington State University*) Prazosin disrupts reconsolidation of appetitive and aversive behavior in rats: discordance between behavior and ultrasonic vocalizations

25 Robinson-Drummer, Patrese; Heroux, Nicholas A.; Stanton, Mark E. (*University of Delaware*) Intra-dorsal Hippocampal antagonism of Muscarinic Acetylcholine receptors disrupts the Context Preexposure Facilitation Effect

26 Rose, Jacqueline; Alfiler, Lauren K.; Pribic, Micaela R. (*Western Washington University*) Pavlovian Conditioning Produced by Activating Two Identified and Distinct Neural Circuits in C. elegans

27 Sangha, Susan (*Purdue University*) Amygdalocortical Circuitry Contributes to Discriminative Reward, Fear and Safety Learning

28 Shipman, Megan L.; Trask, Sydney; Green, John T.; Bouton, Mark, E. (*The University of Vermont*) Inactivation of the Prelimbic Cortex Attenuates Context-Dependent

Excitatory Operant Responding

29 Shors, Tracey J. ; Olson, Ryan; Brush, Christopher J.; Alderman, Brandon (*Rutgers University*) Mental and Physical (MAP) Training: Combining Meditation and Aerobic Exercise Enhances Synchronized Brain Responses during Conflict Monitoring

30 Slaker, Megan; Sorg, Barbara A. (*Washington State University*) Perineuronal nets in the prefrontal cortex contribute to acquisition of drug-associated memory

31 Trask, Sydney; Bouton, Mark A., Carranza-Jasso, R. (*The University of Vermont*) Learning Not to Make the Response During Operant Extinction

32 Williams, Amy R.; Lattal, K. Matthew (*OHSU*) Effects of Acute Ethanol Withdrawal on Extinction and Reconditioning of Fear

Poster Abstracts

Alphabetical by first author.

Abraham, Antony; Land, Benjamin; Soltys, David; Chavkin, Charles (*University of Washington*) Kappa Opioid Receptor Activation Disrupts Behavioral Inhibition in Schedule-Controlled Tasks. Dysregulation of the dynorphin/kappa opioid receptor (KOR) system has been implicated in the pathologies observed in substance use disorders and affective disorders. In humans, pharmacological KOR activation is hallucinogenic and disrupts cognition. A fundamental component of cognitive tasks is the development and maintenance of temporal associations between stimuli to properly guide responding. To measure disruptions in temporal processing and behavioral inhibition induced by KOR activation, male C57Bl/6 mice were trained in a differential reinforcement of low rates of responding (DRL) task for food reward. In the DRL task, mice were required to withhold responding during an unsignaled, fixed time period (15s) following a reinforced response. Optimal responses in the DRL task required interresponse times (IRT) greater than 15s, and premature or 'incorrect' responses in the DRL task led to reset of the 15s wait period. Mice were tested in the DRL task with U50,488 (a prototypical KOR agonist), Salvinorin A (a recreationally used KOR agonist), or following repeated forced swim stress with or without norBNI (a kappa antagonist). Repeated forced swim stress significantly increased the percentage of incorrect responses in the DRL task and this effect was blocked by KOR antagonism. KOR activation with U50,488 and Salvinorin A also significantly increased the percentage of incorrect responses in the DRL task. These results demonstrate that KOR activation, via stress or pharmacological treatment, disrupts performance in the DRL task. These effects are hypothesized to be specific to inhibitory control of responding, rather than time estimation, as most errors occurred in bursts of responding at the beginning of wait period. Decreased behavioral inhibition has been proposed as a mechanism underlying substance abuse disorders, and the present study indicates these cognitive disruptions may be KOR mediated. Future studies will further specify the cellular and neural substrates involved in KOR-mediated disruptions in cognition. SUPPORT: T32DA07278 (AA), KO5DA02570 (CC), NARSAD Young Investigator Award (BBL), and P50MH106428 (CC)

Adkins, Jordan ; Webster, Natalia; Lynch, Joseph III; Vanderhoof, Tyler, T. Gilman, Lee, Jasnow, Aaron (*Kent State University*) Influence of GABAB1 Receptor Deletion in Corticotropin Releasing Factor Neurons on Fear, Anxiety, and Social Stress. Corticotropin releasing factor (CRF) is highly expressed in the paraventricular nucleus (PVN) and throughout the brain, and plays a critical role in neuroendocrine and behavioral responses to stress. Yet how the CRF system is modulated to elicit such responses remains unclear. Previous research in our lab demonstrated CRF-specific disruption of NMDA receptor function sex-dependently enhanced fear, sociability, and social stress responsivity. Furthermore, CRF-specific deletion of GABAAÎś1 subunits also enhanced anxiety and disrupted fear extinction. As a way to further explore the glutamatergic and GABA-ergic regulation of the CRF system, we utilized a floxed GABAB1 transgenic mouse crossed with a CRF-Cre driven mouse, allowing specific GABAB1 deletion in CRF-expressing neurons. CRF GABAB1 knockout mice were trained in context and cued fear conditioning, assessed in anxiety measures, and underwent social defeat to examine social stress sensitivity. Based on our previous findings, we hypothesized CRF-GABAB1 knockout mice would show increased fear and anxiety responses. However, CRF GABAB1 knockout males, but not females, displayed reduced context and cued fear compared to wildtype littermates. CRF-specific deletion of GABAB1 receptors had no effect on anxiety-like behavior in males or females. Additionally, female knockout mice, but not males, trended towards spending less time in the closed arms of the elevated maze plus, suggesting less anxiety-like behavior. Finally, after experiencing social defat, no differences were observed between the two genotypes in a social interaction test. Theses data demonstrate sex-specific effects of GABAB1 receptor deletion within CRF neurons on fear, anxiety-like behavior and social stress responsivity. Contrary to our hypothesis, CRF-GABAB1 knockouts displayed decreased fear to both context and cue, but no differences in anxiety-like behavior or social stress responsivity. Our findings indicate GABAB1- mediated GABA-ergic regulation of CRF neurons is important for appropriately regulating fear, likely functioning to promote survival under specific aversive circumstances.

Alfiler, Lauren K. & Pribic, Micaela R.; Rose, Jacqueline K. (*Western Washington University*) Deletion of CaMKII$_\gamma$ in C. elegans Results in an Associative Conditioning Deficit Without Affecting Mobility, Defecation or Fecundity. . Calcium/calmodulin-kinase II (CaMKII) mod-

ulates the mechanisms of neuronal plasticity that underlie learning. Recently, CaMKII$_\gamma$ has been shown to act as a nuclear transporter, mobilizing from the postsynaptic density to the nucleus to activate gene transcription in response to neuronal firing (Ma et al., 2014). In C. elegans, the CaMKII mutant strain unc-43(gk452) carries a specific deletion for the UNC-43T isoform, a putative ortholog of human CaMKII$_\gamma$, for which there is no published phenotype. Our lab has observed several behaviors and have found no immediately obvious phenotypes of this mutation: unc-43(gk452) worms show normal egg-laying, defection and general mobility. Further investigation revealed that unlike wild-type, unc-43(gk452) worms were unable to demonstrate avoidance to NaCl following pairing of that stimulus with starvation. In addition, unc-43(gk452) worms show a deficit for associative conditioning resulting from a Classical Conditioning protocol. Wild-type worms show an increased avoidance response when a mechanosensory stimulus (NS, vibratory low frequency tone) is paired with a UV or blue-wavelength light stimulus (US). However, unc-43(gk452) worms exhibit either no change in response or a decrease in response to the mechanosensory stimulus alone, thus suggesting no influence of the US. These results suggest that CaMKII$_\gamma$ may be a necessary component for associative conditioning. Current work includes identifying necessary coding regions of the UNC-43T isoform by generating transgenic rescues and examining behavior.

Allen, Todd; Miller, Daniel; Williams; David; West, Kaitlyn; Servatius, Richard, (*University of Northern Colorado; Stress and Motivated Behavior Institute; Carthage College*) US Alone Trials Are Disruptive When Pre-Exposed Prior to CS-US training, but not when Interpolated into CS-US Training. . Recent work has indicated a differential effect of US alone trials on classical eyeblink conditioning dependent on when these trials are presented in training. Holloway et al. (2012) reported enhanced proactive interference of eyeblink conditioning following US alone pre-exposures in individuals with high trait anxiety. More recently, acquisition of CRs did not differ between partial reinforcement with 50% US alone (corneal air puff) trials as compared to 100% CS-US (tone-air puff) trials (Allen et al., 2014). A review of the literature revealed that Kimble et al. (1955) tested a protocol with an interpolated block of 20 US alone trials during CS-US training which did not disrupt CRs. We sought to extend the work with US alone training both when pre-exposed and interpolated with anxiety vulnerability individuals. Undergraduates completed personality inventories including the Adult Measure of Behavioural Inhibition (AMBI). All participants received 60 acquisition trials. Acquisition consisted of either 100% CS-US training, 20 CS-US trials followed by 20 US alone trials, followed by 20 more CS-US trials or 30 US alone pre-exposures followed by 30 CS-US trials. The US alone pre-exposures disrupted acquisition of eyeblink CRs which replicated the findings of Holloway et al. In addition, US alone pre-exposures eliminated the enhanced acquisition normally observed in BI individuals. The interpolated US alone trials did not disrupt CRs. BI individuals exhibited more CRs than non-inhibited individuals in the 100% CS-US training protocol and the Kimble et al. protocol. It appears that BI individuals are not affected by US alone trials once CR acquisition has begun to the US, but are severely disrupted by US alone pre-exposure prior to CS-US training. Our findings will be discussed in the light of the neural substrates of eyeblink conditioning as well as possible factors such as hypervigilance, cue salience and learned helplessness. SUPPORT: University of Northern Colorado Professional Development Funds and the Stress and Motivated Behavior Institute

Asok, Arun; Draper, Adam; Schulkin, Jay; Rosen, Jeffrey B. (*University of Delaware*) Optogenetically Dissecting the Function of Corticotropin-Releasing-Factor Neurons During Contextual Fear Learning. For almost two decades, it has been hypothesized that a corticotropin-releasing-factor (CRF) pathway from the central nucleus of the amygdala (CeA) to the bed nucleus of the stria terminalis (BNST) controls fear to sustained threats. However, the function of CRF in this pathway has remained elusive because of technological limitations of selectively inhibiting or activating CRF neurons. Thus, in the present study, we sought to investigate this CRF pathway by using a cell-type specific optogenetic approach during contextual fear conditioning. First, we developed a plasmid containing archaerhodopsin tp009 (ArchT) fused to enhanced green fluorescent protein (EGFP) flanked by a CRF promoter (CRF-ArchT-EGFP) and packaged it into a serotype-2 adeno-associated virus (AAV2). Immunohistochemical co-labeling with a CRF antibody following viral transfection with AAV2-CRF-ArchT-EGFP showed highly selective expression of the construct in CRF-positive cells. Second, the AAV2-CRF-ArchT-EGFP was injected into the CeA of rats and then fiber optic cannula were bilaterally implanted above the CeA. Four weeks after virus injection, we selectively inhibited CeA CRF neurons with green laser light during contextual fear conditioning. We found inhibition of CRF neurons in the CeA reduced freezing during multi-trial contextual fear conditioning (5 shocks presented 3 minutes apart). Furthermore, freezing remained low during a light-free retention test 24 hours later. This disruption in freezing was not due to rats' inability to respond to the shock during optogenetic inhibition. These studies highlight an important role for CRF neurons in the CeA during contextual fear learning. Ongoing work will examine the effects of optogenetic inhibition of CeA CRF projections to the BNST during contextual fear conditioning. SUPPORT: R01HD075066

Blacktop, Jordan M; Churchill, Lynn; Todd, Ryan P; Slaker, Megan; Sorg, Barbara A. (*Washington State University, Vancouver*) Role of Anterior Dorsal Lateral Hy-

pothalamic Area Perineuronal Nets in the Acquisition of Cocaine-Induced Conditioned Place Preference. Addiction involves drug-induced neuroplasticity of the circuitry of motivated behavior, which includes the medial forebrain bundle and the lateral hypothalamic area. Emerging at the forefront of neuroplasticity regulation are specialized extracellular matrix structures that form perineuronal nets (PNNs) around certain neurons, mainly parvalbumin positive (PV+) fast-spiking interneurons (FSINs), making them a promising target for the regulation of drug-induced neuroplasticity. Despite the emerging significance of PNNs in drug-induced neuroplasticity and the well-established role of the lateral hypothalamic area (LHA) in reward/reinforcement/motivation, very little is known about how PNN-expressing neurons control drug-seeking behavior. The goals of this experiment were: 1) to determine areas of high PNN expression within the LHA, and 2) whether PNN expression within the LHA is necessary for cocaine-induced conditioned place preference (CPP). A discrete region of the anterior dorsal LHA (LHAad) was found to exhibit robust PNN expression, while the rest of LHA exhibited comparatively sparse PNN expression. Compellingly, only PNN removal via chrondroitinase ABC (ChABC) administration within the dorsal anterior LHA prior to conditioning abolished acquisition of cocaine-induced CPP, highlighting the importance and specificity of PNN removal within this subregion of the LHA. It was determined that approximately 87% of LHAad WFA positive neurons are PV+. Removal of LHAad PNNs did not affect locomotor activity, high-fat food intake, sucrose intake, or sucrose-induced CPP in separate groups of cocaine naïve animals. Moreover, preliminary retrograde tracing suggests that the LHAad provides input into the ventral tegmental area (VTA). In summary, our findings indicate that PNN expression in the LHAad: 1) is necessary for acquisition of cocaine-induced CPP, 2) is predominantly co-localized with parvalbumin, and 3) is not necessary for normal locomotor or ingestive behavior or sucrose-induced CPP. These data suggest that PNN-dependent neuroplasticity within the LHAad is critical for the acquisition of cocaine-induced CPP. SUPPORT: Supported by NIH Grant DA033404 to Barbara A. Sorg, WSU-Alcohol and Drug Abuse Research Program Grant 124777 to Jordan M. Blacktop

Calub, Catrina; Furtak, Sharon; Brown, Thomas (*California State University, Sacramento; Yale University*) Autoconditioning Hypothesis for Acquired Fear of Ultrasonic Alarm Cries . Rats commonly emit 22 kHz ultrasonic vocalizations (USVs) in association with pain, fear, or anxiety/distress. As social alarm or distress signals, these 22 kHz USVs are believed to play a key role in group survival. Whereas the capacity to produce these USVs is innate, appropriate reactivity to them requires experience. More specifically, 22 kHz USVs fail to elicit freezing behavior (the classical fear index) in naïve laboratory rats. This fact raises the question: How do rats learn to react fearfully or defensively to these ethologically-important social signals? A possible clue came from a study by Kim et al (2010). In their experiments, laboratory rats did react fearfully to alarm calls (evidenced by USV-elicited freezing behavior) if they received a series of foot shocks 24 hours earlier. Based on several lines of evidence, these authors proposed that acquired fear of 22 kHz USVs depends on "autoconditioning" — an emotional learning process in which self-generated 22 kHz USVs serve as auditory Pavlovian cues that become associated with subsequent foot shocks. The present experiment tested this hypothesis by devocalizing one group of rats by sectioning the recurrent laryngeal nerve. Control animals underwent a nerve-sparing sham operation. Both groups received a series of foot shocks and were subsequently tested, in a novel context, for USV-elicited freezing. Recurrent nerve transection failed to prevent or even diminish USV-elicited freezing. Devocalized animals were statistically indistinguishable from the sham-operated controls, disconfirming an essential prediction of the autoconditioning hypothesis. We consider alternative associative and non-associative mechanisms for acquired USV reactivity. SUPPORT: Social Sciences and Interdisciplinary Studies (SSIS) Faculty Development Award at California State University, Sacramento

Campese, D. Vinn; Kim, T. Ian; LeDoux, E. Joseph (*New York University*) Different Forms of Conditioned Motivation Depend on Different Regions in the Amygdala. Aversive Pavlovian-to-instrumental transfer (PIT) is an effective way to isolate the conditioned motivation produced by an aversive conditioned stimulus (CS). This is seen as an elevation of avoidance responding when an aversive CS is presented. However, different forms of aversive conditioned motivation (i.e., specific versus general) have proven difficult to isolate and attribute to specific neural circuits. In appetitive PIT studies central amygdala (CeA) has been linked to the general form of motivation while basal amygdala (BA) has been linked to the specific form, which involves sensory-specific learning. Here we use a within-subjects design where each subject received aversive Pavlovian threat conditioning (PTC) with both tone-shock and noise-klaxon arrangements. Following unsignaled Sidman active avoidance (USAA) training where subjects were trained to shuttle to avoid shocks, each CS was tested separately for their effect on USAA behavior. Following baseline tests, kappa opioid DREADDs (KORD) were infused into either the CeA or BA. After recovery each CS was tested following treatment with salvinorin-B (Sal-B) as well as the saline vehicle. Comparable to what has been found in appetitive studies, BA inhibition reduced specific PIT, but not general. CeA inhibition reduced general PIT, but also reduced specific PIT. These results suggest that different forms of aversive motivation may depend on different microcircuits in the amygdala. SUPPORT: NIH Grant MH38774

Collins, Sean; Chau, Lily; Galvez, Roberto (*University of Illinois Urbana-Champaign*) Associative learning temporarily increases SHANK neocortical expression consistent with anatomical plasticity. It has been well established that learning induces synaptic modification in the neocortex. To examine the molecular mechanism(s) mediating this process we have utilized the associative learning paradigm whisker trace eyeblink conditioning (WTEB). During WTEB, subjects are presented with a neutral whisker stimulation (CS) which is paired with a mild periorbital eye-shock (US). These stimuli are separated by a stimulus-free trace interval. After multiple CS-US pairings, subjects learn that the CS predicts the US and elicit an eyeblink after the CS but before US onset. Utilization of this paradigm has shown that primary somatosensory cortex is required for both acquiring and retrieval of the learned association. In examining the neuronal mechanisms(s) mediating this learning event, our laboratory has demonstrated that spines in layer IV of the barrel cortex exhibit a transient increase across different phases of learning. The current study set out to determine SHANK's role in mediating this neocortical synaptic plasticity. The SHANK family is of particular interest due to their localization in the post synaptic density (PSD) of excitatory neurons. These proteins indirectly bind to NMDAR, AMPAR, and mGluR; thus playing a role in glutamate receptor organization of the PSD. Knocking-out either SHANK1 or SHANK2 results in behavioral impairments, and dysregulation of spine morphology. These findings suggest that SHANK is involved in learning-induced synaptic plasticity, and thus plays a role in WTEB-induced neocortical spine modification. To explore SHANK's role in WTEB, adult C57BL/6 mice were randomly assigned to one of six groups (acquisition, learned, over-trained, or stimulus matched unpaired controls). Our preliminary findings suggest that SHANK1 and SHANK2 in primary somatosensory cortex exhibit a learning-dependent expression profile that coincides with synaptic changes from our prior anatomical study. These analyses provide an initial understanding into the molecular mechanism(s) mediating neocortical learning-induced synaptic plasticity.

