Annual Pavlovian Society Meeting ~ Sept 18-20, 2014
Hilton Seattle ~ Seattle WA

Program

Thursday (Sept 18)

6:00-10:00PM Welcome Reception ~ (Soundview Room) Cash Bar

Friday (Sept 19)

7:30-8:25AM Breakfast (Pacific Ballroom)

8:25-8:30AM Welcome – Jeansok Kim (UW)

8:30-9:30AM Address – Steve Maren (TAMU) ~ "Noradrenergic blockade stabilizes prefrontal activity and enables fear extinction under stress"

9:30-10:00AM Talk – Mike Morgan (WSU) ~ "Tolerance to the antinociceptive effects of opioids: Methodological issues and parallels with research on learning and memory"

10:00-10:20AM Coffee Break

10:20-12:00PM Symposium ~ "Fear, Avoidance and Extinction" (June-Seek Choi)

- 10:20-10:45 Marie Monfils (U Texas) ~ "Identifying the factors that facilitate or hinder fear memory/expression attenuation"
- 10:45-11:10 Mary Torregrossa (U Pitt) ~ "Neural circuits controlling the context dependence of Extinction"
- 11:10-11:35 Joshua Johansen (RIKEN) ~ "A neural circuit mechanism for setting fear memory strength"
- 11:35-12:00 June-Seek Choi (Korea U) ~ "Brain circuits involved in discriminatory avoidance learning"

12:00-1:30PM Lunch [For EC members]

1:30-3:10PM Symposium ~ "Estrogenic Regulation of Hippocampal Memory Formation throughout the Lifespan" (Karyn Frick)

- 1:30-1:55 Deepak Srivastava (Kings College London) ~ "Deciphering the molecular mechanism underlying estrogenic modulation of dendritic spines"
- 1:55-2:20 Karyn Frick (U Wisconsin) ~ "Cell signaling and receptor mechanisms essential for estrogenic regulation of hippocampal memory consolidation"
- 2:20-2:45 Liisa Galea (UBC) ~ "Estrogens and memory: The good, the bad and the ugly"
- 2:45-3:10 Lori McMahon (U Alabama Birmingham) ~ "The benefits of estrogen replacement on hippocampal physiology: Timing is everything"

3:10-3:30PM Coffee Break

3:30-4:00PM Talk – Frank Krasne (UCLA) ~ "Fraidy rat: A realistic simulation allowing student experiments on the neuroscience of fear conditioning"

4:00-5:00 Address – Peter Balsam (Columbia U) ~ "Aspects of Inhibition that Depend on Time"

5:00-5:30 Break

5:30-7:30PM Poster Session ~ Posters #1 - #30 (Top of the Hilton)(Cash Bar)

Sep 20, Saturday

7:30-8:25AM Breakfast (Pacific Ballroom)

8:25-8:30AM Welcome – Jeansok Kim (UW)

8:30-10:10AM Symposium ~ "Early Life Stress Effects on Learning: Convergence in Humans and Animal Models" (Barbara Knowlton)

- 8:30-8:55 Regina Sullivan (NYU) ~ "Neurobiology of the development of learning: Attachment and fear suppression"
- 8:55-9:20 Dragana Claflin (Wright State) ~ "Early corticosterone exposure affects subsequent trace eyeblink conditioning: Differing magnitudes and durations of elevation yield different behavioral profiles"
- 9:20-9:45 Veronique Bohbot (McGill) ~ "Early adversity, stress response, hippocampal development, and risks of psychiatric diseases"
- 9:45-10:10 Barbara Knowlton (UCLA) ~ "Effects of early life stress on instrumental learning"

10:10-10:30AM Coffee Break

10:30-11:00AM Talk – Mark Bouton (U Vermont) ~ "Contextual control of instrumental behavior and its Inhibition"

11:00-11:30AM Talk – Jee Hyun Kim (U Melbourne) ~ "Adolescent sensitivity to cocaine-associated cues: A dopamine story"

11:30-12:00PM Talk – Fred Helmstetter (U Wisconsin) ~ "Systems consolidation of fear memory in humans"

12:00-1:30PM Lunch [Satellite activity: Women in Learning Lunch]

1:30-3:10PM Symposium ~ "The Affective Substrates of Pro-Social Behavior in Mammals" (Inbal Ben-Ami Bartal)

- 1:30-1:55 Garet Lahvis (OHSH) ~ "Modeling social reward and empathy In mice"

1:55-2:20	Jaak Panksepp (WSU Pullman) ~ "The scientific case for social-emotional feelings in other animals: Do they have affective experiences and are they homologous to our own?"
2:20-2:45	James Burkett (Emory U) ~ "The neurobiology of consoling behavior in prairie voles"
2:45-3:10	Inbal Ben-Ami Bartal (UC Berkeley) ~ "Empathic helping in rats and its modulation by social parameters"

3:10-3:30PM Coffee Break

3:30-4:00PM Talk – Sheri Mizumori ~ "Neural systems analysis of adaptive choice behavior"

4:00-5:00 Address – Ilene Bernstein (UW) ~ "Taste aversion learning: Stimulus convergence in amygdala"

5:00-5:30 Break

5:30-7:30PM Poster Session ~ Posters #31 and greater (Top of the Hilton)(Cash Bar)

7:30-9:00PM ***Banquet*** (Pacific Ballroom)
Talk: Eberhard E. Fetz ~ Bidirectional interactions between the brain and implantable computers
Awards

POSTERS (Friday, 1 – 30; Saturday, 31 – 57)

1	Allen MT, Myers CE, Servatius RJ	The Spacing Effect Faciliates Eyeblink Conditioning in Behaviorally Inhibited Individuals Only When the Inter-Trial Interval Is Varied.
14	Ardiel EL, Giles AC, Rankin CH	Dual Process Theory Revisited: Habituating and Sensitizing components of an optogenetically triggered response
24	Bacharach SZ, Kawa AB, Calu DJ	Sign- and goal-tracking rats learn differently in the face of changing reward value
52	Beeman CL, Pullins SE, Hoogendoorn JJ, Quinn JJ	Complete hippocampus lesions disrupt recent and remote trace fear memories regardless of the lesion-to-test interval.
27	Cai D, Chen S, Pearce K, Glanzman DL	Reinstatement of long-term sensitization memory in Aplysia after reconsolidation blockade or inhibition of PKM
40	Calub CC, Mootz JR, Furtak SC	Medial Temporal Lobe Involvement in Fear Conditioning to a Complex Stimulus
9	Campese VD, LeDoux, JE	General motivational processes drive aversive Pavlovian-to-instrumental transfer in rodents
36	Cullen PL, Ferrara, NC, Helmstetter FJ	Using optogenetics to alter fear and molecular signaling within the amygdala.
21	Eddy MC, Todd TP, Bouton ME, Green JT	Exercise in adolescent rats reduces renewal of extinguished instrumental behavior
12	Eisenreich BR, Szalda-Petree AD	Effects of Fluoxetine on stimulus control of aggressive responding in B. Splendens
55	Eun-hwa Hong, Ji-Hye Lee, June-Seek Choi	Observational fear conditioning: Emotional contagion or Pavlovian conditioning to a social cue?
19	Ferrara NC, Gilmartin MR, Reis DS, Lee JL, Helmstetter FJ	ERK-mTOR interactions in the lateral, basolateral, and central amygdala during fear memory consolidation
13	Fuchs JR, Darlington SW, Morielli AD, Green JT	Measuring Changes in Surface Kv1.2 Expression in Cerebellar Cortex following Eyeblink Conditioning, Unpaired Stimulus or Context Exposure Controls
6	Glenn AM, Morlock EA, Wilson WJ	MK-801 Effect on Escape Behavior in the Earthworm, Lumbricus terrestris
3	Hallock HL, Griffin AL	Spatial working memory deficits accompany reductions in hippocampal-prefrontal synchrony following inactivation of the ventral midline thalamic reuniens and rhomboid nuclei
8	Hegumen T (V.I. Kryukov)	The problem of non-invasive memory erasure and its solution
29	Hitchcock LN, Raybuck JD, Wood MA, Lattal KM	A histone deacetylase 3 inhibitor enhances extinction and attenuates reinstatement of self-administration in rats
48	Hoffman AN, Rajbhandari AK, Tribble JE, Pennington ZT, Perusini JN, Waschek J, Fanselow MS	Amygdala AMPA receptor subunit specificity underlying fear sensitization following acute traumatic stress
15	Hong W, Kim DW, Anderson DJ	Antagonistic Control of Social Behaviors by Inhibitory and Excitatory Neurons in the Medial Amygdala
57	Jie HS, Lee BN, Geiller T, Royer S, Choi J-S,	Modality and spatial configuration of the sensory stimulus modulate