Czerniawski, Jennifer; Guzowski, John F (*University of California - Irvine*) Minocycline Treatment Blocks Cytokine-Induced Alterations in Hippocampal Circuit Activity and Context Discrimination Memory Impairment. Neuroinflammation is implicated in cognitive deficits associated with aging, disease, and trauma. Recently, we demonstrated that systemic administration of the bacterial endotoxin lipopolysaccharide (LPS) elevates mRNA expression of the proinflammatory cytokines IL-1β, TNF-α, and IL-6 in the rat brain and produces specific memory retrieval deficits in context discrimination and altered neural circuit activity in the hippocampus (Czerniawski & Guzowski, 2014; Czerniawski et al., 2015). These cytokines can have a peripheral source, but can also be produced and released centrally by microglia and astrocytes. In order to test that LPS dysregulates neural circuit activity and impairs cognition via cytokines released from microglia, we blocked microglial activation with systemic administration of minocycline, a semi-synthetic tetracycline antibiotic that crosses the BBB. Rats were trained in context discrimination conditioning, in which they were placed into 2 similar behavioral chambers daily, with one of the environments paired with a brief, mild footshock. Upon reaching discrimination criterion, each subject received an intraperitoneal (i.p.) injection of minocycline (50 mg/kg) or saline and a second dose the next day. Thirty minutes after the second dose of minocycline or saline, LPS (150 μg/kg, i.p.) or saline was administered, 6 h prior to testing in each of the contexts. Minocycline treatment blocked the LPS-induced: 1) elevation of IL-1β and TNF-α in dorsal hippocampus, 2) the dysregulation of hippocampal circuit activity and 3) the impairment in context discrimination memory retrieval. In a separate experiment, minocycline (2 μg/side) infused directly into dorsal hippocampus robustly blocked the LPS-induced deficits in context discrimination memory retrieval. Together these findings provide a direct causal link between cytokine expression in the hippocampus and cognitive deficits during neuroinflammation. Lastly, this study provides a strong mechanistic basis for the potential use of minocycline or related compounds in the treatment of human patients with impaired cognitive function resulting from neuroinflammation. SUPPORT: AG00096-31, NIH NRSA Neurobiology of Aging; NIH R01 MH082930

Daniels, W. Carter ; Sanabria, Federico (*Arizona State University*) The Effect of Chronic Nicotine Administration on Sign- and Goal-Tracking: A Pilot Study. Nicotine appears to be a relatively weak primary reinforcer that, nonetheless, enhances the value of non-nicotine rewards. Consistent with this hypothesis, nicotine appears to enhance incentive motivation, facilitating the approach and handling of stimuli associated with rewards. It is possible that chronic exposure to nicotine increases the sensitivity to the enhancing effects of nicotine on incentive motivation, thus amplifying the dependence of individuals to nicotine. To test this hypothesis, 16 adult male Wistar rats received sub-cutaneous (s.c.) administrations of nicotine (NIC: 0.6 mg/kg) or saline (SAL) twice a day, once in the morning and once in the evening, for 12 consecutive days. Two days after the last administration, all rats were trained on a discriminated Pavlovian conditioned approach task, with levers serving as CS+ and CS-, for 12 consecutive sessions. In a two-session nicotine probe (7th and 8th session of training) all rats received one s.c. administration of 0.6 mg/kg of nicotine. Sign- and goal-tracking (lever pressing and head-entries, respectively) in the presence of the CS + and CS- did not differ significantly between groups NIC and SAL until the nicotine probe. Following the probe, rats in group NIC were more likely to sign-track than prior to the probe, and relative to rats in group SAL during

presentation of the CS+ and not the CS- or inter-trial interval (ITI). The probe also increased goal-tracking in the presence of CS- for rats in group SAL and during the ITI for group NIC. These results suggest that chronic exposure to nicotine increases the sensitivity to nicotinic enhancement of incentive motivation.

de Solis, Christopher; Holehonnur, Roopa; Banerjee, Anwesha; Luong, Jonathan, Lella Srihari; Ho, Anthony; Pahlavan, Bahram; Ploski, Jonathan (*University of Texas at Dallas*) Viral Delivery of shRNA to Amygdala Neurons Leads to Neurotoxicity and Deficits in Pavlovian Fear Conditioning. The use of viral vector technology to deliver short hairpin RNAs (shRNAs) to cells of the nervous system of many model organisms has been widely utilized by neuroscientists to study the influence of genes on behavior. However, there have been numerous reports that delivering shRNAs to the nervous system can lead to neurotoxicity. Here we report the results of a series of experiments where adeno-associated viruses (AAV), that were engineered to express shRNAs designed to target known plasticity associated genes (i.e. Arc, Egr1 and GluN2A) or control shRNAs that were designed not to target any rat gene product for depletion, were delivered to the rat basal and lateral nuclei of the amygdala (BLA), and auditory Pavlovian fear conditioning was examined. In our first set of experiments we found that animals that received AAV (3.16E13-1E13GC/mL), designed to knockdown Arc (shArc), or control shRNAs targeting either luciferase (shLuc), or nothing (shCntrl), exhibited impaired fear conditioning compared to animals that received viruses that did not express shRNAs. Notably, animals that received shArc did not exhibit differences in fear conditioning compared to animals that received control shRNAs despite gene knockdown of Arc. The highest dose of shRNA virus examined (3.16E13GC/mL), showed a significant increase in microglia activation as indicated by an increase in IBA1 immunoreactivity. In our final set of experiments, we infused viruses at a titer of 1.60E+12GC/mL (designed to (Arc, Luc, nothing or shRNAs designed to target Egr1 (shEgr1), or GluN2A (shGluN2A) and found that all groups exhibited impaired fear conditioning. However, the shGluN2A group exhibited significantly impaired fear conditioning compared to most of the groups, indicating that gene specific deficits in fear conditioning could be observed utilizing shRNAs. Collectively, these data indicate that viral mediated shRNA expression was toxic to neurons in vivo, under all viral titers examined and this toxicity in some cases may be masking gene specific changes in learning. Therefore, the use of this technology in behavioral neuroscience warrants a heightened level of careful consideration. SUPPORT: RMH096202A, RMH100650A and The University of Texas at Dallas

Elkin, Magdalyn E.; Freeman, John H. (*University of Iowa*) Ontogenetic Changes In Anterior Cingulate Cortical Activity during Trace Eyeblink Conditioning. . The ontogeny of trace eyeblink conditioning is believed to be dependent upon maturation of forebrain structures such as the anterior cingulate cortex (ACC) and hippocampus. (Goldsberry et al., 2015, J. Neurosci., 35:4238). Although neuronal responsiveness in the ACC during trace eyeblink conditioning has been studied in adults, there is a paucity of research concerning its role in the developing animals. The goal of the current study was to examine the development of neuronal activity in the ACC while developing rats were trained in trace eyeblink conditioning. We implanted moveable tetrodes in rat pups on postnatal days (P) 17, 19, 22 and 29. The rat pups were given one of day rest and then trained on trace eyeblink conditioning twice a day for three days, for a total of six sessions. Trace eyeblink conditioning involved presentation of a 250 ms tone conditioned stimulus CS, followed by a 500 ms stimulus-free trace interval which terminated in a 25 ms periorbital shock unconditioned stimulus (US). The rate of acquisition of trace conditioning increased across age groups. The spontaneous firing rate of ACC neurons also increased as a function of age. During conditioning trials there was a substantial developmental increase in the percentage of ACC neurons responsive to individual trial events (i.e., the CS, trace interval, US) and combinations of trial events from the first paired training session. The results indicate that the ACC undergoes developmental changes in neuronal coding of trial events. SUPPORT: NIH #NS038890

Farley, Sean; Freeman, John (*The University of Iowa*) Cerebellar Feedback is Necessary for Learning Related Activity in the Medial Auditory Thalamic Nuclei During Eyeblink Conditioning. We previously hypothesized that the cerebellum facilitates activity in its sensory inputs during learning. In the current study, we examined changes in neuronal firing patterns in the medial auditory thalamic nuclei (MATN) during cerebellar inactivation with muscimol. Rats were trained in delay eyeblink conditioning with alternating sessions of cerebellar inactivation or vehicle infusions with simultaneous tetrode recordings in the MATN. Eyeblink conditioning sessions consisted of a 2 kHz tone conditioned stimulus (CS) paired with a periorbital stimulation unconditioned stimulus (US). Rats were implanted with an infusion cannula directed at the anterior interpositus nucleus (AIN) ipsilateral to the conditioned eye. MATN cells were classified as short latency, long latency, sustained, or no response based upon their firing rates during the CS. After rats reached a conditioned response (CR) criterion, sessions were separated into two 50-trial blocks that were characterized as pre-infusion (1-50) or post-infusion (51-100). After the pre-infusion block saline or muscimol was infused into the AIN. Within-subject data from across rats were combined in a pre- and post-infusion neuronal analysis of the CS period. MATN long latency neuronal activity during the CS period was significantly depressed after muscimol infusions compared to controls. The majority of MATN neurons with long

latency responses to the CS altered their firing patterns to no response with AIN inactivation. Additionally, a significant proportion of sustained response cells changed to short latency responses with AIN inactivation. These firing pattern changes of individual cells with AIN inactivation support the hypothesis that cerebellar learning is driving learning-related activity in the MATN during eyeblink conditioning. SUPPORT: MH080005; NS088567

Furtak, Sharon; Calub, Catrina; Potter, Nicole (*California State University, Sacramento*) Perirhinal Cortex Involvement in Fear Extinction to a Discontinuous Visual Stimulus. The perirhinal cortex (PER) is known to be involved in high level perceptual processing. One hypothesis is that PER functions to unitize stimuli across time or across sensory modalities (Kent & Brown, 2012; Graham et al., 2006). While many findings have supported the role of PER in fear acquisition to discontinuous auditory stimuli and polymodal stimuli (Bucci, Phillips, & Burwell, 2000; Bucci, Saddoris, & Burwell, 2002; Corodimas & LeDoux, 1995; Kholodar-Smith, Allen, & Brown, 2008; Kholodar-Smith, Boguszewski, & Brown, 2008), to date no study has evaluated PER involvement in processing such stimuli during fear extinction. In the present study, Sprague-Dawley male rats were infused with muscimol, a GABA agonist, to temporarily inactivate the PER during extinction training. All subjects were surgically implanted with cannulae targeting PER bilaterally. Following recovery, animals were trained on a three-day fear extinction paradigm. On Day 1, animals underwent fear conditioning, in which they were presented with 5 trials of a discontinuous visual light stimulus (conditioned stimulus; CS) paired with a foot shock (unconditioned stimulus; US). On Day 2, animals received PER infusions of either muscimol or saline prior to extinction training, in which they received 20 trials of the CS alone. On Day 3, extinction recall was assessed by presenting an additional 15 CS trials. Freezing behavior was recorded throughout the experiment. Results showed that bilateral muscimol infusions into the PER during extinction training impaired extinction recall to a discontinuous light CS, as indicated by significantly higher levels of freezing in muscimol infused animals than saline control animals during extinction recall. The results suggest that particular cues may engage brain regions outside what is typically considered the fear extinction neural circuit. Here, for the first time results support a role for PER involvement in fear extinction, perhaps due to the discontinuous nature of the CS. SUPPORT: California State University, Sacramento SSIS/UEI Funding

Gahtan, Ethan; Bishop, Benjamin (*Humboldrt State University*) Light-Evoked Diving Reflex In Zebrafish Larvae. Escape behaviors have been studied in many organisms by neuroscientists seeking detailed, cellular- and synaptic-level descriptions of functional circuits. Escapes are useful because they can be elicited repeatedly and have consistent kinematics. Analysis of escape circuits in fish have revealed neural mechanisms for lateral movements, such as commissural projections and population coding of escape turn angle. However, while most fish navigate in 3 spatial dimensions, few studies have examined neural mechanisms of vertical movements during escapes. We characterized vertical escape swimming in zebrafish larvae using synchronized imaging from two cameras viewing a 10cm3 tank. Escapes elicited by dimming of ambient light consistently elicited downward spiral swimming (dives). At 20s post dimming, larvae showed more vertical (-18.8±2mm) than horizontal (3.2±0.6mm) displacement. The average slope of dives was -1.9±.35, meaning larvae move about twice as fast vertically than horizontally along their spiral paths. In a tubular tank with more vertical (400mm) than horizontal (100mm) range, vertical movement 120s after light dimming was -353±8.39mm (70 body lengths downward). Dives usually began within 1s after light dimming and continued until the tank bottom was reached. Maximum descent rate was 10.75mm/s (at 58s post dimming), and average descent rate was 5.41±0.24mm/s. Auditory taps also elicited rapid escape swimming with equivalent total distance traveled 20s post stimulus but with significantly less vertical and more horizontal displacement than on dimming-evoked escapes. These results suggest that light dimming-evoked spiral diving in larval zebrafish may be a protean escape reflex to specific types of predation threats, and that neural circuit models of escape control must look beyond mechanisms for lateral movements and also consider vertical movement control elements. This result parallels other cases in behavioral neuroscience where limitations of the behavioral testing environment limit possible conclusions about underlying neural mechanisms.

Garr, Eric; Delamater, Andrew R. (*Graduate Center of the City University of New York; Brooklyn College*) Action Sequences are Sensitive to Reward Devaluation. Habitual responses can clearly be dissociated from goal-directed actions both at the behavioral and neural levels. Explanations of how goal-directed actions transition to habitual responses have traditionally focused on the different associations that underlie behavior, with response-outcome associations thought to control behavior early in training while stimulus-response associations dominate later in training. Recent computational modeling from the field of reinforcement learning suggests that habits are the result of automatic, stereotyped action sequences (Dezfouli & Balleine, 2012). To determine if stereotyped action sequences are goal directed, different groups of rats were given the opportunity to earn food rewards under different heterogeneous instrumental chain schedules involving two levers. One group (LLRR) learned to press the left lever twice followed by two right lever presses to earn a grain pellet. Another group (LRRR) was initially unconstrained in which four lever-press sequences earned pellets, but was later shifted to an LRRR schedule. Finally, another group (LR)

learned to press the left lever followed by the right lever for pellets. In all cases, accuracy increased over sessions while variability decreased. A selective satiation manipulation was given at the end of each training procedure in order to assess goal-directedness. Each rat was sated on either the earned grain pellets or equally potent sucrose pellets, after which they were given a five-minute extinction test in which no pellets were earned for pressing the levers. Each rat was sated on both types of pellets over different days. The extinction tests revealed that each group was sensitive to devaluation—rats generally executed less target sequences while sated on the earned outcome compared to when sated on the other outcome. Future studies will address the role of extended training on action sequence sensitivity to reward devaluation and selective PIT tests. SUPPORT: The Graduate Center of the City University of New York; NIDA SC1 DA034995

Gonzalez, Sarah T.; Fanselow, Michael S. (*University of California, Los Angeles*) The Neural Mechanisms of Safety Learning in Mice. Safety learning is the ability to suppress fear in the presence of a stimulus that signals the absence of an aversive event, yet little is known about its neural mechanisms. Here we investigated whether safety learning was associated with altered activity in the medial prefrontal cortex in mice. Mice were divided into three conditions: the Fear Learning condition, in which presentations of a light were immediately followed by a foot shock, the Safety Learning condition in which the light signaled the absence of the shock for at least 2 minutes, and the Random Control condition in which the light provided no information about the occurrence of the shock. Following eight days of training, all subjects received a summation test in which the light was presented in a second context that had been paired with shock.

Activity of the prelimbic and infralimbic regions of the medial prefrontal cortex after the summation test was compared using expression of the immediate early gene arc, a marker of cellular activity. Recent investigations have suggested that the infralimbic region may be involved in fear extinction, a distinct learning process in which a stimulus that previously signaled an aversive event is repeatedly presented alone until it no longer produces a fear response. While safety learning and extinction are distinct learning processes, both are believed to involve the development of a new association in which the stimulus signals the absence of the aversive event, and may therefore involve similar neural mechanisms. Arc protein levels suggested that safety learning may involve increased activity in the infralimbic cortex. SUPPORT: NIH #3RO1MH062122

Goode, Travis; Jin, Jingji; Holehonnur, Roopashri; Ploski, Jonathan; Maren, Stephen (*Texas A&M University*) Combinatorial DREADD Silencing of Ventral Hippocampal Neurons Projecting to Infralimbic Cortex Prevents Fear Renewal. Extinguished fear to a conditioned stimulus (CS) renews outside of the place or context in which extinction training occurred. Here we used designer receptors exclusively activated by designer drugs (DREADDs) to test the hypothesis that projections from the ventral hippocampus (VH) to the infralimbic region (IL) of the medial prefrontal cortex mediate fear renewal. Rats received bilateral VH infusions of inhibitory DREADD virus (AAV-CaMKiiα-hM4D-mCherry), Cre-dependent DREADD silencer (AAV-hSyn-DIO-hM4D-mCherry), or control virus (AAV-CaMKiiα-GFP). In rats receiving Cre-dependent DREADD virus, the IL was infused with a canine adenovirus (CAV) to retrogradely express Cre and inhibitory DREADDs in VH:IL circuits. 2-4 wks later, rats were conditioned and extinguished (in separate contexts) to an auditory CS (10 s, 2 kHz, 80 dB tone; unconditioned stimulus [US] = 2 s, 1 mA footshock). Freezing behavior served as the index of fear. 24 hrs after extinction, and in a counterbalanced within-subjects design, rats were tested to the CS outside of the extinction context on and off CNO (1 or 3 mg/kg, i.p.). As predicted, DREADD-mediated inactivation of either VH or VH neurons projecting to IL disrupted fear renewal as compared to vehicle treatment or reporter-only controls. These data are consistent with the possibility that, during fear renewal, the VH inhibits the IL to limit the suppression of fear to an extinguished CS. SUPPORT: NIH R01MH065961-12A1 to S.M.

Goosens, Ki; Barbara Gisabella, Junmei Yao (*Massachusetts Institute of Technology*) Enhanced Growth Hormone in Amygdala Neurons Dysregulates Associative Fear Memory Allocation. Chronic stress produces a long-lasting vulnerability to excessive fear memory formation following trauma, but the mechanisms by which stress leads to strong fear memories are not understood. One possibility is that individual neurons show greater fear-related plasticity in a stressed brain compared to an unstressed brain. A second possibility is that stronger fear memories, like those observed in stress-exposed individuals, recruit a larger number of neurons during fear memory encoding. We previously found that chronic stress robustly upregulates growth hormone (GH) protein expression in amygdala tissue and hypothesized that GH may be an unrecognized growth factor within the amygdala, where it may contribute to stress-associated disorders that involve an over-encoding of traumatic memories, such as post-traumatic stress disorder (PTSD). Here, we examined the effects of excess GH on neuronal morphology within the amygdala and also on neuronal recruitment during fear memory formation. We found that overexpression of GH within the amygdala nearly doubles spine density on both primary and secondary branches of pyramidal amygdala neurons; this increase was observed for thin, mushroom, and stubby spines. We also found that GH overexpression nearly doubles the number of neurons that express the immediate early gene cFos after auditory fear conditioning, suggesting that stress promotes fear memory formation by increasing the size of the neuronal networks in the amygdala that

encode memory. Additionally, GH-overexpressing neurons had a significantly greater probability of expressing cFos as compared to neighboring neurons that did not overexpress GH. Because activity-dependent release of GH can promote synaptic plasticity, this provides a mechanism by which individual neurons may be predisposed to stronger synaptic plasticity during fear memory formation. Thus, chronic stress may enhance fear memory formation by promoting larger and stronger fear memories through upregulation of GH. This work provides a novel link between GH and psychiatric disease. SUPPORT: NIMH (R01 MH084966), and the U.S. Army Research Office and the Defense Advanced Research Projects Agency (grant W911NF-10-1-0059) to KAG

Gould, Thomas (*Temple University*) Adolescent Nicotine Exposure Alters Adult Contextual Fear Conditioning. Adolescent nicotine use is a serious health issue. The Center for Disease Control and Prevention reports that every day 3,800 adolescents smoke their first cigarette, that e-cigarette use in this age group has tripled in the last year and is now higher than conventional cigarette use, and that nearly 90% of adult smokers initiated smoking by age 18. While it is clear that tobacco product use and nicotine addiction contributes to substantial health problems in the United States, increasing evidence suggests that adolescent nicotine exposure may also cause health problems beyond those commonly associated with tobacco products. Specifically, adolescent nicotine use may produce long-term cognitive deficits. Early adolescent tobacco use was associated with memory deficits and late adolescent smokers had cognitive deficits that emerged after initiation of smoking. These effects were also seen for secondhand tobacco smoke exposure. Children 8-15 years old exposed to secondhand smoke had higher rates of attention-deficit/hyperactivity disorder. Long-lasting deficits in cognition associated with adolescent tobacco use are especially troubling because multiple mental illnesses that have symptoms that include changes in cognition are also associated with higher rates of tobacco use. Thus, adolescent tobacco use may not only lead to addiction but it may contribute to adult cognitive deficits and exasperate cognitive symptoms associated with mental illness. An initial study in mice found that adolescent nicotine exposure is sufficient to produce adult deficits in hippocampus-dependent learning measured with contextual fear conditioning. Preliminary data suggest that these deficits may be associated with altered brain acetylcholinergic function. Thus, this presentation will examine behavioral effects of adolescent nicotine exposure on adult learning, factors that modulate these effects, and cholinergic manipulations that ameliorate associated deficits in contextual fear conditioning. SUPPORT: NIH DA017949

Gray, Michael S.; Lynch III, Joseph F; Winiecki, Patricki A.; Jasnow, Aaron M. (*Kent State University*) Presynaptic GABAB(1a) Receptors Preserve Context Memory During Consolidation. Anxiety disorders, such as PSTD, are characterized by generalization of fear responses to neutral stimuli. GABA-mediated presynaptic inhibition plays a critical role in fear memory specificity, as animals with genetically deleted presynaptic GABAB(1a) receptors cannot discriminate between CS+ and CS- tones. We have previously identified that GABAB(1a) receptors play an important role in maintaining memory precision for context. GABAB(1a)-/- mice show generalized fear to a neutral context 24 hours after training, but not 2 or 6 hours after training (Cullen, et al. 2014). The same pattern is observed with object location and recognition, suggesting that this receptor subtype affected the consolidation and retrieval of contextual and spatial memories. To examine more precisely if GABAB(1a) receptors are involved in consolidation or retrieval of a precise fear memory, we administered infusions of the selective GABAB(1a) receptor antagonist, CGP 36216, intracerebroventricular (ICV), or specifically into the dorsal hippocampus or anterior cingulate cortex (ACC), at different time points during and after context fear conditioning. Post-training ICV or dorsal hippocampal infusions resulted in fear generalization to the neutral context 24 hours later. However, ICV, dorsal hippocampus, or ACC infusions prior to testing did not result in context generalization. These data suggest that presynaptic inhibition through GABAB(1a) receptor activation plays an important role in the consolidation, but not retrieval, of precise contextual memories, as we originally had concluded. Thus, with a combination of genetic and pharmacological manipulations we have more precisely identified the critical role of GABA-mediated presynaptic inhibition in the formation of precise contextual memories. SUPPORT: Whitehall Grant, Farris Family Foundation

Hafenbreidel, Madalyn; Rafa Todd, Carolynn; Smies, Chad. W.; Twining, Robert C.; Mueller, Devin (*University of Wisconsin, Milwaukee*) Blocking Infralimbic Basic Fibroblast Growth Factor (bFGF or FGF2) Facilitates Extinction of Drug Seeking. Stimulant drug use results in structural and functional changes in reward-related brain regions, which may underlie the persistence of compulsive drug seeking and relapse that characterizes drug addiction. Neurotrophic factors, such as basic fibroblast growth factor (bFGF or FGF2), are necessary for neuronal survival, growth, and differentiation, and may mediate drug-induced morphological changes. Following cocaine exposure, bFGF is increased in brains regions such as the infralimbic medial prefrontal cortex (IL-mPFC). The IL-mPFC is necessary for extinction, but if drug-induced over-expression of bFGF in this region affects extinction is unknown. Thus, we aimed to determine if blocking bFGF in IL-mPFC would facilitate extinction following cocaine self-administration (SA). Rats were trained to lever press for i.v. infusions of cocaine prior to extinction. Extinction consisted of four 30 min extinction sessions, in which rats received IL-mPFC infusions

of a neutralizing antibody against bFGF prior to each session. Extinction retention was tested during a subsequent 90 min extinction session. Blocking bFGF in the IL-mPFC decreased lever pressing during the 90 min extinction session, indicating facilitated extinction. In contrast, blocking bFGF and returning rats to their home cage had no effect on subsequent extinction. Next, we examined if bFGF protein expression was altered following extinction. Rats were trained to self-administer cocaine as before with half undergoing extinction or not. Additionally, rats that were reinforced with sucrose and underwent extinction or not, rats that received yoked-saline infusions that were paired with cocaine SA administering rats (extinction or not), and a naïve control were included. bFGF protein expression in the IL-mPFC was only increased following cocaine SA, an effect reversed by extinction. These results suggest that cocaine-induced over-expression of bFGF in the IL-mPFC inhibits extinction, as reducing bFGF expression during extinction permits rapid extinction. Therefore, targeted reductions in bFGF during therapeutic interventions could enhance addiction treatment outcomes. SUPPORT: R01 DA038042; University of Wisconsin-Milwaukee Graduate School

Hanson, Erica E., Mitchell, Suzanne H. (*Oregon Health & Science University*) Fos expression after exposure to an effort discounting procedure. Objective: Apathy is a hallmark of numerous psychiatric disorders. However our understanding of the processes regulating the unwillingness to engage in effortful responses is unclear. This research used the measurement of a marker of neuronal activity to determine the contribution of different brain areas to the decision to work for a food reward.

Methods: 64 Long-Evans rats (N=32 per sex) were used in this study. Rats were divided into three groups: a food-restricted experimental group, a food-restricted group control group, and a baseline control group that wasn't food restricted. Rats in the food-restricted experimental group were trained on an effort-discounting task based upon a modified adjusting amount procedure (Richards, Mitchell et al 1997, J Exp Anal Behav 67: 353) in which the subjective value of 150-μL of sucrose was measured. Discounting in the trained group was established by testing rats on five effort levels (0.01, 0.15, 0.35, 0.6, 0.9 Ns). Un-trained control rats were placed in the operant chambers, but did not complete the task. Rats were euthanized on their last session so that brain tissue could be stained for cFos, a habituating marker of neural activation. Numbers of Fos-positive cells were counted visually, and compared between groups for each region.

Results: For the trained rats, the subjective value of the 150-μL reward decreased as a function of increasing effort. When rats were tested at a single cost magnitude (0.6 Ns) prior to their last session, the subjective value of the 150-μL sucrose was stable across sessions. Preliminary cell-counting suggests an enrichment of cFos-positive cells in accumbens and cingulate, for food-restricted, experimental animals over controls.

Conclusions: These results are consistent with the view that the striatum is involved with the choice to perform effort for a food reward, rather than the value of the reinforce itself. SUPPORT: NIH # T32 DA007262

Heroux, Nicholas; Robinson-Drummer, Patrese A.; Rosen, Jeffrey B.; Stanton, Mark E. (*University of Delaware*) Role of NMDA Receptors in the Acquisition and Retention of the CPFE in Adolescent Rats. The context preexposure facilitation effect (CPFE) is a contextual fear conditioning paradigm in which learning about the context, acquiring the context-shock association, and retrieving/expressing contextual fear are temporally dissociated. The current set of experiments examined the involvement of NMDA receptors across all phases of contextual fear learning in the CPFE in developing animals. Experiment 1 found that systemic injections of .1mg/kg of the non-competitive NMDA receptor antagonist MK-801 given before multiple context preexposure disrupted subsequent 24hr retention test freezing after training. Experiment 2 demonstrated that pre-training MK-801 disrupts not only the acquisition and immediate expression of contextual fear measured by a postshock freezing test, but also subsequently disrupts freezing in a retention test 24 hours later. Experiment 3 employed pre-retention systemic injections of MK-801 and showed that expression of contextual fear via a 24hr retention freezing test does not depend on NMDA receptors and that freezing deficits resulting from MK-801 reflect learning rather than performance effects. Experiment 4 found that the consolidation of contextual information was partially disrupted by post-preexposure MK-801 whereas the consolidation of contextual fear was not disrupted by post-training MK-801 administration. Finally, Experiment 5 reexamined the effects of pre-training MK-801 on subsequent immediate postshock and 24hr retention tests by employing a dose-response design and found that higher doses of MK-801 (.1mg/kg) disrupt both postshock and retention test freezing while lower doses of MK-801 (.025 or .05mg/kg) only disrupt retention freezing. In summary, the acquisition of contextual information during context preexposure and of the context-shock association during the training day of the CPFE is NMDA receptor dependent in adolescent rats. Future experiments will employ DHPC, BLA, and mPFC cannula in an attempt to localize these effects, and will examine the ontogeny of postshock freezing within the CPFE and sCFC. SUPPORT: NIH grant R01 HD075066-01A1 to MES and JBR

Hersman, Sarah; Hoffman, Annie; Hodgins, Lili; Shieh, Shannon; Lam, Jamie; Fanselow, Michael S (*UCLA*) Dissociation of Cholinergic Modulation in Dorsal Hippocampus and Basolateral Amygdala on Stress-enhanced Fear Learning. An inappropriately high fear response can manifest as an anxiety disorder such as post-traumatic stress

disorder (PTSD). While the contextual aspect of the fear memory is dependent on the dorsal hippocampus (DH), the associative emotional memory is dependent on the basolateral amygdala (BLA). In both regions, acetylcholine (Ach) provides an important signal for this learning. Open questions are how these neural substrates change during maladaptive fear learning, and whether inhibiting Ach disrupts maladaptive fear. Using Stress-Enhanced Fear Learning (SEFL), which models some aspects of PTSD in rats, we tested whether Ach in DH and BLA is required for maladaptive fear. To dissociate the effect of Ach in DH and BLA, we infused scopolamine or vehicle into these brain regions immediately before SEFL, and tested fear in both the trauma context and a novel context after a mild stressor in that novel context. The results show that during learning, Ach acting within both DH and BLA is required for sensitization of future fear learning. Rather than simply sensitizing the BLA, SEFL requires functional signaling in both the DH and BLA; this larger circuit, and the requirement for Ach, suggests future research and therapeutic targets for human PTSD. SUPPORT: Ruth L. Kirschstein National Research Service Award (T32 - NS058280), NIMH Grant RO1 62122, the Narsad Distinguished Investigator Award # 18667, and the ARCS Foundation Fellowship.

Huckleberry, Kylie A.; Ferguson, Laura B.; Drew, Michael R. (*University of Texas at Austin*) Behavioral Mechanisms of Context Fear Generalization. There is growing interest in generalization of learned contextual fear, driven in part by the hypothesis that mood and anxiety disorders stem from impaired hippocampal mechanisms of fear generalization and discrimination. However, there has been relatively little investigation of the behavioral and procedural mechanisms that might control generalization of contextual fear. We characterized context fear generalization in two common conditioning protocols—foreground and background contextual fear conditioning—and assessed the relative contribution of different context features to generalization. In Experiment 1, C57bl6/j mice were fear conditioned in A, and then tested for fear both of A and of an alternate context created by varying a subset of A's elements. Generalization was greater when the two contexts differed only in shape than when contexts differed only in odor or floor configuration. The results suggest that floor configuration and odor are more salient features than chamber shape. In Experiment 2, we compared context fear generalization in background versus foreground context conditioning. Generalization to a similar context (A′) was stronger after foreground conditioning than background conditioning. However, foreground conditioning also generated higher levels of contextual fear; when the levels of fear generated by the two procedures were equated by varying the number of conditioning trials, background and foreground procedures produced approximately equivalent levels of generalization. In Experiment 3, we assessed the effects of test order. Generalization between A and A′ was weaker when mice were tested in A first. Data suggest that the test order effect is driven by extinction occurring during the first test. In conclusion, results demonstrate that context generalization is highly sensitive to procedural variations and likely reflects the operation of multiple interacting psychological and neural mechanisms. SUPPORT: R01 M102595; R00 MH083943

Huda, K. Rebecca; Latsko, Maeson S; Gilman, Lee T; Jasnow, Aaron M. (*Kent State University*) Resistance to Social Defeat Stress is Characterized by Poor Behavioral Inhibition; Assessment in Passive Avoidance. Individual differences in vulnerability to social stress have been reported in recent chronic stress models. Following social defeat, mice are characterized as being either resistant (high social approach behavior) or susceptible (low social approach behavior) to social defeat stress as observed in a subsequent social interaction test with a novel conspecific. Traditionally, the resistant phenotype has been thought to be a well-suited model for human resilience to social stress. However, our lab has identified a number of behaviors suggesting that resistant mice have deficits in behavioral inhibition. For example, resistant mice have increased fear to a neutral cue (CS-) compared to susceptible mice. These findings fit our earlier report that resistant mice also have impaired extinction learning compared with susceptible mice. Taken together, these data suggest that resistant mice may be characterized by poor behavioral or response inhibition and social approach behavior. To further test this hypothesis, we are currently examining the relationship between susceptibility and resistance to social defeat and performance on a passive-avoidance task, which requires mice to inhibit their natural tendency to cross into the dark chamber after having experienced footshock. We hypothesize that resistant mice will demonstrate significantly shorter step-through latencies during a retrieval test compared to susceptible and control mice, which might indicate impaired response inhibit in this phenotype. These findings will help continue to characterize behavioral and neurobiological features associated with the resistant phenotype.

Hui, May; Zelikowsky, Moriel; Anderson, David (*California Institute of Technology*) Tac2 mediates chronic stress in mice. Stress is a behavioral response found in virtually all animal species. Recently, Tachykinin 2 (Tac2), which encodes the neuropeptide Neurokinin B (NKB), has been implicated in fear memory consolidation. We were interested in exploring the possibility of a larger role for Tac2 in mediating stress. In particular, we examined whether systemic and local antagonism of the NKB-specific receptor, NK3, with the drug osanetant, could alter the effects of various manipulations to induce stress. Mice were subjected to chronic stress comprised of either social isolation, restraint stress, or footshock, given daily across 14 days. Mice were then tested for stress-induced changes in behavior using a looming assay (as

a measure of innate defensive behavior) and trace fear conditioning (as a measure of cognitive function and stimulus reactivity). At test, mice were treated with a systemic injection (i.p.) of osanetant or microinfusions into the dorsallateral striatum or the dorsal bed nucleus of the stria terminalis. We found that osanetant was able to buffer against the effects of chronic stress. Moreover, we found that systemic administration of the NK3 agonist senktide was able to induce stress-like effects in non-stressed, group-housed control mice. Collectively, our data point to a broad role for Tac2 in the regulation of chronic stress. SUPPORT: Caltech Samuel N. Vodopia and Carol J. Hasson SURF Fellowship

Johnson, Brandon. A.; Wilson, W. Jeffrey (*Albion College*) Light-induced Attenuation of Response to Light in the Earthworm Lumbricus terrestris. Earthworms (Lumbricus terrestris) locomote in response to light. In examining their responses to repeated 10-min lights over an extended period of time (24 or 48 hr) we noted anecdotally that the response to light seemed smaller when the light came soon after another light. We set out to examine this light-induced attenuation more systematically. Earthworm locomotion was recorded as movement of a computerized running wheel; data were not used for a small number of worms that did not move at all or that were dead at the end of the session. In Experiment 1 independent groups (n=16) of worms received multiple presentations of light of duration 1, 2, 5, or 10 min, separated by periods of darkness equal to 1, 2, 3, 6, and 9 times the light duration. Responses were equivalent following all of these intervals of darkness, but were attenuated compared to response to the first light. Cumulative effect of repeated exposure to light might have obscured meaningful differences; examination of the reduced response to the second light compared to the first for each worm suggested that this might be the case. Experiment 2 exposed each worm to light (1-, 2-, 4-, 8-min duration, n=8/group) twice, with an intervening period of dark (2, 8, 32, 128 min). Control worms received light only at the second time. Response to the second light was significantly attenuated for long-duration lights (4 & 8 min), but not short-duration. Duration of the dark interval did not matter. Thus earthworms respond less to a second exposure to a light of sufficiently long duration for up to 128 min after initial exposure. We think this attenuated response reflects a habituation-like phenomenon rather than a sensory or motor effect. SUPPORT: Albion College Dept. of Psychological Science, and Foundation for Undergraduate Research, Scholarship, & Creative Activity

Kennedy, Bruce C; Kohli, Maulika; Maertens, Jamie; Marell, Paulina; Gewirtz, Jonathan C (*University of Minnesota*) Conditioned Object Preference: A Novel Measure of Drug-Seeking in Rodents. Conditioned place preference (CPP) is a commonly used task in rodents in which preference for an environment is acquired through associative learning following pairing/s with drug exposure. Studies in humans have demonstrated that reward-paired objects, such as drug paraphernalia, also elicit conditioned responses. However, the ability of objects to serve as conditioned stimuli has yet to be explored in rodents, despite the greater stimulus control afforded by objects versus environments. We present here initial findings from a novel Conditioned Object Preference (COP) task, in which we measured the preference of adult rats for objects previously paired with cocaine or morphine administration. We first measured investigation of test objects to establish baseline object preference which was followed by alternating saline and drug conditioning days. Rats were injected with saline or drug (cocaine: 20mg/kg, intraperitoneal (ip); morphine: 10mg/kg, subcutaneous) and placed into the test chamber containing two copies of the same object. Different objects were used for saline versus drug conditioning sessions. When tested in a drug-free state, rats investigated the cocaine- or morphine-paired object significantly more than the saline-paired object, indicating acquisition of COP. Cocaine COP was then extinguished via repeated testing without cocaine. Following extinction, object preference returned to preconditioning levels but COP was reinstated with a priming dose of cocaine (10 mg/kg, ip). In a final experiment, we conditioned animals in one chamber (chamber A) and tested them in a second chamber (chamber B). Following extinction in chamber B, cocaine COP was still observed when animals were re-tested in chamber A, indicating "renewal" of COP. These findings demonstrate that, similar to environments, objects can elicit conditioned approach behavior through associative learning mechanisms and that COP is amenable to the phenomena of extinction, drug-primed reinstatement, and renewal. SUPPORT: University of Minnesota Grant-in-Aid