		avoidance learning in a head-fixed preparation
25	Kaganovsky KK, Ahmed S, Marchant NJ	A critical role of Nucleus Accumbens dopamine D1-like receptors in renewal of punished alcohol seeking
45	Keller SM, Schreiber WB, Knox D	Sex differences in fear extinction retention deficits using an animal model of PTSD
56	Kimm SW, Kim DY, Choi JS	Amygdala modulates approach-avoidance but not reflexive withdrawal in a semi-naturalistic conflict situation
44	Kirry AJ, Doncheck EM, Gilmartin MR	Optogenetic stimulation of prelimbic principal cells impairs the formation of trace fear memory
50	Kochli DE, Thompson EC, Fricke EA, Postle AF, Lash KD, Hagerty SL, Quinn JJ	The basal and lateral amygdala nuclei are critical for trace and contextual fear conditioning
43	Latsko M, Dulka B, Lynch III J, Mulvany J, Jasnow AM	Resistance to Acute Social Defeat Results in Impaired Cued Fear Discrimination, but not Contextual Fear Discrimination
11	Leaderbrand KL, Chen HJ, Corcoran KA, Radulovic J	Cholinergic but not glutamatergic antagonism in retrosplenial cortex impairs contextual fear acquisition
10	Lewon M, Parrott Hayes LJ	The Effects of Varying Levels of Food Deprivation on Escape and Avoidance Responding in Mice
31	Li X, Zeric T, Kambhampati S, Bossert JM, Shaham Y	A critical role of the central amygdala nucleus in incubation of methamphetamine craving
34	Lynch III JF, Winiecki PA, Vanderhoof T, Riccio DC, Jasnow AM	Estradiol increases fear generalization through activation of nuclear estrogen receptors and a genomic effect on fear memory retrieval
53	Mahal A, Cushman JD	Sex Differences in Hippocampal Dependent Tasks: Female dominance in Fear Conditioning, Novel Object Recognition, and Morris Water Maze
5	Moench, KM, Miller, DP, Allen, MT, Servatius, RJ	Amygdala lesions impair lever press avoidance acquisition of inbred Wistar-Kyoto rats, but not outbred Sprague Dawley rats
20	Oliver CF, Kutlu MG, Gould TJ	The Effects of Chronic and Withdrawal from Chronic Nicotine on Extinction Learning
38	Pellman BA, Kim ES, Kashima J, Motch O, de la Iglesia HO, Kim JJ	Rats living in a risky environment exhibit threat-entrained anticipatory circadian rhythms
49	Perusini JN, Meyer EM, Rau V, Avershal JA, Rajbhandari A, Hoffman A, Nocera, N, Condro MC, Waschek J, Spigelman I, Fanselow MS	Mechanisms underlying the induction and expression of fear sensitization caused by acute traumatic stress
37	Pierson JL, Pullins SE, Quinn JJ	Dorsal Hippocampus Infusions of CNQX into the Dentate Gyrus Disrupts Expression of Trace Fear Conditioning
22	Pina MM, Cunningham, CL	Chemogenetic inactivation of the bed nucleus of the stria terminalis disrupts ethanol conditioned place preference
18	Pisansky MT, Gewirtz JC	Social fear learning in mice using a novel social conditioned place aversion paradigm
41	Pizzimenti CP, Lattal, KM	The Effects of Prior Aversive Events on Intravenous Self Administration and Reinstatement in Rats
54	Quinn JJ, Sugimoto C, Grainger LM, Kraus JR, Couse MR, Skipper RA, Kochli DE, Oswald BB	Infant stress exposure persistently enhances amygdala-dependent learning
47	Rajbhandari AK, Huang Y, Fanselow MS,	Fear conditioning increases the number of PACAP neurons expressing

	Waschek JA	cFos within the basolateral amygdala
33	Ramsaran AI, Westbrook SR, Stanton ME	Differential Ontogeny of Object, Object Location, Object-in-Context, and Object-Place-Context Recognition in the Rat
35	Reis DS, Sehgal M, Helmstetter FJ	Activity dependent proteolysis in the amygdala modulates protein synthesis in the amygdala and dorsal hippocampus during consolidation of fear conditioning.
7	Rice BA, Keller PS, Akins CK	Classifying Sign and Goal Trackers Using a T-test in a Pavlovian Conditioned Approach Procedure
28	Rose JK, Knauft, S, Hall B, Spearman B	Introduction of a Classical Conditioning Assay to Study Mechanisms of Learning in Caenorhabditis elegans.
17	Schepers ST,Bouton ME	Reducing the resurgence of an instrumental behavior after extinction by altering the temporal distribution of reinforcers during the response elimination phase
42	Sehgal M, Bula TS, Fettinger NB, Moyer JR	Neural circuitry underlying extinction of trace fear conditioning
23	Seo D, Shue F, Nguyen M, Drew MR	Role of adult neurogenesis in trace conditioning: Associative and nonassociative contributions
39	Thrailkill EA, Todd TP, Bouton, ME	Effects of CS duration, intertrial interval, and the I/T ratio on appetitive conditioning
16	Trask S, Bouton ME	Discriminative Role of the Reinforcer in the Inhibition of Operant Behavior
46	Tribble JE, Perusini JN, Zelikowsky M, Fanselow MS	Effects of single- versus pair-housed rats on fear sensitization and baseline anxiety following acute traumatic stress
32	Ulmen AR, Burbules D, Vesia W, Jasnow AM, Riccio DC	MK-801 in the Dorsal Hippocampus Causes State-Dependent Memory Reconsolidation for Passive-Avoidance
30	Winiecki PA, Lynch III JF, Ortiz S, Riccio DC, Jasnow AM	GABAB(1a) Receptors May Be Necessary For The Consolidation and Retrieval of Precise Contextual Fear Memories
4	Wright, C, Bauerle, C, Kruger, A, Sprycha, M, Allen, MT, Servatius, RJ	Reduced Acoustic Startle Response in Behaviorally Inhibited Individuals: Enhanced Responding to Auditory Stimuli Does Not Underlie Facilitated Associative Learning
2	Zelikowsky M, Chang A, Anderson DJ	Dissection of neural circuitry underlying trauma-induced enhancements in fear and aggression