Kim, Earnest; Lattal, Matthew K. (*Oregon Health & Science University*) Amygdala Modulation of Cocaine-seeking Behavior. Persistent drug use despite harmful consequences is a hallmark characteristic of addiction. Human and animal studies have suggested that compulsive drug-seeking behavior to be the result of prefrontal cortical dysfunction and subsequent loss of "top down" inhibitory control of striatal circuity. However, it remains unclear how affective information over time becomes disregarded with chronic drug use. Here we investigate the role of the amygdala in compulsive drug-seeking behavior using a rat intravenous self-administration model in which cocaine-seeking is procured at the risk of receiving mild fooshocks. Male Long Evans rats underwent cocaine self-administration training under a seeking-taking chain schedule of reinforcement. Daily self-administration sessions consisted of a maximum of 30 trials. After one press on the seek lever and a variable interval (5 to 10 sec), one additional press resulted in a retraction of the seek lever and insertion of a take lever. Subsequently one take lever press resulted in an intravenous cocaine infusion (0.89 mg/kg at rate of 0.088 mL/5 sec). Af-

ter >5 weeks of self-administration training, rats received 9 days of shock sessions in which 30% of the seek lever ended with a mild footshock (0.4 mA, 0.5sec). Sham rats quickly learned to suppress their drug taking and seeking responses during shock. In contrast, animals with electrolytic lesion to the amygdala (BLA + CeA), continued to press both take and seek levers during punishment. These results suggest that amygdaloid processing is critical for compulsive drug seeking behavior and highlights the need to understand further how "bottom up" limbic-cortical and limbic-striatal circuits contribute to the development of compulsive drug-seeking behavior. SUPPORT: NIH grants T32DA007262 and R01DA025922

Knox, Dayan; Stanfield, Briana R; Staib, Jennifer; Keller, Samantha M; DePietro, Thomas. (*University of Delaware*) Using Measures of c-Jun Activity to Determine Mnemonic Processes Through which SPS Exposure Disrupts Extinction Retention. Single prolonged stress (SPS) is a rodent model of post traumatic stress disorder that leads to extinction retention deficits. However, it is unknown if SPS disrupts extinction retention by enhancing fear memory and/or disrupting extinction memory. We previously attempted to use behavioral manipulations to address this question, but the results of these experiments were inconclusive. In this study, we examined mnemonic processes through which SPS exposure results in extinction retention deficits by assaying c-Jun levels in fear and extinction circuits after fear conditioning, extinction training, and extinction testing in SPS and non-stressed rats. Groups of SPS and non-stressed rats were either fear conditioned (CS-fear) or presented with CSs in the absence of any footshock (CS-only). Subsets of rats were then euthanized after fear conditioning, extinction training, or extinction testing. A separate set of rats were euthanized after removal from their housing colony in order to establish basal levels of c-Jun. c-Jun levels were measured in the medial prefrontal cortex (mPFC), dorsal hippocampus (dHipp), ventral hippocampus (vHipp), lateral amygdala (LA), and basal amygdala (BA). In replication of previous studies, SPS induced extinction retention deficits. Preliminary findings suggest that SPS decreased baseline c-Jun levels in the Hipp. No changes in c-Jun levels during fear conditioning were observed with any of our treatments. During extinction training there were increases in c-Jun levels in the mPFC of non-stressed rats, and this effect was attenuated in the infralimbic region of SPS rats. SPS exposure also attenuated changes c-Jun levels in the Hipp and BA during extinction training. While the study is ongoing, the results support the hypothesis that SPS-induced changes in c-Jun transcriptional activity in the mPFC, Hipp, and BA during extinction training contribute to extinction retention deficits in the SPS model. SUPPORT: NIH COBRE grant - 1P20GM103653; UDRF grant awarded to D.K.

Koraym, Adam; Gallimore, Darci; Kidd, Tara; Dodd, Maria; Hennessy, Michael B.; Claflin, Dragana I. (*Wright State University*) Effects of Early-Life Stress and Social Buffering on Learning in Juvenile Rats. It is well known that separation from the mother can increase HPA activity and the presence of the mother can reduce glucocorticoid levels in the presence of stressors. Changes in glucocorticoids can, in turn, influence learning processes. The current study examined the interaction between early-life stress and the presence or absence of the mother on circulating corticosterone levels (Experiment 1). The impact of these effects on the development of subsequent learning processes were then assessed using delay classical eyeblink conditioning (Experiment 2). In 17-day-old rat pups, the absence of the mother greatly elevated glucocorticoid levels over a 60 minute period whereas the presence of the mother suppressed this response. In Experiment 2 rat pups experienced 3 sessions of shock exposure on Day 17 and were randomly assigned to one of 3 social manipulation groups. Immediately after each shock exposure session pups were either returned to the dam (social buffering), kept separate in a novel environment for 90 minutes (maternal separation), or returned to the dam for 45 minutes but then separated for 90 minutes (delayed maternal separation). On Day 24, rats received 3 sessions of delay eyeblink conditioning. At this time they had undergone weaning on Day 21 and were individually housed. Preliminary data suggest that acquisition of eyeblink conditioning for the maternal separation and social buffering groups was not significantly different from a naïve control group that did not experience any stressors at Day 17. However, the delayed maternal separation group may have been facilitated in acquiring delay eyeblink conditioning. It appears that a second stressor during the early consolidation period may enhance acquisition. These data are discussed in terms of recent findings demonstrating improved cognitive performance following oscillations in circulating glucocorticoid levels. SUPPORT: NIH/NIGMS, R25GM090122, IMSB BioSTAR and the Department of Psychology, Wright State University

Kwapis, Janine L.; Alaghband, Yasaman; Kramar, Eniko A.; Matheos, Dina P.; Rhee, Diane; Lopez, Alberto, J.; Wood, Marcelo A. (*University of California, Irvine*) HDAC3: An Epigenetic Key to Ameliorating Synaptic Plasticity and Memory Deficits in the Aging Brain. Aging is accompanied by cognitive impairments, including difficulty forming long-term memories. Long-term memory formation requires gene expression, a process that may be disrupted with age (Rowe et al., 2007; Berchtold et al., 2008). Epigenetic alterations (changes in gene expression that occur through alterations in chromatin structure) may therefore contribute to age-related impairments in both gene expression and long-term memory. One major epigenetic mechanism important for memory is histone acetylation, in which acetyl groups are added or removed from histone tails by acetyltransferases (HATs) or histone deacetylases (HDACs),

respectively. Increasing histone acetylation by blocking HDAC activity generally enhances both gene expression and long-term memory. In particular, histone deacetylase 3 (HDAC3) appears to be a key negative regulator of long-term memory formation, as blocking HDAC3 produces persistent object location memory following subthreshold training in young mice. Here, we tested whether HDAC3 activity also contributes to age-related impairments in long-term memory and synaptic plasticity. First, we deleted HDAC3 in the dorsal hippocampi of aging (18-month-old) mice before training them in the hippocampus-dependent object location memory (OLM) task. As predicted, aging mice showed severe impairments in OLM. Deleting HDAC3 in the hippocampus rescued this deficit; aging mice lacking hippocampal HDAC3 showed robust memory for OLM. We next tested whether HDAC3 also limits synaptic plasticity in the aging hippocampus. A single train of 5 theta bursts failed to produce stable LTP in slices from 18-month-old mice, confirming that synaptic plasticity is also impaired with age. Blocking HDAC3 activity with a dominant negative mutant (HDAC3Y298H), however, ameliorated this deficit, resulting in robust and stable potentiation. Together, these results indicate that HDAC3 is a key negative regulator of both long-term memory and synaptic plasticity in aging mice. SUPPORT: NIH grants MH81004, MH101491, DA025922, DA036984, DA031989 to M.A. Wood and NIH T32 AG000096-31 to J.L. Kwapis

Lacagnina, Anthony F; Drew, Michael R (*University of Texas at Austin*) Enhanced Context Fear Memory with Spaced Context Exposures. Contextual fear conditioning (CFC) is an associative learning paradigm that requires an animal to form a mental representation of the surrounding context before an aversive stimulus may become associated with it. Theories of CFC posit that such mental representations can be acquired during passive exploration of an environment and are essential for conditioning, but make no predictions about how variation in the temporal interval between context preexposure and conditioning will affect learning. Because the synaptic and anatomical substrates of memory change over time, we predicted that the interval between context preexposure and conditioning would affect the strength of conditioning. We found that context preexposure 72h or 24h before single-shock CFC produced higher conditioned fear than did preexposure 1min before or contiguous with conditioning. Next we asked whether the effect of preexposure timing was mediated through enhanced fear acquisition or increased resistance to extinction. To do so, we tested whether variation in the timing of context preexposure affects sensitivity to postshock context exposure, a manipulation that potently extinguishes context fear. Again, increased preexposure-to-conditioning intervals were associated with higher freezing; however, the timing of context preexposure and postshock duration did not interact, suggesting that these manipulations independently affect the strength of conditioning. We hypothesized that the superiority of 24h or 72h preexposure intervals relates to refinement of the hippocampal context representation during memory consolidation. To characterize the effect of preexposure interval on hippocampal context representations, we performed activity-dependent neural tagging using transgenic ArcCreERT2 mice. In summary, CFC acquisition is enhanced when sufficient time elapses between context preexposure and conditioning. We hypothesize that CFC acquisition is enhanced when it involves reactivation of a consolidated context memory as opposed to simultaneous acquisition of a context memory and a context-shock association. SUPPORT: NIH 5R01MH102595-02

Latsko, Maeson S.; Farnbauch, Laure A.; Jasnow, Aaron M. (*Kent State University*) Enhanced juvenile glucocorticoid response is associated with an adult resistant phenotype following juvenile social defeat. Adolescence is a critical developmental period during which stress reactivity is exacerbated compared to adulthood, and when the experience of stress can severely compromise appropriate adult stress responsiveness. Targeting specific mechanisms underlying long-term effects of early life stress is important yet challenging, given the significant individual variability in the response to stress. To begin to understand predictors of individual differences in response to social stress, we examine immediate and enduring effects of periadolescent social stress in mice focusing on identifying predictive markers and mechanisms underlying two phenotypic behavioral responses that emerge as animals transition into adulthood. Specifically, we subject periadolescent mice to a mild repeated social defeat paradigm followed by a social interaction test. We then examined the effects of periadolescent social defeat on social interaction at several developmental stages. We hypothesized that neurobiological markers and physiological responses to periadolescent social defeat stress may predict adult phenotypic responses to social stress. One day following periadolescent social defeat, all mice display a resistant phenotype. When tested 8, 21, and 30 days later, two phenotypic responses emerge; most mice display a susceptible phenotype, whereas a small percentage remains resistant. Baseline and adult post-interaction corticosterone levels were not associated with either phenotype. However, mice that continued to display the resistant phenotype into adulthood had significantly elevated periadolescent post-interaction serum corticosterone levels compared with mice that developed the susceptible phenotype and control mice. These data suggest that the presence of elevated corticosterone during periadolescence may confer some protection against the delayed effects of early life social defeat. These findings and future studies will help determine the role of stress hormones on the ontogeny of differential response to adolescent social stress. SUPPORT: Farris Family Foun-

dation

Laude, R. Jennifer; Fillmore, T. Mark (*University of Kentucky*) Activities Engaged in while Drinking Alcohol Affect Perceived Intoxication and Liking of Effects. The effect of different experiences had while intoxicated on the appetitive properties of alcohol is a relatively unexplored area of study. It may be that the constellation of positive effects is enhanced by a history of reward while drinking. Using a sample of moderate drinkers, we tested whether engaging in rewarding activities while intoxicated would increase the appetitive properties of the drug relative to another condition in which subjects engaged in a boring activity throughout the drinking episode. Drinkers were brought into the laboratory for two sessions, each on a different day. They were then assigned to either a placebo or active dose condition. In session one, participants consumed a beverage and then played stimulating computer games (rewarding condition). During the second session, the same dose of alcohol received in session one was consumed but this time, a boring task was performed (boring condition). Changes in the subjective liking of alcohol effects, and perceived intoxication were measured using a visual analog scale after each treatment (rewarding vs. boring). Drinkers liked the effect of their drinks most when they had experienced a history of reward, regardless of dose. They also felt most intoxicated when engaging in a rewarding activity following a low dose of alcohol. Surprisingly, those who had a history of being bored following the active dose perceived little to no intoxication. These data have implications for the limited success of behavioral interventions aimed at reducing drinking that fail to consider the role of the environment and subjective responses to alcohol. SUPPORT: NIAAA F31AA023694 and R01AA018274

Lee, Ji-Hye; Kimm, Sunwhi; Choi, June-Seek (*Korea University*) Effect of Emotional Trauma during Adolescence on subsequent Aversive Memory Formation in rats. Psychological trauma experienced during adolescence has lasting effect on an individual's emotional life. We developed an animal model of traumatic event during adolescence: a rat placed in an inescapable, donut-shaped maze was chased by a fast-approaching "bullying robot". Effect of the trauma was examined in two ways: specific memory formation and regulation of new emotional memory. Adolescent male Sprague Dawley rats were exposed to the trauma at postnatal day 33 to 35. They received 20 trials per day for 3 days which consisted of a warning signal (5-s tone) and overlapped 2.5-s of being chased by the robot at every 15-20 second. After 3 weeks, animals were re-exposed to the context and tone to test memory retention. Rats were also tested in the elevated-plus-maze and received Pavlovian threat conditioning with 3 pairings of tone CS (20-s, 75 dB) and footshock US (1-s, 0.6-mA). Following conditioning, memory tests for context and cue were conducted. Additionally, rats received two sessions of extinction and test for retention of extinction. We found that rats that had experienced the initial traumatic event showed a higher level of fear response both to the context and the cue used in the traumatic experience. In the elevated-plus-maze test, rats that had experienced trauma displayed reduced entries to open arms. Rats that had received the trauma showed a greater level of fear response during fear conditioning. They also showed increased fear response to the tone CS and the conditioning context. In addition, they showed retardation in the subsequent extinction learning. Taken together, the current results suggest that the "bullying" robot is an effective new method for inducing traumatic stress using adolescent rats, which seems to up-regulates general anxiety and the formation of aversive memory in subsequent emotional events, as well as preserving the memory of initial trauma per se. SUPPORT: NRF 2013M3C7A1056734

Lee, Yeon Kyung; Hong, Eun-Hwa; Kim, Sunwhi; Jie, Hyesoo; Choi, June-Seek Choi (*Korea University*) Uncontrollable stress effects on discriminatory avoidance learning. Choosing an appropriate defensive response in the face of danger constitutes an important survival strategy. Two different types of behavioral paradigm, namely the active and passive avoidance learning, have been employed to study brain circuits involved in learning of defensive response. However, little is known about defensive decision-making between the two conflicting responses. Here we used the discriminatory avoidance learning (DIAL) in which two conditioned stimuli (CS1 and CS2) signal the same aversive stimulus (footshock unconditioned stimulus, US) but different types of avoidance are required to rats. There is a strong clinical relationship between exposure to profound stress and deficits in adaptive behavior, and one of the key brain area modulating this relationship is the medial prefrontal cortex (mPFC). In the previous study, we showed the lesion of mPFC in early phase of DIAL impaired the discriminatory performance. In this experiment, we assessed the ability of rats to select appropriate defensive behavior in threatening situations with two types of uncontrollable stress. In the first experiment, 14 rats (8 stress vs. 6 control) were trained in a shuttle box with CS1 (4 kHz, 10 s) that requires a "go" response and CS2 (500Hz, 10 s) that requires a "no-go" response. The rats trained 2 days of DIAL, and then they were exposed to restraint stress for next 4 days. After total 6 days of training, both groups of rats showed equal level of discriminatory responding. In the second experiment, 13 rats (8 stress vs. 5 control) were exposed by cold water immersion stress. The results showed that the stress group was impaired in learning of discriminatory avoidance compared to the control group. In sum, our data showed that DIAL is a useful paradigm for investigating how different responses are selected in aversive situations and that the different types of stress interfere the process where the CS information must be utilized to select an optimal defensive response. SUPPORT: NRF-2013M3C7A1056734

Loh, Ryan; Galvez, Roberto (*University of Illinois at Urbana-Champaign*) Neocortical Kappa Inhibition Attenuates Forebrain-Dependent Associative Learning. There have been several findings indicating a primary role for the opioid receptor system in the modulation of learning and memory. Spatial tasks, fear conditioning, and y-maze spontaneous alternation have all demonstrated that opioid modulation can impair task acquisition. The current literature investigating opioid effects in learning and memory have focused on the mu-opioid receptor; however, several reports have indicated that there is a likely role for the kappa-opioid receptor in various behavioral learning paradigms. We have recently demonstrated that systemic administration of the kappa specific opioid antagonist, NorBNI, prior to training delays acquisition for whisker-trace eyeblink (WTEB) associations in mice. In investigating the specific brain region mediating this effect, it is well known that primary somatosensory neocortex (S1) is required for the acquisition and consolidation of the association. The following study utilized intra-S1 injections of NorBNI with the behavioral paradigm WTEB to determine if the kappa-opioid receptor is mediating its learning induced effect through S1. In WTEB, animals are trained to associate whisker stimulation (CS) with a salient unconditioned stimulus (US) that causes an unconditioned response. After several trials in which the CS is paired with the US, the animal begins to exhibit a conditioned response in anticipation of the US. Separating the CS and US with a stimulus free trace interval makes this paradigm forebrain dependent by recruiting higher brain structures such as the hippocampus and most importantly, the neocortex. The current study utilized localized S1 injections of 10μg and 20μg of NorBNI paired with WTEB behavioral training. Local injections of NorBNI significantly retarded learning relative to saline controls, suggesting that the kappa-opioid receptor is neocortically involved in the acquisition of forebrain-dependent trace associative learning. Further research will focus on determining the specific downstream effects of the kappa-opioid receptor that is mediating this effect in learning. SUPPORT: Internal

Lynch III, Joseph; Gray, Michael; Ouellette, Emily; Adkins, Jordan; Winiecki, Patrick A.; Riccio, David C.; Jasnow, Aaron M.; (*Kent State University*) Estradiol-induced Fear Generalization is Blocked by Inactivation of NMDA or AMPA Receptors Prior to Retrieval . Generalization of fear responses is a symptom of many anxiety disorders and we have previously demonstrated that females generalize fear to a neutral context at a faster rate than males. This effect is due in part, to activation of ERβ and modulation of memory retrieval mechanisms resulting in fear generalization. Given that the effects of estradiol on fear generalization required approximately 24 hours, our data suggested possible genomic actions on fear generalization. To determine whether these actions were due to cytosolic versus membrane bound receptors, female rats were given infusions of ICI 182,780, a cytosolic estrogen receptor antagonist, into the lateral ventricle or dorsal hippocampus simultaneously with estradiol treatment or with an ERβ agonist (DPN). Infusions of ICI into the dorsal hippocampus blocked the generalization induced by treatment with estradiol or DPN, suggesting that estradiol acts through cytosolic ERβ. In a complimentary experiment, animals were infused with bovine serum conjugated estradiol (E2-BSA) to test the effects of membrane bound estrogen receptor activation on fear generalization. E2-BSA infusions into the dorsal hippocampus did not induce fear generalization. Moreover, rats receiving intra-hippocampal infusions of the ERK/MAPK inhibitor, U0126, continued to display estradiol-induced generalization, again suggesting that membrane-bound estrogen receptors do not contribute to fear generalization. These data suggest that estradiol-induced enhancements in fear generalization are mediated through activation of cytosolic/nuclear receptors. We hypothesized that estradiol induced fear generalization in the hippocampus through downstream enhanced glutamatergic signaling, Thus, in the final experiment, animals were given peripheral injections of estradiol 24 hours before a retention test and an infusion of low concentrations APV or NBQX into the dorsal hippocampus 5 minutes prior to testing. Infusions of either antagonist attenuated estradiol-induced generalization, but had no effect on learning or generalization when administered alone, suggesting that estradiol-induced generalization is due, in part, to enhanced glutamatergic signaling. SUPPORT: Whitehall Grant; Farris Family Foundation

Murawski, Nathen J.; Asok, Arun (*Center for Neuroscience, UC Davis (NJM); Department of Psychology and Brain Sciences, University of Delaware (AA)*) Digital Context Fear Conditioning in Rats: Utilizing LCD-Based Visual Context Manipulations During Conditioning. Contextual fear conditioning (CFC) is a powerful behavioral assay for examining the neurobiology of learning and memory. The simple behavioral setup and robust long-lasting memories formed during conditioning have made CFC an ideal paradigm for incorporating methodological innovations in behavioral neuroscience. CFC is highly context-specific in that higher levels of conditioned freezing are observed when training and testing occur in the same, versus a different, context. Typically, one context is made distinct from another by manipulating one or more sensory features of a context (e.g., visual). Developing new methodologies to systematically alter contexts can improve experimental control over context features and help better identify the neural substrates that support CFC. We examined if adult male rats show the context pre-exposure facilitation effect (CPFE) by altering visual information provided by four LCD screens surrounding a chamber. Rats received context pre-exposure on Day 1, an immediate 1.5 mA foot shock on Day 2, and a con-

text test on Day 3. Rats were pre-exposed to Context A or B on Day 1. Context A and B only differed in the visual image projected onto the LCD monitors. Controls included rats pre-exposed to a distinct context and those without pre-exposure. Immediate shock and context test occurred in Context A. Rats pre-exposed to Context A showed the CPFE with significantly higher levels of freezing compared to controls. Rats pre-exposed to Context B failed to show the CPFE, with freezing that did not differ significantly from any group. The results suggest that 1) visual features contribute to CFC in rats and that 2) visual components of the context can be parametrically controlled via LCD screens. Quantitative control and temporal precision of contextual features afforded by digital context presentations can greatly aid researchers in their understanding of discriminating and generalizing fear to aversive contexts. SUPPORT: 1 R01 HD075066-01A1 (ME Stanton and JB Rosen, University of Delaware)

Noble, J. Lindsey; Gonzalez, Ian J; Ngo, Tiffany T, Malhotra, Simran; Meyers, Eric; Bleker, Nathaniel, Carrillo, D'angelique D; Ramananthan, Karthik R; Rennaker, Robert L; Kilgard, Michael P; McIntyre, Christa K* (*The University of Texas at Dallas*) Vagus Nerve Stimulation Enhances Extinction of Conditioned Fear in an Animal Model of PTSD. Trauma-related disorders, such as post-traumatic stress disorder (PTSD) are typically treated with cognitive behavioral therapy (CBT), as failure to extinguish conditioned fear is a core symptom. Exposure therapy is a form of cognitive behavior therapy where patients are repeatedly exposed to the cues that elicit maladaptive conditioned responses. After repeated exposures to conditioned cues in the absence of reinforcement, conditioned responses should be extinguished. However, in humans with PTSD this often is ineffective. An adjunct therapy that could be administered with CBT to increase the effectiveness of the therapy would be beneficial. Optimal adjuncts should enhance memory consolidation, as extinction learning requires memory formation, while reducing anxiety to the stimuli presented during therapy. Vagus nerve stimulation (VNS) is an FDA-approved treatment for the prevention of seizures. Recent research indicates that VNS can enhance memory consolidation in rats and humans, and research from our lab has shown that VNS can enhance fear extinction in healthy animals. Additionally recent research from our lab shows that VNS can reduce anxiety. This evidence suggests that VNS has the rare combination of effects that are needed in an adjunct to CBT. The single prolonged stressor (SPS) is an animal model of PTSD that exhibits many symptoms of PTSD seen in humans, including failure to extinguish conditioned fear. The purpose of these experiments is to determine if VNS administered during extinction can enhance extinction learning, even in a population that is resistant to fear extinction. We have found that VNS-paired extinction not only enhances extinction in SPS-treated animals vs. SPS-treated animals receiving sham stimulation during extinction, but also enhances extinction to the same level as controls not subjected to SPS. These results suggest that VNS could be effective as an adjunct to CBT. SUPPORT: NIH MH0869600101A1, DARPA

Ouellette, B. Emily; Lynch III, F. Joseph; Riccio, C. David; McEwen, S. Bruce; Jasnow, M. Aaron (*Kent State University*) Corticosterone Attenuates Context Fear Generalization in Male Rats. A characteristic of many anxiety disorders, including PTSD is generalization of fear responses to neutral stimuli. A counterintuitive feature of PTSD is that individuals with lower cortisol levels tend to be more susceptible to develop the disorder. Recent evidence in rodents suggests that administration of corticosterone protects against the delayed behavioral and cellular effects of acute restraint stress. Given these data, we wanted to see if the administration of corticosterone would also attenuate fear generalization. We assessed the effects of corticosterone administration on fear generalization by training male Long-Evans rats in passive avoidance, and injecting them with 25 mg/kg of corticosterone or vehicle either 30 minutes prior to, or 30 minutes following training. Animals were then tested 14 days later in either the training context or a neutral context. Animals that were given pre-training injections of corticosterone displayed significant fear generalization. However, rats receiving post-training injections of corticosterone displayed significantly attenuated fear generalization compared to rats receiving vehicle control. These data suggest that increased corticosterone during the consolidation period may help enhance memory precision for context and attenuate fear generalization. These data have particular relevance to PTSD, as they are consistent with recent reports of protective effects of glucocorticoids on behavioral and cellular symptoms triggered by traumatic stress. SUPPORT: Harris Family Foundation; Whitehall Grant

Pennington, Zachary T.; Avershal, Jacob Z.; Anderson, Austin S.; Fanselow, Michael S. (*University of California, Los Angeles*) Broadening the stress enhanced fear learning (SEFL) model: acute shock exposure augments subsequent startle responses and contextual fear of a startle-paired context. Exposure to traumatic events is able to produce an enduring sensitization of several stress processes, among them, the acquisition of novel fear associations. Our laboratory has previously developed a model of this phenotype in which acute exposure to 15 unsignaled shocks in one context subsequently increases fear of a novel CS paired with a single shock. However, because both learning experiences utilize the same unconditional stimulus (US), it has been unclear whether heightened learning within this preparation is US-specific. Furthermore, the extent to which this model captures other aspects of the stress sensitization process has not been thoroughly explored. Here, we assessed whether the same 15 shock stressor we have traditionally used would similarly increase fear learning about a different US: an auditory

startle stimulus. Open field activity was also assessed. Animals previously exposed to 15 shocks showed an increased auditory startle response and also displayed enhanced freezing in the context in which the startle stimulus was presented. However, these animals did not display altered open field activity. These findings demonstrate that enhanced fear learning in our model is not limited to a single US, although general US processing may be altered. Furthermore, these findings show that this model can simultaneously capture multiple phenotypes associated with traumatic experience. Lastly, this data demonstrates that both startle responses and freezing can be measured using the same automated, video-based, software. SUPPORT: NIH R01 #2R01MH062122-05A1 to MSF; NRSA 1F31MH108257-01 to ZTP

Pina, Melanie M.; Cunningham, Christopher L. (*Oregon Health & Science University*) Ethanol-Seeking Behavior is Expressed Directly through an Extended Amygdala to Midbrain Neural Circuit. Drug-associated cues produce profound psychological and physiological effects, driving craving, drug seeking and ultimately relapse. Previous research has indicated that drug seeking is modulated by the bed nucleus of the stria terminalis (BNST) of the extended amygdala. Anatomically, the BNST sends dense projections to the ventral tegmental area (VTA), a midbrain region essential for motivation and reward. Behaviorally, disconnection of BNST from VTA has significantly attenuated cue-induced cocaine seeking, as measured by conditioned place preference (CPP). Though indirect, this evidence suggests that a BNST-VTA circuit may underlie cue-induced drug seeking. In the present experiments, we used selective manipulations to directly establish a role for the BNST to VTA neural circuit in cue-elicited ethanol seeking in adult male DBA/2J mice. An unbiased CPP procedure was used, where ethanol (2 g/kg, IP) was paired with a distinct tactile cue and an expression (seeking) test was given 24 h after the final conditioning session. We used a retrograde intersectional strategy to drive expression of inhibitory designer receptors (hM4Di-DREADD) in an intermixed yet distinct subpopulation of BNST neurons that project to VTA. To selectively silence BNST-VTA neurons, hM4Di was activated by clozapine-N-oxide (CNO; 10 mg/kg, IP) injection 30 min before the CPP test. Silencing of BNST-VTA neurons by CNO blocked ethanol CPP expression. Control studies showed this was not due to CNO or viral infection alone, as CPP was not affected in vehicle-administered hM4Di mice or CNO-treated GFP mice. Our findings suggest that a BNST-VTA projection is critically involved in ethanol seeking, as indexed by CPP. Moreover, our work complements and expands upon previous findings and provides novel evidence for a direct BNST input to VTA in cue-induced ethanol seeking. With the existing literature, these studies indicate that BNST and its VTA projections are promising targets for treatments directed at reducing craving and relapse. SUPPORT: NIH-NIAAA R01AA007702; Vertex Scholar Award; OHSU Brain Institute Fellowship; APA Dissertation Research Award; Psi Chi Graduate Research Grant; N.L. Tartar Trust Fellowship

Pisansky, Marc T.; Gewirtz, Jonathan C. (*University of Minnesota - Twin Cities*) Intranasal oxytocin enhances socially transmitted fear behavior in mice. Oxytocin is an evolutionarily conserved neuropeptide implicated in social cognition processes. The oxytocin system is dysregulated in psychiatric diseases characterized by impaired social cognition (e.g., autism, schizophrenia), and intranasal oxytocin enhances emotional recognition (a component of "empathy") in both normal and psychiatric populations. In order to investigate the neurobiological effects of oxytocin on social cognition, we have developed paradigms for measuring socially transmitted fear, in which an observer mouse views a demonstrator mouse undergoing Pavlovian fear conditioning. Observer mice exhibit freezing or escape, and—importantly—these fear behaviors occur more robustly when the observer-demonstrator pair are familiar (i.e., siblings) with one another. We hypothesized that oxytocin would enhance socially transmitted fear behaviors. Therefore we administered oxytocin intranasally (20μg/kg) to non-sibling observer mice using both acute (1 day) and sub-chronic (5 days) regimens. Compared to saline controls, sub-chronic oxytocin-treated observer mice exhibited significantly more freezing (during) and escape (on the day following) conditioning of demonstrator mice. Observer freezing behavior also correlated with demonstrator distress vocalizations recorded during conditioning, but only for oxytocin-treated observer mice. Acute administration of oxytocin 30mins prior to conditioning had no effect on freezing. These data suggested that sub-chronic intranasal oxytocin produced neuroadaptive changes, perhaps in endogenous oxytocin signaling mechanisms. To test this hypothesis, we conducted quantitative PCR analyses of oxytocin receptor (OXTR) expression in observer mouse brain tissue. Although unaffected in the anterior cingulate cortex, an area associated with the affective component of pain processing, expression of OXTR mRNA was significantly reduced in the amygdala of oxytocin-treated observer mice. This study elucidates one mechanism by which intranasal oxytocin enhances social cognition and therefore promises to contribute to our understanding of psychiatric diseases in which these processes are compromised.

Pizzimenti, Christie; Lattal, K. Matthew (*Oregon Health & Science University*) Context-independent effects of shock on drug-seeking. Even following long periods of abstinence individuals with anxiety disorders have high rates of relapse to drugs of abuse. Many current models of relapse examine stressful experiences that occur in close temporal and physical (i.e., within the same context) proximity to the reinstatement test. Therefore, little is known about how potentially stressful or fearful experiences in other contexts can cause persistent changes in drug-seeking behavior.

In three experiments we examined the effects of fear conditioning in one context on drug-seeking for methamphetamine in another context. In Experiment 1, animals were trained to self-administer intravenous methamphetamine, followed by extinction. Animals then received either a battery of 15 shocks in a distinct environment or exposure to that context only. Twenty-four hours later animals received a single shock in the self-administration context, and while this failed to produce reinstatement, animals that received a battery of shocks the day before froze significantly more than controls. In Experiment 2 the battery of shocks was administered during acquisition of self-administration. Animals that received shock reinstated significantly more than controls to drug-associated cues and took significantly longer to extinguish lever pressing following drug-cue-induced reinstatement. In Experiment 3 the battery of shocks were administered before acquisition of self-administration. Animals that received shock lever pressed for methamphetamine significantly more than exposure only controls during acquisition. In a separate group of animals, the battery of footshocks was also shown to significantly elevate plasma levels of corticosterone relative to exposure only controls to levels that are consistent with physiological stress. Taken together, these results suggest that a history of fear conditioning may induce greater rates of reinstatement to drug-related cues and confer resistance against extinction following reinstatement. SUPPORT: NIDA, R01DA025922; NIDA, T32DA007262

Pochiro, Joseph M; Goodfellow, Molly J; Lee, Michael A; Lindquist, Derick H. (*The Ohio State University*) Trace Fear Conditioning and Hippocampal NR2B-NMDA Receptor Signaling in Adult Rats Administered Ethanol During the Third Trimester-equivalent Period. Modeling fetal alcohol spectrum disorders (FASD) in rats, our lab administers ethanol (5 g/kg/day) over postnatal days (PD) 4 to 9, corresponding to the third trimester 'brain growth spurt'. As adults, cognitive function is assessed by trace fear conditioning (TFC), whose acquisition depends on a distributed forebrain circuit (Raybuck & Lattal, 2014). Interestingly, the dorsal hippocampus (DH), and NR2B-containing NMDA receptors, become necessary only when the CS-US trace interval exceeds 5-10 sec (Chowdhury et al. 2005; Gao et al. 2010). Consistent with such data, Dupont et al. (2014) found impaired TFC in FASD rats trained with a 15 but not 5 sec trace interval, whereas the expression of phosphorylated ERK1/2—activated downstream of the NMDA receptor—was significantly reduced in CA1 and CA3 neurons at both intervals. Immunoblot results also indicate the DH of FASD rats contains significantly fewer NR2A and NR2B membrane-bound subunits (Goodfellow et al., In press). Altogether, postnatal ethanol is hypothesized to disrupt NMDA receptor signaling during TFC, limiting or perturbing the intracellular cascades required to consolidate new learning.