ABSTRACTS

~~~~~~~Talks~~~~~~~

Alphabetical by author

Balsam, Peter

Aspects of inhibition that depend on time ~ Inhibition develops when a cue signals a decrease in the likelihood of an unconditioned stimulus (US). In error prediction models inhibition develops because a cue signals the omission of an expected reward. In contingency models inhibition develops when the rate of US presentation in the presence of a cue is lower than the rate in the absence of that cue. In several experiments we found that even though a cue signals a perfect negative contingency its ability to inhibit ongoing behavior does not depend on the fact that it is a signal for non-reward rather it depends on the relative increase in the delay to the next reward signaled by that cue. This fits with a general view that the learning of temporal relationships determines the speed of emergence, vigor and form of conditioned behavior. The speed with which both excitatory and inhibitory learning emerges is proportional to the temporal informativeness of the predictive cue (CS). In excitatory learning the informativeness depends on the reduction in the average time to the next US signaled by the CS relative to the background expected time between US's. The informativeness of an inhibitor depends on the increase in the average time to the next US signaled by the CS relative to its background expected time to the next US. In contrast, extinction seems to depend on trial number rather than time adding to the data suggesting that the learning that regulates extinction is qualitatively different than the learning underlying other forms of inhibition.

Ben-Ami Bartal, Inbal

Empathic helping in rats and its modulation by social parameters ~ Emotional contagion, the sharing of affective states between individuals, is a powerful motivator for pro-social behavior in humans. In the helping behavior test, rats demonstrate pro-social behavior by releasing a cagemate trapped inside a restrainer. Once learned, this behavior is intentional, consistent, and does not require social reward. Releasing a cagemate is as rewarding as accessing chocolate, yet rats do not help strangers of an unfamiliar strain. Moreover, helping does not occur when distress is pharmacologically blocked in either the free or trapped cagemate with a benzodiazepine. Corticosterone measurements indicate that successful helping abolishes stress induced by testing. Thus, emotional contagion of distress between the free and trapped rats plays a crucial role in motivating pro-social behavior in the helping behavior test, indication that a common affective mechanism underlies certain expressions of helping behavior in humans and rodents.

Bernstein, Ilene L.

Taste aversion learning: stimulus convergence in amygdala ~ Identification of neurons which respond to coincident inputs during associative learning in awake and behaving animals has been elusive. Arc catFISH (Cellular compartmental Analysis of Temporal activity by Fluorescence In Situ Hybridization) allowed us to visualize convergence of CS and US information within individual neurons and do this in a single trial learning paradigm – taste aversion learning - where animals were awake and learning at the time of stimulus exposure. Convergence occurred in a region of the amygdala implicated in another adaptive, defensive associative learning task, cued fear conditioning. Coincident activation in these neurons was selective for stimulus presentations which are supportive of learning. Coincident activation was NOT evident when CS-US exposures were NOT supportive of learning. A simple model which accounts for our results involves potentiation by novel CS presentation of US responses as key to coincident activation.

Bohbot, VD

Early adversity, stress response, hippocampal development and risks of psychiatric diseases ~ A larger hippocampus has been associated with healthy cognition in normal aging and with a reduced risk of numerous neurological and psychiatric disorders such as Alzheimer's disease, Schizophrenia, Post-Traumatic Stress disorder and Depression. The hippocampus is implicated in only one of two navigation strategies utilized when finding one's way in the environment. The spatial strategy involves remembering the relationship between environmental landmarks to form a cognitive map, whereas the response strategy relies on making a series of stimulus-response associations (e.g. right and left turns) from a given position. Participants who spontaneously

use the spatial strategy show increased fMRI activity and grey matter in the hippocampus relative to those spontaneously using the response strategy. Response learners, on the other hand, show increases in activity and grey matter in the caudate nucleus as well as atrophy in the hippocampus relative to spatial learners. Recent results from our laboratory show that spatial and response learners differ in terms of cortisol levels in adulthood, childhood and infancy. They differ in terms of their cortisol response to a psychological and physical stressor and their brains develop in different ways throughout childhood. Factors modulating spatial and response strategies include genes, prenatal stress, reward, and experience. Importantly, spatial memory training was shown to reverse hippocampal atrophy associated with normal aging, potentially reducing risks of neurological and psychiatric disorders.
CIHR

Bouton ME
Contextual control of instrumental behavior and its inhibition ~ After years of studying the context's role in Pavlovian learning, my colleagues and I have begun to study its role in instrumental (operant) learning, the animal laboratory's model of voluntary behavior. Consistent with what we know about Pavlovian learning, the context plays an especially important role in extinction and in other paradigms that retroactively inhibit the instrumental response (punishment, differential reinforcement of an alternative behavior, and negative contingency). However, in instrumental extinction, the organism appears to learn to inhibit a specific response in a specific context. Meanwhile, the instrumental behavior itself can also be surprisingly easy to disrupt by context change. The results suggest that the context is important in both the evocation and the inhibition of instrumental behavior.
NIDA RO1 DA033123

Burkett J, Curry D, Andari E, & Young L
The Neurobiology of Consoling Behavior in Prairie Voles ~ Consolation is a common human expression of sympathy for another's pain. Consoling behavior has also been naturally observed in several animal species, including some great apes, canids, corvids, and elephants. However, no laboratory model has been established to study this behavior. Here I present experimental data showing that male prairie voles (Microtus ochrogaster) express consoling behavior under laboratory conditions. Males significantly increase their partner-directed allogrooming toward stressed female partners, as compared to baseline and to unstressed controls. Promiscuous meadow voles do not show this stress-evoked increase. Prairie males who observe their stressed partners show an increase in self-grooming and an elevation of plasma corticosterone that is highly correlated to the corticosterone of their female partner, suggesting that consolation is based on an empathy mechanism. Finally, injection of an oxytocin receptor antagonist (OTA) into the cerebral ventricle prevents the stress-evoked increase in allogrooming. We are also exploring the role of OTA in specific brain regions in modulating consolation. These experiments are the beginning of a foundation for an animal model that will inform us about the neurobiology of consolation and empathy.

Choi J-S, LEE YK
Brain circuits involved in discriminatory avoidance learning ~ Facing the challenge of predicting and avoiding harmful consequences in a variety of situations, an organism could utilize two response strategies: passive and active defensive responses. In most laboratory experiments with small rodents, the former is often associated with innate defensive reactions and the latter with learned fear actions. Combining passive and active defensive response systems, we developed a novel protocol, the discriminatory avoidance learning (DIAL), to investigate response selection in a threat situation. DIAL is a modified 2-way active avoidance conditioning with Go/No-go response selection. Rats were placed in a shuttle box with two identical compartments and allowed to move freely. They were then presented with one of the two auditory cues: one signals a Go response (action: crossing over to the adjoining compartment) and the other No-go response (inaction: staying in the current compartment). Failure to perform the appropriate avoidance response before termination of the cue would result in footshock punishment. Response selection was heavily biased toward No-go response during the early phase of learning. However, the rate of Go response gradually increased as the training continued. Using micro-PET imaging technique, we found that the acquisition and expression of of DIAL involves activation of the medial prefrontal cortex (mPFC), dorsal striatum, and amygdala. Finally, we found that bilateral lesions of the mPFC during the

early phase of training interfered with normal acquisition of DIAL. Taken together, the current results suggest that prefronto-amygdala interaction might be essential for regulation of defensive response selection, perhaps due to their critical role in elicitation and inhibition of fear.

This research was supported by the Brain Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (NRF-2013M3C7A1056734)

Claflin, DI, Jensen, SJ, Greenfield, LR, Wentworth-Eidsuane, CL, Kraszpulski, M, Schmidt, KD, and Hennessy, MB

Postnatal Administration of Corticosterone Produces Lasting Effects on Eyeblink Classical Conditioning in Developing Rat Pups ~ It is well established in adult laboratory animals and humans that increased stress, leading to elevated levels of glucocorticoids, can influence cognitive performance, sometimes positively, sometimes negatively. It is becoming increasingly evident that even mild to moderate elevations of glucocorticoids can produce detrimental effects in developmentally immature and vulnerable organisms that may persist into adulthood. Because tasks engaging the hippocampus are particularly sensitive to glucocorticoid actions, and the hippocampus has a critical role in learning and memory, this interplay between glucocorticoids and the development of learning can be examined using trace eyeblink classical conditioning. We present here a series of studies in which we administered the glucocorticoid corticosterone (CORT) to Long-Evans rat pups on postnatal Day 15 and assessed learning on Day 28 using trace eyeblink classical conditioning. High pharmacological doses of CORT causing elevations in the blood for at least 5 days (subcutaneous pellets) yielded the most severe results with significantly poorer acquisition of trace eyeblink conditioning that was most notable for males compared to placebo controls. Moderate CORT elevations for at least two full days (subcutaneous osmotic minipumps) was sufficient to impair trace eyeblink conditioning in males and females. Interestingly, CORT injections (twice daily, subcutaneous) which delivered a very high dose for less than 4 hours at a time, facilitated learning, again preferentially in males. Together, these data suggest that the overall level, duration and pattern of elevation of corticosterone play a role in the lasting behavioral effects observed.

Supported by NIH grant # R15MH081257 and the WSU Comprehensive Neuroscience Center to D. I. Claflin

Fetz EE

Bidirectional interactions between the brain and implantable computers ~ Brains and computers are complementary information processing systems that have developed many symbiotic bidirectional interactions. Technology has reduced the size of computers to the limits of our ability to interact through normal sensory and motor channels. Even smaller computers can now interact directly with the brain through implanted electrodes. We are investigating the consequences of direct bidirectional connections produced by an autonomous implantable recurrent brain-computer interface [R-BCI connected to electrodes that record the activity of motor cortex cells. It operates continuously during free behavior and generates activity-dependent stimulation of the brain or spinal cord. The neural activity is processed by a programmable computer chip and can be converted in real-time to activity-contingent stimuli. Given sufficient time to adapt to an implanted R-BCI the brain could learn to incorporate such artificial recurrent connections into normal behavior, as demonstrated for cortically controlled electrical stimulation of paralyzed forearm muscles and intraspinal stimulation. A second application of the R-BCI is to produce Hebbian plasticity through spike-triggered stimulation; this can strengthen neural connections, as demonstrated in several recent studies in cortex and spinal cord. A third application is to deliver intracranial reinforcing stimuli contingent on patterns of neural or muscular activity, thus implementing prolonged periods of operant conditioning during free behavior. The R-BCI paradigm has numerous potential applications, depending on the input signals, the computed transform and the output targets.

Frick KM

Cell signaling and receptor mechanisms essential for estrogenic regulation of hippocampal memory consolidation ~ The classical mechanism through which sex steroid hormones regulate gene expression involves the binding of a hormone-receptor complex to a hormone response element on the DNA, thereby requiring a direct interaction between hormone receptors and nuclear DNA. However, it has become increasingly well accepted that 17beta-estradiol and other sex steroid hormones can rapidly influence cellular function without direct receptor-DNA interactions. For example, we have shown that the effects of 17beta-estradiol on hippocampal function require interactions with neurotransmitter receptors, activation of cell signaling cascades,

and facilitation of epigenetic processes such as histone acetylation and DNA methylation. This talk will briefly review our data showing that these mechanisms are necessary for 17beta-estradiol to enhance hippocampal-dependent object recognition and spatial memory in ovariectomized mice, and will provide a synthesis of these findings to date.

Galea, Liisa AM

The good, the bad and the ugly: How different estrogens influence hippocampus dependent learning, memory and neurogenesis in adult females ~ Women are more likely to be diagnosed with Alzheimer's disease but not other forms of dementia. Estrogens are thought to be neuroprotective and hormone therapies (HTs) have been used to various degrees of effectiveness in the treatment of cognitive decline in postmenopausal women. One factor that has not received a lot of attention is the form of estrogens in HTs. HTs differ by in their composition of estrogens. There are three forms or estrogens: estradiol, estrone and estriol. Estradiol is the most potent estrogen and is the predominant estrogen in younger women, while estrone is a weaker estrogen and is the predominant estrogen in older postmenopausal women. Meta-analyses indicate that estradiol-based HRTs may have more of a beneficial effect, while estrone-based HRTs may have more of a detrimental effect, on cognition and dementia risk. My talk will focus primarily on how these different estrogens affect hippocampus-dependent neuroplasticity and cognition and how reproductive experience can moderate those effects in aging. Estradiol can dose-dependently facilitate different forms of hippocampus-dependent learning and memory, while estrone has more limited and often impairing effects on hippocampus-dependent learning and memory. Both acute estradiol and estrone dose-dependently upregulate the synaptic protein, synaptophysin, in the hippocampus and increase cell proliferation (the production of new cells) in the dentate gyrus in young adult female. Chronic estradiol, but not estrone, significantly increases neurogenesis (the survival of new neurons) and expression of the immediate early gene (IEG) product, zif268, in new neurons after spatial memory retrieval in young adult female rats. Furthermore estradiol, but not estrone, is correlated positively with performance in the water maze. Both ERα and β agonists upregulate cell proliferation but G1, the GPER (formally GPR30) membrane ER agonist, decreases cell proliferation in the hippocampus. Finally reproductive experience (pregnancy and mothering) modulates the ability of the hippocampus to respond to estrogens in middle age, such that both estradiol and estrone increase cell proliferation in reproductively-experienced, but not inexperienced, older female rats. This suggests that reproductive experience permanently alters the hippocampus to respond to estrogens in older female rodents. Together these studies show that estrogens differentially affect neuroplasticity, cognition and the response of new neurons to spatial memory in both younger and older female rodents. These findings have implications for the treatment of age-related neurodegenerative disorders in women.
CIHR, NSERC

Fred J Helmstetter

Systems consolidation of fear memory in humans ~ Memory "consolidation" is inferred primarily from time dependent alterations in nervous system function and behavioral changes in susceptibility to disruption. A major confound that limits our ability to monitor the development of a given memory's stability over time relates to the fact that memory use or retrieval changes its state and likely its content. We took advantage of the recent observation (Schultz, et al, 2012) that fear conditioning alters resting-state functional connectivity (RSFC) during the post training period and applied this technique to monitor alterations in functional connections within networks of brain areas that occurred between 1 day and 1 week after learning. Human volunteers were trained with trace or delay fear conditioning. In addition to stimulus-evoked responses during training and retrieval, we monitored spontaneous low frequency alterations in BOLD signal during several "resting" scans when no CSs were presented. RSFC was increased between the amygdala and several other regions for both the delay and the trace group at 24 hours after acquisition. We found increased RSFC for the trace group relative to the delay group in several brain regions thought to support trace conditioning. The most robust differences in RSFC were apparent twenty-four hours following acquisition and most of those increases had diminished by seven days after conditioning. The results generally support the idea that time-dependent changes in functional connectivity

support memory consolidation in the post training period and that alterations in brain activity related to specific training protocols can be detected in the absence of the explicit presentation of cues used during training.
NIMH

J.P. Johansen
A neural circuit mechanism for setting fear memory strength ~ Aversive experiences are powerful triggers for neural plasticity and memory formation and the intensity of these experiences controls the strength of the memory. To trigger memories, aversive experiences activate neural 'teaching signal' circuits which engage plasticity in brain regions involved in learning and memory. Fear conditioning is an ideal model system for studying these processes because a site of plasticity mediating memory formation has been identified in the lateral nucleus of the amygdala. Using a combined optogenetic, behavioral and physiological approach, we examined how aversive teaching signals are encoded within the fear teaching signal circuit, identified a neural circuit which regulates this neural coding and explored the functional implications of this neural coding for setting the strength of fear memories. These studies reveal a concerted neural circuit and coding mechanism for how aversive experiences initiate and control the strength of associative fear memories.

Kim JH
Adolescent vulnerability to addiction: A dopamine story ~ It is well-known that adolescence is a particularly vulnerable age for drug addiction. We show that this is at least partly due to extinction deficits during adolescence. Extinction forms the basis of exposure therapies that inhibit the salience of the cues and the environments associated with drug use. When P34 (adolescent) and P69 (adult) rats received extinction to a cocaine-associated cue following a period of cocaine self-administration, it proved effective in reducing cue-induced reinstatement in adults, but not in adolescents. A likely explanation for the differential extinction observed in adolescents relates to how dopaminergic signaling in the adolescent PFC is dominated by D1R compared to D2R. Indeed, when we increased D2R activity via intra-infralimbic cortex infusion of quinpirole, or via systemic injection of the selective D2 partial-agonist Aripiprazole prior to cue extinction in adolescent rats, cue-induced reinstatement was significantly reduced the next day. These behavioral findings were also accompanied with changes in D1R vs D2R mRNA in the prefrontal cortex.