In the current study, adult (~PD70) rats were trained with 10 CS-US pairings, separated by a 30 sec trace interval; over the next two days freezing was measured during context and CS retention tests. Only during the trace interval, following tone CS offset, was freezing significantly reduced in FASD rats. During TFC, synaptic NMDA-gated calcium influx stimulates CaMKII translocation and NR2B binding, activating plasticity-related signaling molecules essential to LTP maintenance and long-term memory consolidation (Halt et al. 2012). A second group of rats were therefore sacrificed 20 min post-training in order to image (confocal, 60X) and quantify NR2B and phosphorylated αCaMKII immunofluorescent signal intensity, normalized to Hoechst-stained cell counts, in areas CA1, CA3, and the dentate gyrus. Results will be discussed in terms of putative treatment group differences. SUPPORT: ABMRF, grant 60034780

Rafa Todd, Carolynn; Hafenbreidel, Madalyn; Otis, James, M.; Twining, Robert, C.; Mueller, Devin (*University of Wisconsin, Milwaukee*) Infralimbic NR2A-containing NMDA Receptors are Necessary for the Reconsolidation of Cocaine Self-administration Memory. Addiction is characterized by high relapse susceptibility, which can be triggered by drug-associated cues. Cue presentation results in retrieval of the original drug-cue memory that becomes labile and must be reconsolidated back into long-term storage. Repeated unpaired cue presentation, however, induces extinction. Thus, cue-reactivity can be reduced by blocking reconsolidation or facilitating extinction. Systemic blockade of NMDA receptors (NMDArs) disrupts reconsolidation of drug-cue associations in a modified self-administration (SA) paradigm or extinction in a standard SA paradigm. To further characterize these processes, we examined the effects of post-extinction injections of an NMDAr antagonist (CPP) on drug seeking following SA. Rats acquired cocaine SA followed by extinction. Extinction consisted of four 45-min extinction sessions in which rats were administered CPP after each session. Extinction retention was then tested during a subsequent 90-min session. CPP treatment decreased lever pressing during subsequent extinction sessions, suggesting either disrupted reconsolidation or facilitated extinction. Next, we targeted the infralimbic medial prefrontal cortex (IL-mPFC), a structure implicated in extinction. Using the same procedure, CPP infusions before or after four brief extinction sessions resulted in a similar reduction in lever pressing across subsequent days. To determine the NMDAr subtype involved, we infused either the NR2A-selective antagonist NVP or the NR2B-selective antagonist Ro25 after four 45-min extinction sessions. Similar to the effects of nonspecific NMDAr blockade, blocking NR2A- but not NR2B-containing NMDArs reduced lever pressing across subsequent days. Finally, to dissociate if blocking NR2A-containing NMDArs disrupts reconsolidation or facilitates extinction consolidation, NVP was infused

into IL-mPFC after four 10-min reactivation trials or in the absence of behavioral testing. Memory retention was tested during a subsequent 90-min session, revealing that blocking NR2A-containing NMDArs after memory reactivation reduced lever pressing. Overall, these results indicate that blocking NR2A-containing NMDArs in IL-mPFC disrupts reconsolidation of the original drug-cue memory rather than facilitates extinction. SUPPORT: R01 DA038042; University of Wisconsin-Milwaukee Graduate School

Rankin, Catharine H.; Giles, A.C., Ardiel, E. A., Yu, A. (*University of British Columbi*) Response Based Analyses of Behavior Overlook Other Important Behavioral Changes: Integrating Habituation into Ongoing Behavior. Traditionally researchers who study habituation have focused on a single dimension of the behavior (i.e., response probability or magnitude). Our high throughput behavioural analyses of habituation of two different responses for wild-type and mutant strains of C. elegans have changed this view. First we have shown that there are a number of independent components (habituation rate and final level for probability, duration and speed) of habituation of the tap response that show different forms of plasticity and, for the most part, are mediated by different genes. In addition, for both tap and photoactivation of the ASH neurons the response does not occur in a vacuum- there are changes in ongoing behavior that complement the response decrement. Interestingly, as some aspects of behavior decrement others appear to sensitize. When the changes in the components of behavior are integrated it facilitates dispersal allowing the animal to move away from the area. This offers a new way to think about the role of habituation and sensitization in the context of overall behavioral strategies. These findings also have implications for other response-based measures of learning and memory. SUPPORT: NSERC grant #122216-2013

Reyes, Kyrie-Anne E.; Kudva, Priya S; Todd, Ryan P; Sorg, Barbara A. (*Washington State University*) Prazosin disrupts reconsolidation of appetitive and aversive behavior in rats: discordance between behavior and ultrasonic vocalizations. Reconsolidation of memory is the process whereby memories are recalled (reactivated) and then become susceptible to disruption by certain pharmacological agents if they are given shortly after the memory is reactivated. In this study, we examined the effects of the alpha-1-adrenergic antagonist, prazosin, for its ability to disrupt the reconsolidation of an appetitive memory (cocaine-induced conditioned place preference, CPP) and an aversive memory (fear conditioning). Using a CPP model, we found that rats given prazosin immediately after memory reactivation did not show CPP for their cocaine-paired side 1 day or 1 week later compared with rats given saline. In contrast, prazosin had no effect on CPP in rats not given memory reactivation, suggesting that prazosin prevented CPP memory reconsolidation. We also examined 50 kHz (positive affective) ultrasonic vocalizations (50 USVs) associated with the cocaine memory. Contrary to the suppressed CPP in rats given memory reactivation + prazosin, the number of 50 USVs was not different between treatment groups when tested 1 day after memory reactivation: 50 USVs were elevated in both groups on the cocaine-paired vs. saline-paired side. In fear conditioning studies, rats given prazosin immediately after memory reactivation decreased freezing behavior 1 day and 1 week later compared with rats given vehicle. Prazosin had no effect on freezing in rats not given memory reactivation, suggesting that prazosin prevented fear memory reconsolidation. However, the number of 50 USVs was equally suppressed in both treatment groups. Taken together, prazosin appears to disrupt the reconsolidation of both an appetitive and aversive memory. The number of 50 kHz USVs did not mimic the behavior for CPP or fear conditioning, indicating that its correlation with behavior is not straightforward. USVs may be a more sensitive index of memory or of positive affect than is motor output. SUPPORT: Washington State Initiative 171 (Alcohol and Drug Abuse Research Program)

Robinson-Drummer, Patrese; Heroux, Nicholas A.; Stanton, Mark E. (*University of Delaware*) Intra-dorsal Hippocampal antagonism of Muscarinic Acetylcholine receptors disrupts the Context Preexposure Facilitation Effect. Cholinergic dysfunction produced by neonatal alcohol exposure can be mitigated by developmental cholinergic enhancement (Monk, et al., 2012, Hippocampus 22:1750–1757). This dysfunction also contributes to learning impairments in juvenile rats tested in a variant of context fear conditioning known as the context preexposure facilitation effect (CPFE) (Dokovna et al., 2013, Behavioral Brain Research, 248:114–120). However the specific brain regions subserving cholinergic effects on the CPFE during development are not known. Scopolamine, a muscarinic acetylcholine receptor antagonist, disrupts the CPFE in juvenile rats, when administered systemically before preexposure, training and testing, or before any single phase alone (Dokovna, & Stanton, 2012, ISDP Abstract). The current experiment extended these findings by locally infusing scopolamine into the dorsal hippocampus on the preexposure, training and testing day. On PD 31, one group of juvenile rats received bilateral infusions of scopolamine (35μg) or PBS 10min before preexposure to the training context (Pre group) or an alternate context (Alt-Pre group). Twenty-four hours later, a second set of animals received infusions before receiving two immediate 2s, 1.5mA shocks in the training context. Finally, 24hr after training, a third set of animals received infusions 10min before testing in the training context. For all infusion days, the Pre group given PBS exhibited significantly more fear to the training context than the Alt-Pre group. However, Pre group animals given scopolamine were not significantly different from the Alt-Pre group. This finding illustrates a specific role of dorsal hippocampal cholinergic function in contex-

tual fear conditioning in juveniles. Enhancing hippocampal cholinergic function may reverse deficits in the CPFE produced by neonatal alcohol exposure. SUPPORT: NIH grant R01 HD075066-01A1 to MES; Office of Graduate and Professional Education Graduate Scholarship

Rose, Jacqueline; Alfiler, Lauren K.; Pribic, Micaela R. (*Western Washington University*) Pavlovian Conditioning Produced by Activating Two Identified and Distinct Neural Circuits in C. elegans. Pavlovian Conditioning has led to several discoveries into mechanisms of learning and memory. After determining a stable alteration in behavior following stimulus pairing, researchers have been able to uncover brain regions, neural circuits and cellular mechanisms involved. Using the C. elegans model, with its well-described nervous system that includes circuits between 302 identified neurons total, we have been able to take the reverse approach, selecting US and CS based on the known neurons activated by these stimuli and then measuring an increase in responsiveness after these mapped neural circuits are activated close together in time. To examine Classical Conditioning in C. elegans, a mild mechanosensory stimulus (vibration of worm substrate produced by a low-frequency, 100 Hz auditory tone) activating the 'touch circuit' served as the NS/CS. For the US, exposure to either UV or blue-wavelength light (450 nm) activating the recently described 'light circuit' was employed. C. elegans show reliable avoidance to UV and blue-wavelength light as their outer cuticle is transparent making them vulnerable to damaging light rays. When these stimuli were paired in a delayed pairing protocol (4 sec tone whereby US light onset occurs at a 2 sec delay), an increase in responsiveness was seen to the CS (tone-alone) stimulus at two minutes after pairing. When no light is presented, a slight decrease in responding to the tone-alone can be seen, similar to habituation. Early data suggest that increasing the number of pairings does not immediately increase the response to tone. Additional studies are underway to test the possibility of 'circuit' plasticity by determining if motor response activation or inhibition can become associated to the tone-alone stimulus by pairing the activation of channelrhodopsin expressed in the excitatory, as well as inhibitory, motor output neurons.

Sangha, Susan (*Purdue University*) Amygdalocortical Circuitry Contributes to Discriminative Reward, Fear and Safety Learning. Accurate discrimination of environmental cues predicting reward, fear or safety is important for survival. The amygdala, prelimbic and infralimbic cortices are implicated in regulating reward-seeking and fear behavior; however, no studies have examined their roles in discriminating among reward, fear and safety cues. Using a discriminative conditioning task that includes presentations of a reward cue (paired with sucrose), fear cue (paired with footshock) and a compound fear+safety cue (no footshock) within the same sessions allowed us to assess the flexibility and precision of fear and reward-seeking behaviors to these cues. Single unit recordings made in the rat basal amygdala showed neurons discriminate among reward, fear and safety cues. One particular population of neurons showed a selective response to only the safety cue, demonstrating safety selective neurons in the basal amygdala. Local, reversible inactivations of the prelimbic or infralimbic cortex yielded differential results: inactivating the prelimbic cortex blunted discriminatory reward seeking whereas infralimbic cortical inactivation impaired discrimination between the fear and safety cues. Together these results imply that the amygdalocortical circuitry contributes to precise discriminative reward, fear and safety learning.

Shipman, Megan L.; Trask, Sydney; Green, John T.; Bouton, Mark, E. (*The University of Vermont*) Inactivation of the Prelimbic Cortex Attenuates Context-Dependent Excitatory Operant Responding. In operant renewal, extinguished operant behavior can recover when tested outside the context in which it was extinguished. Previous work (Eddy et al., submitted) has shown that inactivation of the prelimbic (PL) region of the medial prefrontal cortex (mPFC) by baclofen/muscimol (B/M) during testing attenuates renewal of an extinguished response when testing occurs in Context A (e.g., ABA renewal). One explanation for this attenuated renewal is that the PL may play a role in context-dependent excitatory responding (e.g., Thrailkill & Bouton, 2015). Two experiments tested this prediction. In Experiment 1, rats learned to lever press for a sucrose-pellet reward. Once the behavior was acquired, animals received an infusion of either B/M or saline vehicle into the PL and were tested in the acquisition context, Context A, or a different context, Context B. Although both groups showed a decrement in responding in Context B (a typical context-switch effect), inactivation of the PL decreased responding in Context A relative to the vehicle controls. Given that PL inactivation decreased behavioral control by the acquisition context, other types of renewal in which testing occurs outside the acquisition context should not be affected by PL inactivation. Therefore, in Experiment 2, the same rats again responded for the sucrose reinforcer in Context A. Responding was then extinguished in a new context, Context C. Animals then received an infusion of either B/M or saline into the PL before being tested in the extinction context, Context C, or another context, Context D. As predicted, both groups showed ACD renewal that was unaffected by PL inactivation. A final test of ABA renewal verified that the cannulae were still functional and replicated Eddy et al. (submitted). Rather than affecting renewal generally, inactivation of the prelimbic cortex attenuates ABA renewal by reducing context-dependent excitatory responding in the conditioning context. SUPPORT: NIH RO1 DA 033123 to MEB

Shors, Tracey J. ; Olson, Ryan; Brush, Christopher J.; Alderman, Brandon (*Rutgers University*) Mental and

Physical (MAP) Training: Combining Meditation and Aerobic Exercise Enhances Synchronized Brain Responses during Conflict Monitoring. Feelings of fear and anxiety can interfere with our ability to monitor and ultimately resolve conflict in our lives. In this study, we provided a clinical intervention known as MAP Training which stands for Mental and Physical Training. The intervention was inspired by and translated from laboratory studies that relate mental and physical training activities to neurogenesis in the adult hippocampus (Shors et al., Neurobiology of Learning and Memory, 2014). The intervention was provided to individuals who were either clinically depressed (n=23) or otherwise healthy (n=33). Participants engaged in twice-weekly sessions, beginning with 30 minutes of "mental" training with focused-attention silent meditation followed by 30 minutes of "physical" training with supervised aerobic exercise at a moderate intensity. Before and after MAP Training, EEGs and event-related potentials (ERPs) were recorded during the Flanker Test, as a measure of cognitive control during conflict monitoring.

Before MAP Training, individuals with clinical depression displayed less robust ERP responses (indexed by N2 and P3) during the flanker task when compared to healthy individuals (Alderman et al., Frontiers in Human Neuroscience, 2015). After 8 weeks of MAP Training, depressed individuals exhibited similar responses to healthy individuals and these neurocognitive improvements were accompanied by significant decreases in depressive symptoms, reflected by a nearly 40% decrease in scores on the Beck Depression Inventory (BDI; Alderman et al., submitted). These data suggest that MAP Training improves neural correlates of depression. Surprisingly, even healthy individuals reported significantly fewer symptoms of depression following MAP Training. Depressed individuals also reported fewer ruminations, as measured by the Rumination Response Scale (RRS), and less self-judgment as suggested by responses to the Five Facet Mindfulness Questionnaire (FFMQ). These data suggest that a combination of mental training with silent meditation and physical training with aerobic exercise can increase cognitive control processes during conflict monitoring, which may thereby improve mental states associated with depression while decreasing the rehearsal of fearful memories of the past.

Slaker, Megan; Sorg, Barbara A. (*Washington State University*) Perineuronal nets in the prefrontal cortex contribute to acquisition of drug-associated memory. Perineuronal nets (PNNs) are unique structures of extracellular matrix (ECM) that surround primarily fast-spiking, parvalbumin-containing, GABAergic interneurons. PNNs have been implicated in learning and memory within the amygdala, hippocampus, and medial prefrontal cortex (mPFC). Removal of PNNs with chondroitinase-ABC (Ch-ABC) enhances extinction learning when removed from the amygdala, impairs context-induced reinstatement of fear conditioning when removed from the hippocampus, and impairs cue-induced reinstatement of fear conditioning when removed from the mPFC. Recent work from our laboratory indicates that Ch-ABC treatment in the prelimbic region of the mPFC impairs acquisition and maintenance of a cocaine-induced conditioned place preference (CPP) memory. Accompanying this impairment was a decrease in the expression of c-Fos, an immediate early gene that corresponds to neuronal activation, specifically within neurons surrounded by PNNs. The goal of this study was to investigate the role of PNNs within an additional region of the PFC, the orbitofrontal cortex (OFC), on the acquisition of cocaine-induced CPP. The OFC is important for decision making involving delayed reinforcement and is implicated in compulsivity and craving that accompanies drug addiction. Adult, male Sprague-Dawley rats were treated with Ch-ABC in the OFC and trained for cocaine-induced CPP. Preliminary results indicate that rats treated with Ch-ABC show impaired acquisition of CPP compared to rats treated with vehicle. Additional studies will need to verify these findings, and future studies will examine the role of PNNs within the OFC on the maintenance of a CPP memory. SUPPORT: NIHDA 033404

Trask, Sydney; Bouton, Mark A., Carranza-Jasso, R. (*The University of Vermont*) Learning Not to Make the Response During Operant Extinction. In operant extinction, an operant behavior decreases in strength or frequency when it is no longer reinforced. Historically, the main explanations of operant extinction have emphasized the loss of stimulus or motivational support when the reinforcer is withdrawn. Here we present evidence, however, that operant extinction involves actively learning to inhibit the response. The experiments used a discriminated operant procedure in which rats were reinforced for lever pressing or chain pulling in the presence of a discriminative stimulus (S), but not in its absence. In one experiment, extinction of the response (R) in the presence of S weakened responding in S, but equivalent extinction exposure to S without the opportunity to make R did not. In other experiments, rats learned to perform several different combinations of stimulus and response (S1R1, S2R1, S3R2, and S4R2). Extinction of a response in one stimulus (i.e., S1R1) transferred and weakened the same response, but not a different response, when it was tested in another stimulus (i.e., S2R1 but not S3R2). The transfer of response inhibition also occurred when S1 and S2 set the occasion for R's association with different food reinforcers. The results suggest that the organism needs to make the response in extinction to achieve effective operant extinction, and that the response inhibition that results can inhibit the response when it is occasioned by other discriminative stimuli. SUPPORT: NIH RO1 DA 033123 to MEB

Williams, Amy R.; Lattal, K. Matthew (*OHSU*) Effects of Acute Ethanol Withdrawal on Extinction and Recondition-

ing of Fear. Memory can be affected by a variety of illicit substances, including alcohol. Research has shown that acute and chronic ethanol use can either enhance or perturb memory processes. The alteration of memory by ethanol is particularly relevant to Post-traumatic stress disorder (PTSD), as alcohol use disorder is highly comorbid with PTSD. Previously research from our laboratory has shown that acute ethanol withdrawal (6 hr following a single 4 g/kg i.p. injection of 20% ethanol) caused impairments in both strong and weak conditioning of contextual fear. The effect of acute ethanol withdrawal upon memory processes following conditioning remains unknown. Therefore, the focus of this research was to assess how acute ethanol withdrawal affects extinction and reconditioning in a mouse contextual fear conditioning procedure. Acute ethanol withdrawal did not significantly affect the formation of either an extinction memory or a reconditioning memory relative to a saline control, but there was an ordinal trend that acute ethanol withdrawal caused a loss of rapid reacquisition following reconditioning that has been demonstrated by ethanol naïve animals. This suggests different effects of ethanol withdrawal on processes underlying the formation of initial conditioning, extinction, and post-extinction reconditioning memories. Further study on the interaction of alcohol withdrawal and fear memory could inform treatment and prevention of the overlap of these two disorders. SUPPORT: NIDA DA025922; DOD W81XWH-12-2-0048; ARCS Roche Scholar; T32 NIAAA 5T32AA007468-28

Talk Abstracts

Alphabetical by first author. (Abstracts not available for all speakers.)