Australian Research Council/ National Health and Medical Research Council

Knowlton BJ, Patterson TK
Actions, Habits, and Early Life Stress ~ Early life stress is strongly associated with a host of negative health consequences in adulthood, including addiction and obesity, but the mechanisms underlying these relationships are poorly understood. We describe a hypothesized mechanism linking behavioral and neural mechanisms of habit vs. goal-directed learning with early life stress. In support of this idea, we have demonstrated that young adults who report experiencing early life adversity exhibit differences in an appetitive instrumental behavior compared to individuals who did not report major early-life stressors. While subjects in the non-stress condition showed a strong partial reinforcement extinction effect, this effect was attenuated in subjects reporting early life adversity. There was a dose effect, with greater early life stress leading to greater attenuation. We interpret this finding as evidence that people who experienced early life stress have a tendency to base instrumental responses on habit strength under circumstances in which controls based responding on memory for trial outcomes during learning. The idea that the partial reinforcement extinction effect is based on episodic memory is rooted in Capaldi's sequential hypothesis. In further support, we find that providing a challenge to episodic memory during learning in the form of a secondary task reduces the partial reinforcement extinction effect further in people who experienced early-life stress. We suggest that stress during early life alters hippocampal function to the extent that it influences the competition between declarative memory and habit. A propensity toward habit learning may predispose individuals to behaviors such as substance abuse and overeating that are associated with early life stress.

Krasne FB
Fraidy Rat: A realistic simulation allowing student experiments on the neuroscience of fear conditioning ~ This presentation will describe and illustrate the use of a computer program called Fraidy Rat. Fraidy Rat emulates many aspects of rodent fear conditioning and allows students to design fear conditioning experiments

and investigate neural mechanisms underlying this form of learning. The mental processes students must go through to investigate Fraidy Rat are identical to those researchers engage in when doing real research. In-effect students are doing "real" research, but on an imaginary (virtual) animal. Fraidy Rat exhibits both cue and context conditioning and extinction, blocking, renewal, consolidation, and other standard phenomena. The model underlying the program incorporates various current ideas about relevant mechanisms. Fraidy Rat has a two-dimensional brain with atlas, and probes can be implanted stereotaxically that can record single unit activity, electrically stimulate, infuse various drugs, and make lesions. It is planned to make Fraidy Rat available for instructors who wish to use it for teaching, and both lab manual and instructor manual material will be available.

Lahvis GP

Modeling social reward and empathy in mice ~ In the natural setting of human and non-human animals, learning typically occurs within a social context, whether it's with a mentor or peers or it's between a parent and offspring. Communication, in all of its forms (vocalizations, gestures, facial expressions, etc.), provides a medium for learning in a social context, yet we know little about its features, particularly the neurobiological structures that engender motivation for social learning. Using laboratory mice, we ask basic questions about the nature of this process. Can access to social interactions be a form of reward? Do mouse vocalizations convey emotional information? Can a mouse acquire fear of a conditioned stimulus by hearing the vocalizations of others? We'll explore these questions and attempts at partial answers.

Maren, S

Noradrenergic blockade stabilizes prefrontal activity and enables fear extinction under stress ~ The extinction of conditioned fear depends on the interval between conditioning and extinction. Interestingly, delivering extinction trials relatively soon (15 min – 6 hr) after conditioning yields only a transient reduction in conditional freezing that largely recovers the next day. This so-called 'immediate extinction deficit' may involve stress-induced dysregulation of amygdala-prefrontal circuits involved in the acquisition and consolidation of extinction memories. I will present new data suggesting that medial prefrontal neurons exhibit dramatic changes in spontaneous firing immediately after fear conditioning. These changes are stabilized by systemic administration of propranolol, a beta-adrenergic antagonist; propranolol, in turn, rescues the immediate extinction deficit. These data suggest that propranolol might be particularly effective in facilitating fear reduction when prevailing stress counteracts extinction learning.

McMahon L

The benefits of estrogen replacement on hippocampal physiology: Timing is everything ~ Elevated plasma 17 b estradiol levels is associated with improved learning and memory in many women, and declining hormone levels in menopause is often linked with memory impairment. Estrogen replacement therapy (ERT) is effective in some, but not all women, in the treatment of post-menopausal cognitive decline. Growing evidence suggests this variability in response to ERT may be due in part to a critical period, or window of opportunity, during which time ERT must be initiated to remain beneficial for cognition. During proestrous in female rats, spine density on hippocampal CA1 pyramidal cells is increased, synaptic plasticity is heightened, and learning and memory are enhanced. Treating aged female rats, ovariectomized (OVX) as young adults, acutely with exogenous proestrous levels of 17b estradiol mimics the effect of proestrus on hippocampal structure and function in cycling rats by increasing CA1 spine density, the NMDAR/AMPAR ratio, GluN2B-mediated NMDAR current, and LTP at CA3-CA1 synapses. However, this beneficial effect only occurs if 17b estradiol is administered by 15, but not at 19, months post-OVX, defining the critical window of opportunity. Importantly, when rats are aged with ovaries intact until undergoing ovariectomy at 20 months of age, hippocampal E2 responsiveness is maintained, indicating the deficit at 19 months post-OVX is a consequence of the duration of hormone deprivation and not chronological age. In this presentation will we discuss whether the beneficial effect of exogenous proestrous-like E2 levels on novel object recognition in OVX rats is constrained by the same critical window. Furthermore, we will discuss the impact of chronic low level E2 replacement, using subcutaneous capsules, and the timing of the replacement on hippocampal responsiveness in aged rats. Our findings help to define the dynamic nature of the critical window by showing that chronic replacement with physiological E2 levels within a certain period post-OVX can lengthen the beneficial window.

Mizumori SJY, Penner MR, Tryon V, Jo YS, Heide S

Behavioral economic analysis of ventral tegmental and hippocampal neural activity ~ Adaptive behavioral choices are based on an evaluation of the costs and benefits of expected outcomes. Traditionally, decision making that is based on this sort of economic analysis of future behaviors has been attributed to computations within midbrain-striatal-frontal networks of the brain. In natural environments, situation-specific (i.e., contextual) information likely plays an important role in biasing the impact of an economic analysis of decisions about future behaviors. Given the well-known role of the hippocampus in forming event or context-dependent memories, our current research investigates the functional relationship between midbrain structures and the hippocampus when animals perform tasks that require the flexible deliberation of the risks and benefits of choices. It will be shown that when rats perform a hippocampal dependent (spatial working memory) task, dopaminergic neurons of the midbrain ventral tegmental area (VTA) not only encode the properties of reward such as the actual and expected magnitude of rewards, but they do so depending on the location of the reward and depending on the current contextual conditions. Further, when rats perform a probability discounting (maze-based) task of the sort known to involve VTA dopaminergic neurons, hippocampal place fields show dramatic responses to changes in the economic conditions under which choices are made: Place field properties were found to depend on the actual and expected probability of future rewards, and whether rats are forced to make a specific choice or are allowed to freely choose amongst options. These data show that hippocampal processing modulates midbrain contributions to an economic analysis of behavior, and that conducting an economic analysis of behavior alters hippocampal processing. We will discuss the proposal that memory and decision neural circuitry necessarily overlap so that our memories enable better decisions, and so that optimal decision processing can facilitate efficient memory function.

NS076416

Marie, M-H

Identifying the Factors that Facilitate or Hinder Fear Memory/Expression Attenuation ~ We previously showed that combining principles of reconsolidation and extinction leads to a persistent reduction in fear responses (retrieval+extinction). The precise conditions under which this paradigm may be effective remain to be determined. In the present study, we aimed to minimize return of fear by manipulating parameters of inter-trial intervals (ITIs) between the conditioned stimuli (CSs) during extinction in rats. We manipulated 2 factors on the day of extinction: the interval between the first and second CSs, and the variability of the extinction ITIs (no variability, small variability, or large variability) while keeping the average ITI length constant. We demonstrate that preventing the return of fear is dependent upon the presence an isolated retrieval, as well as variability in ITI presentation.

Morgan MM

Tolerance to the Antinociceptive Effects of Opioids: Methodological Issues and Parallels with Research on Learning and Memory ~ Understanding the molecular mechanisms underlying behavior is a major goal of neuroscience research. One result of this research is the identification of over fifty distinct mechanisms for tolerance to the antinociceptive effect of morphine. Unfortunately, these studies have not resulted in an integrated theory of opioid tolerance. The goal of this presentation is to describe the problems that prevent a more complete understanding of the molecular mechanisms underlying tolerance to opioids with the expectation that these problems, and the solutions, will be similar for learning and memory. The molecular mechanisms underlying tolerance vary both with the type of tolerance (e.g., behavioral, associative, non-associative) and part of the nervous system engaged. Thus, distinct signaling pathways must be described for each type of tolerance at each site. However, even when a distinct type of tolerance is localized to a specific neural structure, numerous molecular changes are produced by chronic drug administration. There are many reasons for the wide range of putative mechanisms for tolerance. These include poorly designed experiments, lack of distinction between correlation and causation, complex signaling pathways, the questionable relevance of in vitro data, and distinct ligand-biased mechanisms. Attempts to address these issues using repeated microinjections of opioids into the periaqueductal gray will be discussed.

NIDA

Panksepp J

The scientific case for social-emotional feelings in other animals: Do they have affective experiences and are they homologous to our own? And Psychiatric Implications! ~ Because of the basic scientific value of skepticism, there has been a sustained scientific denial or disregard of consciousness in other animals from Rene Descartes to the present day. This remains so in scientific circles, because there has never been any agreed upon way to fathom the subjective experiences of animals, since they do not have cognitive tools comparable to human language, which allows us to communicate about our inner states. This has discouraged most scientists, especially neuroscientists and animal behaviorists, from accepting animal sentience as a scientifically workable topic. However, from an affective neuroscience perspective, the current "weight of evidence" affirms that all other mammals and birds are sentient creatures. I will summarize how the aforementioned, long-standing "scientific" biases in the field can finally be overcome, based on evidence as opposed to argumentation, and how the resulting knowledge can help illuminate, and thereby greatly benefit, our understanding of the affective foundations of human nature. Enormous advances have been made in the past half century in understanding how primal emotions are organized within mammalian brains, and how primary-process emotions (e.g., SEEKING, RAGE, FEAR, LUST, CARE, PANIC and PLAY, control secondary-process learning and memory processes of the brain, providing essential ingredients for higher-order cognitive activities, which, unlike emotional states, remain more difficult to study scientifically in animals. Thus, this talk will summarize the evidence for the conclusion that other animals do have emotional feelings, and implications for understanding of the nature of human consciousness. It also yields new perspectives for the better understanding and treatment of human psychiatric disorders. Tthree new treatments will be briefly summarized.

Srivastava DP

Deciphering the molecular mechanisms underlying estrogenic modulation of dendritic spines ~
There is increasing evidence that the regulation of structure and function of neuronal circuits is an essential component of normal cognitive function and behaviour. Indeed, multiple studies have demonstrated concurrent changes in synaptic connectivity between neurons during and following the acquisition of learned behaviours. Estrogens have repeatedly been shown to have powerful influences over cognitive function and behaviour, which is believed to be, in part, driven by estrogenic-regulation of neuronal connectivity. It is now becoming clear that estrogens have two modes of actions. In addition to the classic mode of action, which takes hours to days to manifest and relies on gene transcription, it is emerging that estrogens can act in a rapid manner, with effects occurring within minutes to hours. These rapid effects can result in the initiation of signalling pathways, leading to a number of cellular events, many of which are independent of gene transcription. Moreover, it is becoming clear that estrogens can also rapidly regulate specific behaviours. However, the molecular and cellular mechanisms that underlie this rapid regulation of behaviour have yet to be fully elucidated. As the remodelling of cortical connectivity is believed to be an essential component of cognitive function, we have focused on understanding how estrogens can regulate dendritic spines, the site for the majority of excitatory synapses, on cortical neurons. We are currently investigating the signalling pathways that are initiated by acute estrogenic treatment, and are interested in how these pathways drive estrogen-dependent spine formation. In addition, we are interested in understanding which estrogen receptor(s) mediate the rapid (acute) actions of estrogens to the remodelling of dendritic spines on cortical neurons. By further elucidating the molecular underpinnings of rapid estrogenic-modulation of cortical connectivity, we hope to add to the growing understanding of how estrogens rapidly regulate behaviour.

Sullivan RM

Enduring memory of infant pavlovian fear conditioning ~ We explored the enduring olfactory memory induced by infant odor-shock conditioning. Pups were conditioned daily from postnatal (PN) days 8-12. In infancy, this conditioning produces a preferred odor that can substitute for the natural maternal odor. This odor memory is retained into adulthood and continues to be preferred. However, this infant conditioning also produces later-life depressive-like behaviors as characterized by the Forced Swim Test (FST), sucrose consumption and social behavior. Here we explore how the learned infant odor alters amygdala function and interacts with the adult depressive-like behaviors. We found that the presence of the infant odor altered amygdala function and rescued adult depressive-like behavior. A causal role of glucocorticoid/5-HT in the later-life rescue was demonstrated by

blocking amygdala 5-HT, which prevented the odor-mediated rescue, whereas increasing amygdala 5-HT and blocking CORT mimicked the odor rescue effect in the FST. Taken together, these results suggest that the infant odor has properties reminiscent of safety signals, which can be acquired in infancy, modulate the adult amygdala LFP and rescue adult depressive-like behavior by modulating amygdala 5HT and glucocorticoids.
NSF GFRP (DGE-1137475) to MRC and NIH-MH091451 to RMS

Rich MT, Gordon J, Sanchez H, Taylor JR, Torregrossa MM

Neural Circuits Controlling the Context Dependence of Extinction ~ Extinction of maladaptive associative memories, including those associated with traumatic experiences or substance abuse, may improve treatment outcomes for these disorders. However, expression of extinction learning is highly context dependent, with a reduction in the undesirable memory observed only in the same context where extinction training occurs. Thus, for extinction based therapies to be effective, extinction would either need to occur in all possible contexts, or contextual encoding during extinction would need to be disrupted. To date, most studies exploring contextual encoding during extinction have used conditioned fear paradigms and have identified the hippocampus and hippocampal inputs and outputs as being critical for context appropriate expression of extinction memory. However, the context dependence of extinction of drug-based Pavlovian associations, and identification of the circuits that regulate contextual encoding during extinction of drug memories has been less well studied. We tested the hypothesis that extinction of memories for discrete cues associated with cocaine infusions during self-administration can reduce future cue-motivated cocaine seeking in a context-dependent manner, and that the anterior cingulate cortex (ACC) is critical for encoding context during extinction. Male Sprague-Dawley rats were trained to press a lever to receive cocaine infusions (1 mg/kg/infusion) that were each paired with an audiovisual conditioned stimulus (CS; stimulus light + tone). Following at least 10 days of self-administration, the instrumental response was extinguished to achieve low levels of responding for later cue-induced reinstatement/renewal testing. Then, the CS was extinguished in a Pavlovian manner, by presenting it non-contingently either in the same context as training (context A) or a novel context (context B: different size, shape, floor, odor). Rats were then tested for cue-induced reinstatement in context A to determine if rats would express extinction learning or demonstrate renewal. In some experiments, rats were tested the following day in context B for acquisition of a new response for conditioned reinforcement to assess expression of extinction or renewal. In the final experiment, the ACC was inactivated by infusion of the GABAergic agonists baclofen and muscimol during Pavlovian extinction training to determine if the ACC regulates contextual encoding of extinction memories. We found that Pavlovian extinction of cocaine cues reduces subsequent cue motivated responding in a context and cue exposure duration dependent manner, similar to results observed with conditioned fear. We also found that inactivation of the ACC during extinction training disrupted later expression of context-appropriate renewal, but did not interfere with extinction learning itself. Thus, Pavlovian extinction of cocaine cues translates to a reduction in instrumental responding reinforced by that cue, and the context dependence of cocaine cue extinction can be reduced by inactivation of the ACC during acquisition of extinction learning. Therefore, future studies should determine if combining extinction training with manipulations that reduce ACC activity could improve the effectiveness and context-independence of exposure-based therapies.
K01DA031745