Barrientos, Ruth M. (*University of Colorado Boulder*) Neuroinflammation in the Normal Aging Hippocampus: Causes, Effects, and Therapeutic Interventions. Neuroinflammation is a normal and adaptive response following challenging life events such as infection, surgery, or injury. In normal aging, however, this neuroinflammatory response is exaggerated and prolonged, particularly in the hippocampus, causing greater susceptibility to memory impairments in this population. Sensitized, or primed, microglia have been identified as a primary source of this exaggerated neuroinflammatory response and appear to be a hallmark of the normal aging brain. Dysregulation of the neuroendocrine system may be an important cause of normal aging-induced microglial sensitization, as regulating corticosterone in the aged hippocampus prevents microglial sensitization and the downstream inflammatory sequelae. Several mechanisms by which long lasting elevations in pro-inflammatory cytokines in the hippocampus produce memory impairments have been described, and include impairments to long-term potentiation (LTP), and blunting of brain-derived neurotrophic factor (BDNF). Pharmacological, dietary, and behavioral (physical exercise) therapeutic approaches have been effective at attenuating the sensitized microglial phenotype, the exaggerated neuroinflammatory response following an insult, and the cognitive impairments that ultimately follow. SUPPORT: NIA 028271

Baxter, Mark; Alvarado, Maria (*Icahn School of Medicine at Mount Sinai; Yerkes National Primate Research Center and Emory University*) Cognitive and Socioemotional Development After Postnatal Anesthetic Exposure. Exposure to general anesthesia early in life is associated with neurotoxicity and long-term cognitive impairment in rodents. Combined with the observation that humans that undergo surgical procedures before the age of 4 years are at a greater risk of learning disabilities at school age, this raises the concern that general anesthetics may cause enduring neurocognitive impairments in humans. Nonhuman primate models are uniquely positioned to shed light on this question because of the protracted neural and behavioral development of monkeys. We have been studying behavioral development in rhesus monkeys that were anesthetized with the inhalation anesthetic sevoflurane as infants. These monkeys have impaired memory and altered emotional behavior compared to controls. These findings contribute to concerns about the impact of anesthesia on cognitive development in humans, and establish a model system in which potential mitigating treatments for this unintended effect of anesthesia may be studied. SUPPORT: NIH R01-HD068388

Bevins, Rick; Charntikov, Sergios; Pittenger, Steven; Swalve, Natashia; Barrett, Scott (*University of Nebraska - Lincoln*) Conditioned Enhancement of the Nicotine Reinforcer. According to the World Health Organization, "No other consumer product is as dangerous, or kills as many people. Tobacco kills more than AIDS, legal drugs, illegal drugs, road accidents, murder, and suicide combined" (The Tobacco Atlas [p.36]). Approaches to drug addiction that include conditioning processes have led to important advances in our understanding of drug use in general, and nicotine dependence specifically. These approaches typically treat nicotine as an unconditioned stimulus. That is, the biological effects of nicotine (reward, psychomotor stimulation, cognitive enhancement, analgesia) enter into an association with stimuli that reliably co-occur with these effects (cigarette, throat irritation, situational cues). Also of import, but far less studied, is the pharmacological effects of nicotine serving as an interoceptive stimulus for other reinforcing events such as peer acceptance, alcohol, work breaks, stress relief, etc. Consequently, over time a user develops a rich appetitive conditioning history with the interoceptive stimulus effects of nicotine. This acquired appetitive value likely contributes to the tenacity of the nicotine addiction. An important prediction from theories of conditioned reinforcement and incentive motivation is that an excitatory conditioned stimulus, nicotine in our

case, should more readily support behavior. In this talk, we will present a set of studies testing this prediction. Using rats, we merge the discriminated goal-tracking task (interoceptive Pavlovian conditioning) with the intravenous nicotine self-administration task (nicotine reinforcement). In each study, rats had nicotine repeatedly paired with intermittent access to sucrose. This excitatory interoceptive conditioning history increased the later self-administration of nicotine. This finding held whether the schedule of reinforcement was a CRF, a VR, or a PR; it also persisted in extinction. Further, this effect occurred at 0.01 and 0.03 mg/kg/infusion of nicotine. Finally, control comparisons decrease the feasibility of alternative accounts. SUPPORT: DA034389

Chamizo, Victoria D. (*Universitat de Barcelona*) Trying to Understand the Differential Use of Spatial Information in Male and Female Rats of Different Age, Juveniles and Adults. There is good evidence that male and female rats often rely on different cues to solve spatial problems. Males tend to use geometric information while females use more visual features or landmarks. The same claim has been made in the human literature, while using a variety of tasks. Are there specific conditions or requirements that determine this differential use of spatial information in males and females? Recent research shows that this seems to be the case (Torres et al., 2014, Chamizo et al., 2014). However, the initial claims are not confirmed with younger female rats (Rodríguez et al., 2013). Why is this? Nick Mackintosh and I, working with rats, addressed these issues in recent years. In my talk I will present a set of challenging experiments, unfortunately not all of them with his collaboration, each of them opening a new line of research. It was my privilege for 30 years to work in collaboration with Professor Mackintosh. I still wonder what I did to deserve such a magnificent gift!
References:
Chamizo, V.D., Rodríguez, C.A., Torres, I., Torres, M.N., & Mackintosh, N.J. (2014). What makes a landmark effective?: Sex differences in a navigation task. Learning and Behavior, 42, 348–356. Rodríguez, Clara A., Chamizo, V.D., & Mackintosh, N.J. (2013) Do hormonal changes that appear at the onset of puberty determine the strategies used by female rats when solving a navigation task? Hormones and Behavior, 64, 122–135. Torres, M.N., Rodríguez, C.A., Chamizo, V.D., & Mackintosh, N.J. (2014). Landmark vs. geometry learning: Explaining female rats' selective preference for a landmark. Psicológica, 35, 81–100.

Cunningham, Chris (*Oregon Health & Science University*) Deconstructing the Elements of Context in Pavlovian Drug Conditioning. Abused drugs are frequently paired with contextual cues to gain a better understanding of the conditioned motivational processes that contribute to drug-seeking behavior. Although such cues typically consist of elements from several modalities (e.g., visual, tactile, spatial), relatively little is known about the associative control gained by these elements or possible interactions among them (e.g., overshadowing, blocking). We have recently explored this issue in the conditioned place preference (CPP) procedure using an apparatus that allows independent manipulation of contextual elements. Our studies compared control of CPP when tactile (T) and visual (V) cues were conditioned individually or in compound (TV). In all studies, male DBA/2J mice were conditioned with ethanol (2 g/kg, IP) using an unbiased (counterbalanced) two-compartment CPP procedure. Contextual elements were manipulated by varying the floor texture (grid vs. hole) and wall coverings (stripes vs. dots). Results showed that both elements acquired control of CPP after VT conditioning, but CPP was generally stronger to T than to V, suggesting that T was more salient. However, there was no evidence of overshadowing since element tests after TV training showed CPP levels similar to those elicited by each element conditioned alone. Furthermore, compound tests after element training did not provide evidence of summation, suggesting instead that performance was controlled largely by the T element. Finally, conditioning of V alone before TV conditioning failed to interfere with conditioning to T despite strong conditioning to V, indicating an absence of associative blocking. Overall, these findings suggest that the elements of static contextual cues do not interact in the same way as the elements of phasic conditioned stimuli commonly studied in other conditioning models, raising the possibility of a fundamental difference in the nature of stimulus control by such cues. SUPPORT: NIH/NIAAA AA007702

Drew, Michael R.; Bernier, Brian E.; Ayoub, A.; Kim, H.J.; Zemelman, B.V. (*University of Texas at Austin*) Dentate Gyrus Controls Extinction of Contextual Fear Memory. Research on fear extinction has focused primarily on tone-shock conditioning. Less is known about the neural circuitry mediating extinction of contextual fear, a distinct form of conditioning that recruits both cortical and subcortical plasticity. Based on previous studies implicating the hippocampal dentate gyrus (DG) in context fear acquisition, we used optogenetic and pharmacogenetic methods to assess the role of DG in context fear expression and extinction. To rapidly and reversibly manipulate DG neural activity during distinct phases of training, recombinant adeno-associated virus (rAAV) was used to express optogenetic or pharmacogenetic neural actuators in DG cell populations. Halorhodopsin (eNpHR3.0) was used for optogenetic inhibition, and the Gs-DREADD GPCR (rM3Ds) was used for pharmacogenetic excitation. Optogenetic inhibition of dorsal DG during the context-shock pairing impaired context fear acquisition. Silencing DG during repeated re-exposures to the context without shock did not alter fear expression but attenuated fear extinction, suggesting that neural activity in DG is required for acquisition and extinction of context fear but not for its expression. Enhancing plasticity and/or excitability of dentate granule cells via rM3Ds during context re-exposure af-

ter acquisition had no effect on expression of fear memory but led to a significant reduction in freezing during a subsequent drug-free context test, suggesting enhanced extinction or impaired consolidation. In summary, our data indicate that DG controls acquisition and extinction of context fear memory but is not required for expression of context fear or context fear extinction. DG may provide a teaching signal that supports acquisition- and extinction-related plasticity in a downstream region. Furthermore, the data suggest that context fear extinction recruits neural circuits partially distinct from those controlling extinction of tone-shock associations. Whereas extinction of tone-shock learning is mediated by plasticity in amygdala and prefrontal cortex, our studies identify DG as a critical locus for context fear extinction. SUPPORT: NIH MH102595

Esber, Guillem; Haselgrove, Mark; LePelley, Mike (*City University of New York, Brooklyn College*) Reconciling the Effects of Predictiveness and Uncertainty on Attention in Associative Learning. The study of attention in associative learning has been dominated by two contrasting proposals. On the one hand, predictiveness theories such as that advanced by Mackintosh (1975) state that organisms will pay preferential attention to stimuli that have reliably signaled important events in the past. On the other hand, uncertainty theories such as the Pearce-Hall (1980) model posit that organisms would be better served by attending to (and thereby learning about) stimuli whose consequences are uncertain, such as novel or inconsistently reinforced stimuli. Although both proposals have received ample empirical support, they often make conflicting predictions. Thus, the question remains whether predictiveness- and uncertainty-tracking attentional mechanisms coexist in the brain as suggested by recent hybrid models or, rather, whether a common mechanism might reconcile the apparent paradox in the data. Esber and Haselgrove (2011) proposed a predictiveness-based mechanism which is able to account not only for predictiveness- but also for uncertainty-related attentional phenomena. Interestingly, a slight modification of the uncertainty-based model advanced by Pearce, Kaye and Hall (1982) is equally able to accommodate both kinds of phenomena. Thus, an integration of the extant attentional evidence is possible in terms of either a predictiveness- or an uncertainty-tracking mechanism. The relationship between these mechanisms will be explored.

Escobar, Martha; Bhattacharya, Subhrajit; Bhattacharya, Dwipayan; Suppiramaniam, Vishnu (*Oakland University; Auburn University*) Synaptic Plasticity Changes After Retrieval of Contextual Fear Memories. Retrieval of a previously-acquired memory renders it 'labile' until the memory is once again consolidated. Although much is known about the cellular processes that underlie this reconsolidation process, little is known about how synaptic plasticity changes through the reconsolidation period. Using rats in a conditioned fear preparation, we investigated changes in long-term potentiation (LTP) and depression (LTD), as well as the associated changes in glutamate receptor expression on a time-dependent manner through the reconsolidation period. Retrieval of the fear memory resulted in decreased LTP and increased LTD in the Schaffer Collateral pathway of the hippocampus, slowly recovering to control levels over a 7h period. Expression of N-Methyl-D-Aspartate receptors (NMDARs) and α-Amino-3-hydroxy-5-Methyl-4-isoxazole-Propionic Acid receptors (AMPARs) changed along with fEPTPs. GluR1 and GluR2 subunits of AMPARs were downregulated shortly after retrieval, but returned to control levels 4h after retrieval. NR2B levels were above normal immediately after retrieval, but decreased to control levels 4h after retrieval. LTP/LTD were at control levels after retrieval if normal levels of GluR2 were maintained or NR2B receptors were antagonized, which was accompanied by maintenance of the conditioned memory through an amnestic treatment. These results suggest that retrieval-induced alterations of conditioned memories may be mediated by the interplay of GluR2/NR2B activity and their effects on synaptic plasticity. SUPPORT: Auburn University Internal Grant Program

Frick, Karyn (*University of Wisconsin-Milwaukee*) Neural Mechanisms Through Which Progesterone Regulates Hippocampal Memory. Although much recent attention has been paid to the role of sex steroid hormones in regulating memory, the vast majority of research has focused on estrogens. Progesterone is a potent neurosteroid that can facilitate memory formation on its own, yet little is known about the molecular mechanisms underlying the cognitive effects of this hormone. This talk will review data showing that intrahippocampal infusion of progesterone enhances object memory in a manner dependent on activation of ERK, mTOR, and canonical Wnt signaling. In particular, the role of membrane and intracellular progesterone receptors will be highlighted, as these discrete types of receptors appear to employ different molecular mechanisms to enhance hippocampal memory. SUPPORT: Univ. of Wisconsin-Milwaukee, Ellison Medical Foundation/AFAR, NIH AG022525

Guzowski, John; Lewandowski, Gail; Miyashita, Teiko; Czerniawski, Jennifer (*University of California, Irvine*) Cytokine Modulation of Hippocampal Circuit Activity and Memory Function. Cytokines—originally identified as signaling molecules of the peripheral immune system—can directly impact neuronal function and synaptic plasticity. At present, little is known about how cytokines modulate activity of neural circuits underlying specific cognitive processes. With ever-increasing evidence implicating cytokines in cognitive dysfunction associated with neurodegenerative and neuropsychiatric disorders, it is imperative to gain a deeper understanding of how cytokines alter neural circuit functions supporting cognition. To that end, we are studying the impact of acute neuroinflammation on memory

retrieval processes at behavioral, neural circuit, and molecular levels in young adult male rats. In these experiments, we use systemic injection of bacterial lipopolysaccharide (LPS; 150 micrograms / kg, i.p.) to cause transient neuroinflammation, which peaks at 6 hours post injection as determined by elevated levels of proinflammatory cytokines in hippocampus and other brain regions. Recently, we have shown that acute neuroinflammation preferentially disrupts retrieval in contextual discrimination memory tasks, but not all hippocampus-dependent memory tasks (Czerniawski et al., Brain Behav Immun, 2015). Moreover, such impairment in context discrimination memory is accompanied by altered neuronal ensemble activity in hippocampal CA3 and CA1 circuits, consistent with impaired pattern separation processes (Czerniawski & Guzowski, J Neurosci, 2014). Ongoing experiments demonstrate that blocking microglia activation in LPS-treated rats with the systemic injection of minocycline attenuates proinflammatory cytokine expression in hippocampus, rescues context discrimination memory retrieval, and restores hippocampal circuit activity. Moreover, local microinfusion of minocycline into the dorsal hippocampus of LPS-treated rats is sufficient to rescue context discrimination memory retrieval. We are now using microarray and proteomic approaches to better understand the cellular and synaptic mechanisms altered by cytokine expression that produces the disruption of pattern separation processes in hippocampus critical for contextual discrimination memory function. SUPPORT: R01 MH08930 (JG); T32 AG00896 (JC); James L. McGaugh Chair in the Neurobiology of Learning and Memory (JG)

Kim, Jeansok (*University of Washington*) Place and Time of Fear. Fear is an adaptive mechanism evolved to influence the primal decisions of foragers in 'approach resource-avoid predator' conflicts. To survive and procreate, animals must attain the basic needs (such as food, water, shelter, mate) while avoiding the ultimate cost of predation. Consistent with this view, ecological studies have found that predatory threats cause animals to limit foraging to fewer places and/or to restricted times in their habitat. However, the neural mechanisms through which animals alter their foraging location and time when confronted with danger remain largely unknown. I will present data from two 'ethological' fear preparations indicating that the amygdala-coded fear stamps the psychological fear content onto the hippocampal representation of physical location, and that the time-specific fear can act as a non-photic entraining stimulus for the circadian system. The clinical implications of fear spatially and temporally organizing defensive behavior to human fear disorders will be discussed. SUPPORT: NIH MH099073

Knox, Dayan (*University of Delaware*) Examining the effects of stress on fear extinction using the single prolonged stress paradigm. Recently, there have been attempts to systematically examine the effects of stress on fear extinction. A number of studies have used established stress protocols or the stress inherent during fear conditioning to examine this relationship. In this talk, I describe how the single prolonged stress (SPS) paradigm can be used to examine the effects of stress on fear extinction. SPS refers to serial exposure to restraint, forced swim, and ether. The effects of this acute stress protocol are typically observed seven days later. SPS is different to other stress protocols in that it was designed to specifically mimic behavioral and neuroendocrinological effects observed in post traumatic stress disorder. To this end SPS enhances fast negative feedback of the HPA axis, arousal, glucocorticoid receptor (GR) levels in the hippocampus, and decreases excitatory tone in the medial prefrontal cortex. When SPS is conducted prior to fear and extinction memory formation, deficits in fear extinction retention are consistently observed, though the exact memory processes through which this occurs remains unknown (e.g. enhanced fear memory or deficit in extinction memory). While it was initially thought that enhanced GR expression and extinction retention deficits in the SPS model are linked, recent research in my lab suggests that SPS-induced changes in GR function inhibits the extent of extinction retention deficits in the SPS model. The observation that SPS exposure consistently induces extinction retention deficits suggests that SPS exposure must induce maladaptive neurobiological effects that lead to this outcome. However, it would also appear that SPS exposure elicits neurobiological effects that counteract the maladaptive effects induced by SPS. In a more general sense, this raises the possibility that acute stress can induce maladaptive effects that are offset by adaptive effects induced by the same acute stress. Put another, way acute stress can be dualistic in nature.

Malvaez, Melissa; Greenfield, Venuz y.; Ochoa, Jesus; Wood, Marcelo A.; Kennedy, Pamela J.; Wassum, Kate M. (*University of California, Los Angeles*) Histone Acetylation in the Dorsolateral Striatum Selectively Mediates the Formation of Behavioral Habits. Considerable evidence suggests that instrumental behavior depends on two distinct learning processes; one cognitive, in which the relationship between actions and their consequences is encoded, and one habitual, involving the formation of stimulus-response associations. These processes rely on dissociable neural circuits, but beyond this very little is known about the mechanisms required to form these associative memories. Given the recently ascribed role for epigenetics in memory formation, we examined the role of one such mechanism, histone acetylation, in instrumental learning. Rats were trained to lever press for a food reward and were administered the non-specific histone deacetylase (HDAC) inhibitor sodium butyrate (NaB) immediately following each training session. Following training, devaluation of the food outcome was used to probe cognitive versus habitual con-

trol of instrumental behavior. Although both groups learned the task, those rats treated with NaB showed insensitivity to outcome devaluation earlier in training than vehicle-treated rats, suggesting that HDAC inhibition potentiated habit formation. Insensitivity to degradation in the action-outcome contingency was also observed and the potentiation of habit occurred regardless of training schedule (i.e., random interval v. ratio). Systemic HDAC inhibition increased histone H4 lysine 8 acetylation specifically in the dorsal striatum, a major component of the instrumental learning circuit. In follow-up experiments, selective modulation of HDAC3 activity in the dorsolateral striatum was found to be sufficient to modulate the sensitivity of instrumental action to devaluation. These data identify chromatin modification by histone acetylation as an important mechanism in the development of stimulus-response associative memories. Moreover, the results suggest that HDAC inhibition can potentiate habitual over cognitive instrumental learning processes, a finding with important implications for the therapeutic application of HDAC inhibitors. SUPPORT: NIDA; UCLA Life Sciences; UCLA Faculty Career Development Award

McGaughy, Jill A. (*University of New Hampshire*) Do changes in Prefrontal Norepinephrine Transporter density across Adolescence influence the Development of Cognitive Control in rats? Adolescence is a period of major behavioral and brain reorganization. Different aspects of cognitive control mature at different rates in humans and in rodent models of it. These different developmental trajectories are hypothesized to reflect region specific maturation within the prefrontal subregions. Converging evidence has shown that norepinephrine is critical to attentional set-shifting and some forms of distractibility. As a result, we assessed two noradrenergic markers at early, mid- and late adolescence in five functionally distinct subregions of the rat prefrontal cortex. We found that the density of norepinephrine transporters (NET), but not dopamine beta hydroxylase (DBH), changed across adolescence in a regionally selective manner. The prelimbic cortex, which is critical to cognitive flexibility and the lateral orbitofrontal cortex, critical to response inhibition, showed higher levels of NET at early than mid- to late adolescence. Additionally, we used a rat model to study the effect of standard ADHD treatments, atomoxetine and methylphenidate on cognitive control during early adolescence. While both of these drugs act as norepinephrine reuptake inhibitors, higher doses of atomoxetine and all doses of methylphenidate also block dopamine transporters (DAT). Low doses of atomoxetine, selective for NET blockade, were effective at remediating cognitive rigidity found in adolescents. In contrast, methylphenidate improved performance in rats unable to form an attentional set due to distractibility but was without effect in normal subjects. These data support the critical role of cortical noradrenergic maturation in cognitive control throughout adolescence. SUPPORT: R21MH087921

McNally, Gavan P. (*UNSW Australia*) Selecting danger signals: Attention, CS associability, and fear learning. Mackintosh described some of the important behavioural conditions revealing variations in the associability of stimuli with reinforcement, and also a theoretical approach to understanding these. Such issues remain fundamental in Pavlovian learning and have important implications for understanding the neural circuitries of fear learning. I will describe the results of several experiments, some inspired by and others directly 'borrowed' from this tradition, attempting to understand the brain mechanisms for learned changes in attention to danger signals.