~~~~~~~*Posters*~~~~~~~

By Poster Number

1.

Allen, MT., Myers, CE., & Servatius, RJ.

The Spacing Effect Faciliates Eyeblink Conditioning in Behaviorally Inhibited Individuals Only When the Inter-Trial Interval Is Varied ~ Recent work has found that behaviorally inhibited individuals acquire conditioned eyeblinks faster than non-vulnerable individuals. Facilitated eyeblink conditioning has also been found in behaviorally inhibited individuals using partial reinforcement protocols that included either CS or US alone trials inter-mixed with CS-US paired trials. These CS or US alone trials actually spaced the paired CS-US trials across a longer time period. It is possible that the enhancement effects observed in these protocols were partially due to a trial spacing effect. We tested the hypothesis that spacing trials over a longer period of time by extending the inter-trial interval or ITI would facilitate learning overall. We compared training with a standard ITI with both a fixed longer ITI and a longer ITI that varied according to the pattern of trials previously tested

with partial reinforcement schedules. Ninety five completed the Adult and Retrospective Measures of Behavioural Inhibition (AMBI and RMBI). Participants were grouped as behaviorally inhibited (BI) and non-behaviorally inhibited (NI) based on a median split of the AMBI score. All participants received 3 US alone trials and 30 acquisition trials presented in one session. Conditioning stimuli were a 500 ms tone conditioned stimulus (CS) and a 50-ms air puff unconditional stimulus (US). All participants were randomly assigned to receive standard ITI training with a fixed 30 sec ITI, spaced training with a variable longer ITI (mean = 57 seconds, range 25-123 seconds), or spaced training with a fixed longer ITI of 57 s. Overall, anxiety vulnerable individuals exhibiting behavioral inhibition acquired conditioned eyeblink responses at a faster rate than those non-anxiety vulnerable individuals. Behaviorally inhibited individuals exhibited significantly higher rates of conditioned eyeblinks than non-inhibited individuals in the variable longer ITI condition, but not the short ITI or longer fixed ITI condition. This facilitated acquisition of CRs was statistically significant only for spaced training with variable ITIs. These findings indicate that facilitated acquisition in partial reinforcement protocols are due to a trial spacing effect but also due to the uncertainty of when the next CS-US paired trial will occur.

2.

Zelikowsky M, Chang A, Anderson, DJ

Dissection of neural circuitry underlying trauma-induced enhancements in fear and aggression ~ Fear organizes and orchestrates a host of species-specific defense reactions that enable an animal to adaptively respond to threat. However, fear can be maladaptive when elicited in non-threatening situations or in excess, and the persistence of such inappropriate fears is thought to contribute to the formation of anxiety disorders and phobias. One poignant example of this is post-traumatic stress disorder (PTSD), wherein following an extremely traumatic event, animals display a host of maladaptive behaviors. Thus far, scientific research has largely focused on PTSD symptoms such as the propensity to acquire new fears, exaggerated fear responses to mild stressors, increased substance abuse, depression, and anxiety, while relatively fewer studies have examined the effects of trauma on social behaviors such as sex and aggression. Moreover, most rodent models of PTSD that have examined aggression report that trauma produces an overall reduction in aggression. Thus, current rodent PTSD models fail to capture the increases in aggressiveness, violence, and anger that often characterize humans with PTSD. Using mice, we adapted a model of PTSD in which mice received a single traumatic event comprised of multiple unsignaled, unpredictable footshocks in a novel environment. We found that following trauma, mice showed enhanced fear learning, increased aggression and alterations in adaptive mating behavior. Importantly, we found that these disruptions in adaptive behavior persisted despite extensive extinction of the context in which the trauma occurred. Lastly, using behavioral, pharmacogenetic, optogenetic and pharmacological techniques, we investigated and characterized the neural circuitry involved in trauma-enhanced fear and aggression.