Meyer, Heidi C.; Bucci, David J. (*Dartmouth College*) Behavioral and Neurobiological Substrates of Negative Occasion Setting During Adolescence. The ability to inhibit an inappropriate response is an essential aspect of adaptive behavior and one that is still developing during adolescence. Successful inhibition depends not only on motoric stopping behavior, but also on cognitive processes that include the detection and use of environmental cues that signal that a response is inappropriate. Here we present evidence that adolescent rats have difficulty using a class of these cues, known as negative occasion setters, to guide behavior. Briefly, rats received trials in which a "target" stimulus was presented by itself and followed immediately by the delivery of food reinforcement. On other trials a "feature" stimulus was presented just before the target and on those trials food was not delivered. Adolescents required twice as many training sessions as adults or pre-adolescent rats to exhibit more responding to the target during reinforced trials than during non-reinforced trials. In a second set of studies we probed the behavioral underpinnings of this delay and found that adolescents can learn the dual meaning of the target cue like adults, but cannot express that learning until they are at least 53 days old, which is when the prefrontal cortex is thought to reach maturity. Moreover, we found that although adolescent and adult rats exhibit similar rates of acquisition of Pavlovian approach behavior, adolescents experience difficulty learning a secondary inhibitory meaning of same cue during extinction. Finally, we probed the neural basis for the delay in negative occasion setting using chemogenetics and found that temporarily silencing neuronal activity in the prefrontal cortex of adult rats, while simultaneously increasing activity in the nucleus accumbens, resulted in the behavioral phenotype exhibited by adolescents. Together, the findings inform the nature of the behavioral and neural substrates that contribute to immature behavioral control in adolescents. SUPPORT: F31MH107138 and R01DA027688

Miller, Ralph R. (*State University of New York at Binghamton*) The Construct of Attention and Beyond: Homage to NJ Mackintosh. For 50 years, NJ Mackintosh (1935-2015) was among the world's leading students of animal cognition. He radically changed the way in which we think about at-

tention, he formulated three important models of associative learning, he mentored several generations of notable researchers, and he was the field's foremost spokesman. I will discuss associability as a modifiable perceptual process and alternatively as a modifiable orienting response. Five conversations with Mackintosh over a span of 40 years will be described along with how they altered research and theorizing in my laboratory. (1) The implications of cue-to-consequence effects for stimulus associability. (2) Two types of memory interference, one a function of recency between target training and the interfering event, and the other a function of the similarity in content of the target association and interfering association. (3) The development of the comparator hypothesis to address failings of prevailing models such as Mackintosh (1975) and Rescorla & Wagner (1972). (4) Causal reasoning in rats (and by extension, humans) as a bottom-up process. (5) Perception as a learning process as exemplified in inhibitory perceptual learning. SUPPORT: NIH MH33881

***Parker, Linda A.; *Sticht, Martin A., *Limebeer, Cheryl L., *Rafla, Benjamin R., #Abdullah,Rehab A., #Poklis, Justin L., @Ho Winnie, +Niphakis, Michah J., +Cravatt, Benjamin F., @Sharkey, Keith A, #Lichtman, Aron H.** (**University of Guelph, #Virginia Commonwealth University, @University of Calgary & #Scripps Institute, La-Jolla*) Endocannabinoid Regulation of Nausea is mediated by 2-Arachidonoylglycerol (2-AG) in the Rat Visceral Insular Cortex. Manipulations that elevate the endogenous cannabinoids (eCBs), anandamide and 2-AG, have previously been found to interfere with nausea-induced conditioned gaping (a selective measure of nausea) in rats. Although the precise brain mechanisms underlying nausea have yet to be fully uncovered, the visceral insular cortex (VIC) appears to play a critical role in mediating its sensation. In fact, the synthetic cannabinoid agonist, HU210 (Limebeer et al 2012) and exogenous 2-AG (Sticht et al, 2015) have both been found to disrupt the establishment of lithium chloride (LiCl) induced conditioned gaping in rats following intra-VIC administration, suggesting an important role for the VIC eCB system. Therefore, to further investigate the nature of eCB suppression of nausea we assessed whether pharmacological inhibition of eCB catabolic enzymes within the VIC interferes with acute nausea-induced conditioned gaping. Moreover, we quantified VIC eCB levels following these manipulations, and assessed their effects on VIC neuronal activity using the functional activation marker, c-Fos.

Rats received bilateral intra-VIC infusions of: 1) the dual FAAH/MAGL inhibitor, JZL195 (10 μg); 2) the selective FAAH inhibitors URB597 (0.01 μg) or PF3845 (10 μg); or 3) the selective MAGL inhibitor MJN110 (2 μg) prior to receiving an intraoral saccharin infusion and systemically administered LiCl. They were subsequently re-exposed to saccharin 72 hr later in a drug-free taste reactivity test, in which conditioned gaping was assessed. It was found that an acute LiCl injection resulted in a selective increase in VIC 2-AG levels relative to saline-treated control animals, whereas anandamide content in the VIC remained unchanged. Furthermore, MAGL inhibition selectively increased VIC 2-AG levels following systemic or intra-VIC administration of MJN110 and suppressed conditioned gaping, as did intra-VIC infusion of the dual FAAH/MAGL inhibitor, JZL195. On the other hand, FAAH inhibition following either systemic or intra-VIC administration of URB597 or PF3845 did not elevate VIC AEA levels, and neither compound had an effect on nausea-induced gaping. Lastly, MAGL inhibition by MJN110 reduced LiCl-induced c-Fos expression in the VIC.

Taken together, these findings suggest that VIC eCB system modulation of nausea may be driven primarily by the ligand, 2-AG. More over, manipulations selectively targeting 2-AG may have therapeutic potential in reducing nausea, likely by reducing neuronal activation in this brain region during an episode of nausea.

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Pattwell, Siobhan S; Liston, Conor; Jing, Deqiang; Ninan, Ipe; Yang, Rui R; Witzmun, Jonathan; Murdock, Mitchell, H; Casey, BJ; Deisseroth, Karl; Lee, Francis S. (*Fred Hutchinson Cancer Research Center*) Leveraging Dynamic Changes in Neural Circuitry During Adolescence to Persistently Attenuate Fear Memories . Fear can be highly adaptive in promoting survival, yet it can also be detrimental when it persists long after a threat has passed. Malleability of the fear response may be most advantageous during adolescence when there is an increased prevalence to explore novel, potentially threatening environments. Two opposing adolescent fear-related behaviors—diminished extinction of cued fear and suppressed expression of contextual fear—may serve this purpose, but the neural basis underlying these changes is unknown. Using microprisms to image prefrontal cortical spine maturation longitudinally and retrograde tracing of neurons in prelimbic and infralimbic cortices across development, we delineate dynamic BLA-hippocampal-mPFC circuit reorganization associated with these behavioral shifts. Exploiting this sensitive period of neural development, we modified existing behavioral interventions in an age-specific manner to attenuate adolescent fear memories persistently into adulthood by incorporating combined contextual and cued extinction elements. These findings may define a strategy for leveraging dynamic neural changes during adolescence to extinguish pathological fears implicated in anxiety and stress related disorders. SUPPORT: NIH HD055177; Behavior & Brain Research Foundation/NARSAD

Ryabinin, Andrey E.; Smith, Monique L; Hostetler

Caroline M, Heinricher Mary M, Ryabinin Andrey E. (*Oregon Health & Science University*) Mice transfer increased pain sensitivity via olfactory cues. Social environment has a strong impact on human behavior and pathological disorders. Early studies by Pavlov replicated this phenomenon in dogs by demonstrating that organism's responses to environmental stimuli depend on social circumstances. Recent studies identified complex social behaviors in rodent species, including prosocial and empathy-like behaviors. These complex social behaviors have the ability to affect the preclinical understanding of many pathological conditions, including chronic pain and alcohol use disorders. Since chronic pain, alcohol dependence and social behaviors are interrelated, we examined pain during voluntary alcohol drinking in adult male C57BL/6J (B6) mice. We found that withdrawal from voluntary alcohol drinking in B6 mice increased sensitivity to mechanical and chemical pain stimuli. Remarkably, we observed hypersensitivity not only in alcohol-withdrawn mice, but also in water-drinking "bystander control" mice housed in the same room. Additional experiments determined that this abnormal pain state is communicated to bystander mice via olfactory cues within the bedding. The increased sensitivity in bystander mice did not generalize to all types of sensory stimulation, as it was not accompanied by increased startle response or anxiety-like behavior in the elevated plus maze. However, the pain experience in bystander mice was accompanied by increased c-Fos expression in cortical areas known to contribute to pain and empathy in humans. Finally, we demonstrated that this phenomenon also generalizes to other hyperalgesic states, as evidenced by the social transfer of morphine withdrawal-induced or inflammatory pain. These studies demonstrate that hyperalgesia induced by a variety of stimuli can be socially transferred to conspecifics housed in the same room, which leads to substantial concerns about how we house and test our experimental animals. Finally, these results suggest that social variables should be carefully considered when designing and interpreting animal experiments. SUPPORT: NIH AA016647 and AA10760

Sharpe, Melissa; Killcross, Simon (*Sharpe: Princeton Neuroscience Institute, Princeton University/National Institute on Drug Abuse, Baltimore ; Killcross:School of Psychology, UNSW, Australia*) The Prelimbic Cortex Directs Attention Toward Predictive Cues During Pavlovian Conditioning. Theories of functioning in the prelimbic (PL) cortex are distinct across aversively- and appetitively-motivated procedures. In the appetitive domain, the PL cortex is argued to use higher-order cues to modulate learning and performance when the experimental circumstance changes. In contrast, the dominant theory in the aversive literature is that the PL cortex is important for generating responses to fearful cues. Here, we present data which demonstrates that the PL cortex uses a cue's predictive power to direct a preferential degree of attention towards the best predictors of an outcome in line with Mackintosh's (1975) attentional process. Using an aversive overshadowing procedure, we demonstrate that this role of the PL cortex is necessary during the learning phase but not for the expression of these fear associations once they have been formed. In a second set of experiments, we demonstrate that the PL cortex is only recruited for fear expression when attentional modulation is required to resolve competition between discrete and contextual cues (i.e. when competition between contextual and discrete cues is high). We interpret these data as an example of the PL's role in using higher-order information, here the associative history of a stimulus, to direct attention towards the best predictors of an outcome to overcome stimulus competition. These data show that the role of the PL cortex in attention generalises across both appetitive and aversive procedures.

Sorg, Barbara A.; Slaker, Megan; Todd, Ryan P.; Blacktop, Jordan M.; Zuloaga, Damian G; Raber, Jacob; Darling, Rebecca A.; Brown, Travis E.; Churchill, Lynn (*Washington State University*) Role of Perineuronal Nets in Cocaine-Associated Memory. The medial prefrontal cortex (mPFC) plays a significant role in cognitive function and contributes to cocaine-seeking behavior in both humans and rodents. Exposure to cocaine and cocaine-associated cues increases the activity of pyramidal output neurons from the mPFC. The activity of these neurons is significantly modulated by GABAergic, parvalbumin (PV)-containing, fast-spiking interneurons. The majority of these interneurons are enveloped by unique structures of extracellular matrix called perineuronal nets (PNNs) that are integral to the maintenance of many types of plasticity. Using a conditioned place preference (CPP) procedure, we found that removal of PNNs primarily within the prelimbic (PL) region of the mPFC of adult male Sprague-Dawley rats impaired the acquisition and reconsolidation of a cocaine-induced CPP memory. This impairment was accompanied by a decrease in the number of c-Fos-positive cells surrounded by PNNs. Following removal of PNNs, the frequency of inhibitory currents on mPFC pyramidal neurons was decreased, but following cocaine-induced CPP, both frequency and amplitude of inhibitory currents were decreased, suggesting that removal of PNNs may prevent some of the cocaine-induced changes in pyramidal neurons. Consistent with these findings, preliminary data indicate that repeated cocaine exposure increases PV and PNN intensity in the PL mPFC. Previous work indicates that the increase of PV and PNNs intensity may represent maturation of these interneurons that is associated with reduced plasticity. Together, our findings indicate that cocaine-induced plasticity is impaired by removal of PNNs in the PL mPFC and that repeated cocaine exposure may reduce plasticity of PV+/PNN+ interneurons in the PL mPFC. Our studies suggest that PNNs may be a therapeutic target for disruption of cocaine CPP memories. SUPPORT: Sup-

ported by NIH Grant DA 030647 and DA033404 (BAS); NIDA T32DA007262 (DGZ); NASA NNJ12ZSA001N (JR); and P30 GM 103398-32128 (TEB and RAD).

Stanton, Mark E. (*University of Delaware*) The Ontogeny of Learning in Context. This presentation briefly reviews the history and some key lessons of developmental studies of learning as a context for the current symposium on adolescent cognition. It then describes recent developmental studies of the context preexposure facilitation effect (CPFE) in the rat. The discovery that rats show infantile amnesia—poor long-term retention of infant learning (Campbell & Campbell, 1962)—stimulated rapid expansion of research on the ontogeny of learning. The initial goal to demonstrate learning at progressively younger ages (1970s–1980s) gave way to an interest in multiple memory systems research (1990s–present) and in cognition that develops at later ages, including adolescence (2000—present). Some key issues emerging from this history include continuity (or not) of development, ontogeny as a series of "ecological niches," and the role of parameters, sensory-motor-motivational processes, acquisition vs. expression, and memory system interactions in the developmental emergence of learned performance. The main lesson is to never be surprised by anything that happens at any stage of development.

My colleagues and I have recently begun to study the ontogeny of the CPFE, a variant of contextual fear conditioning in which (incidental) context learning on one day enables conditioning to immediate shock on the next day (Fanselow, 1990). We have found that the CPFE develops gradually between postnatal day (PD) 17 and 24, at which point it depends on conjunctive learning of context features and on hippocampal NMDA receptors (Schiffino et al., 2011; Jablonski et al., 2012). By early adolescence (PD31), the CPFE differentially drives prefrontal egr-1 expression on the training day (Asok et al, 2013; Schreiber et al., 2014); and long-term memory of context learning increases during the adolescent period (Robinson-Drummer & Stanton, 2015). We are just beginning to learn how different processes and mechanisms of contextual fear conditioning develop from infancy through adolescence to adulthood. SUPPORT: NIH grant R01 HD075066-01A1

Tronson, Natalie C. (*University of Michigan*) Persistent Memory Deficits and Histone Modifications after a Systemic Inflammatory Event. Neuroimmune signaling is increasingly identified as a critical component in normal neural processes including learning and memory, and synaptic plasticity. Activation of immune or inflammatory signaling in the periphery robustly activates neuroimmune processes including microglia and elevation in levels of cytokines in the brain, resulting in modulation of synaptic function. Furthermore, inflammatory events including myocardial infarction, illness or major surgery, commonly lead to cognitive deficits and depression-like behavior lasting months or years after the event. Whereas acute activation of these processes has been the focus of many studies, the mechanisms by which transient inflammatory events cause persistent changes in cognitive function, memory and mood, remain unknown. Here we used a surgical model of myocardial infarction in mice to compare short- and long-term changes in learning and memory after inflammation and determine the downstream mechanisms by which neuroimmune signaling causes changes in memory formation that persist long after resolution of inflammation. We observed impaired context fear conditioning 8 weeks following surgically induced myocardial infarction in both male and female mice, and these alterations in memory processes persist beyond the duration of cytokine activity in the brain after MI. One candidate mechanism for mediating such effects is via epigenetic changes as a consequence of cytokine-dependent signaling. We observed dysregulation of histone acetylation and phospho-acetylation in the hippocampus eight weeks after MI, although differential modifications were observed in males compared with females. These findings identify histone modification as one mechanism that may mediate lasting modulatory influence on memory after a systemic inflammatory event, and suggest novel targets for prevention and treatment of persistent cognitive deficits after MI, illness or major surgery. SUPPORT: NIH MH093459

Wiltgen, Brian (*UC Davis*) Retrieving memory with the hippocampus. The hippocampus is assumed to retrieve memory by reinstating patterns of cortical activity that were observed during learning. To test this idea, we monitored the activity of individual cortical neurons while simultaneously inactivating the hippocampus. Neurons that were active during context fear conditioning were tagged with the long-lasting fluorescent protein H2B-GFP and the light activated proton pump ArchT. These proteins allowed us to identify encoding neurons several days after learning and silence them with laser stimulation. When tagged CA1 cells were silenced, we found that memory retrieval was impaired and representations in the cortex (entorhinal, retrosplenial, perirhinal) and the amygdala could not be reactivated. Importantly, hippocampal inactivation did not alter the total amount of activity in most brain regions. Instead, it selectively prevented neurons that were active during learning from being reactivated during retrieval. These data provide functional evidence that the hippocampus reactivates specific memory representations during retrieval. SUPPORT: McKnight Memory & Cognitive Disorders Award

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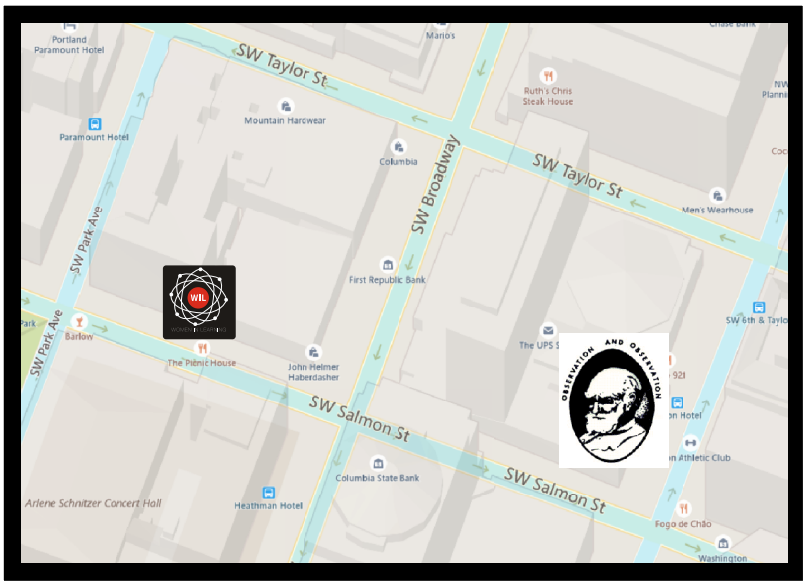

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