NIH, HHMI, NSF

3.

Hallock HL, Griffin AL

Spatial working memory deficits accompany reductions in hippocampal-prefrontal synchrony following inactivation of the ventral midline thalamic reuniens and rhomboid nuclei ~ A growing body of evidence suggests that different types of learning and memory processes are distributed across specialized neural circuits consisting of two or more anatomically- and functionally-connected brain areas. One such neural circuit consists of the dorsal hippocampus (dHC) and the medial prefrontal cortex (mPFC). This circuit is thought to be critically important for spatial working memory (the ability to flexibly maintain and use trial-specific spatial information within a testing session). dHC-mPFC interactions have been shown to correlate with spatial working memory-guided task performance in rodents; however, there are no direct anatomical connections between the dHC and mPFC. The reuniens and rhomboid (RE/Rh) nuclei of the ventral midline thalamus are bi-directionally connected with the infralimbic, prelimbic, and anterior cingulate sub-regions of the mPFC, as well as the CA1 subfield of dHC. The efferent and afferent connections of the RE/Rh suggest that these thalamic nuclei may support working memory by modulating interactions between the dHC and mPFC. If RE/Rh support working memory by gating the flow of information between dHC and the mPFC, then functionally inactivating the RE/Rh should cause reduced synchrony in the dHC-mPFC network and concomitant performance impairments in spatial working memory tasks. We directly tested this prediction by simultaneously recording single units and

local field potentials (LFPs) from CA1 of the dHC and the mPFC while rats performed a working memory-dependent delayed spatial alternation (DA) task in a T-maze. Prior to the recording session, RE/Rh were functionally inactivated by an intracranial infusion of the GABAA receptor agonist muscimol. Our results show that RE/Rh inactivation caused alterations in theta power, theta phase coherence, and theta-gamma phase-amplitude coupling in the hippocampal-prefrontal network that co-occurred with severe performance impairments. These results provide a novel characterization of the mechanisms underlying memory-guided decision making by directly examining the relationship between thalamic gating of cortico-limbic interactions and spatial working memory performance.
NIH COBRE 1P20GM103653 – 01A1

4.

Wright, C, Bauerle, C, Kruger, A, Sprycha, M, Allen, MT, & Servatius, RJ

Reduced Acoustic Startle Response in Behaviorally Inhibited Individuals: Enhanced Responding to Auditory Stimuli Does Not Underlie Facilitated Associative Learning ~ The acoustic startle response (ASR) of behaviorally inhibited and non-inhibited individual was explored. We investigated possible physiological differences in responsivity to auditory stimuli in anxiety vulnerable individuals that may underlie our previous findings of facilitated classical eyeblink conditioning in anxiety vulnerable individuals. Ninety eight college-aged undergraduate students voluntarily participated in the study in exchange for research credit for psychology coursework. All participants completed the Adult Measure of Behavioral Inhibition (AMBI). We measured ASR through eyeblink-related EMG activity as well as heart rate. The session consisted of 180 seconds of pre and post baseline monitoring. The acoustic stimuli were a 50 ms white noise bursts (82, 92, or 102 dB) presented pseudo-randomly eight times each for a total of 24 trials. As expected, the number and intensity of startle responses increased as the volume of the auditory stimulus increased ($p < .0001$). ASR also habituated across the eight trials for each volume. Overall, behaviorally inhibited individuals exhibited a reduced acoustic startle response to auditory stimuli as compared to non-inhibited individuals ($p < .05$). There was also a significant effect of gender such that females exhibited more startle responses ($p < .05$) and higher amplitude responses ($p < .0005$) than males. There were no significant differences in heart rate between behaviorally inhibited and non-inhibited individuals ($p > .05$). The current ASR findings support the hypothesis that the facilitated learning previously observed in anxiety vulnerable individuals with various forms of eyeblink conditioning is not based heightened sensitivity or responsivity to the auditory stimuli.

5.

Moench, KM, Miller, DP, Allen, MT, & Servatius, RJ

Amygdala lesions impair lever press avoidance acquisition of inbred Wistar-Kyoto rats, but not outbred Sprague Dawley rats ~ We have proposed the Wistar-Kyoto (WKY) strain as a model for anxiety vulnerability due to their observed behavioral inhibition coupled with their enhanced acquisition of signaled avoidance and resistance to extinction. The amygdala is critical for Pavlovian fear and threat, and instrumental conditioning with aversive outcomes. In our initial efforts to understand the role of the amygdala in signaled lever press avoidance, we demonstrated that lesions of the amygdala disrupted, but did not prevent, the acquisition of avoidance behavior in both WKY and SD rats. In that work, a lever press during the warning signal (WS) was reinforcement by an immediate cessation of the warning signal (WS), prevention of shock and initiation of the safety signal. These multiple reinforcement contingencies did not allow for an unambiguous interpretation of the role of the amygdala in learning. In this study we removed the contingency between the lever press and WS termination. Avoidance prevented foot shock delivery, but only after the completion of the 60 sec tone signal regardless of when the avoidance occurred. Similar to previous reports, WKY sham rats showed the fastest acquisition and highest rate of avoidance whereas the SD sham rats displayed a balance between avoidance and efficient escape responses. In the absence of a response contingency in WS termination, amygdala lesions in SD rats did not appear to alter avoidance learning at all. In contrast, amygdala lesions impaired, but did not prevent avoidance acquisition of WKY rats. Anxiety disorders are best understood as a diathesis of inherent vulnerabilities and risk. The role of the amygdala in anxiety is illustrated in these strain differences in avoidance acquisition.

6.

Glenn AM, Morlock EA, & Wilson WJ

MK-801 Effect on Escape Behavior in the Earthworm, Lumbricus terrestris ~ Wilson and colleagues previously found that earthworms are able to learn to escape from an aversive stimulus by making a locomotor response. We administered the NMDA receptor antagonist MK-801 and studied the ability of Lumbricus terrestris to learn to escape from an aversive bright light by moving in a running wheel. "Master" worms controlled the bright light. "Yoked" control worms received the same exposure to the light but had no control over it; their lights were controlled by the Master worm to which they were yoked. Master worms exposed to either a low- (0.1 mg/ml) or high- (1.0 mg/ml) dose of MK-801 via absorption through the skin were found to move less than those receiving no drug, indicating that the ability to recognize the contingency between a response and its effect is sensitive to MK-801. This suggests a) that although the literature is silent on this issue earthworms must have an NMDA receptor, and b) that the functional role of this receptor in learning has been evolutionarily conserved.

Albion College FURSCA

7.

Rice BA, Keller P.S., & Akins, C.K

Classifying Sign and Goal Trackers Using a T-test in a Pavlovian Conditioned Approach Procedure ~ Sign and goal tracking behaviors categorize individual differences in incentive salience attribution. Sign trackers (ST; high incentive salience) spend the majority of the time engaging with the conditioned stimulus (CS) while goal trackers (GT; low incentive salience) spend the majority of the time with the unconditioned stimulus (US). Various methods have been used to classify STs and GTs, many utilizing a rank order split, resulting in subjects that demonstrate either low or high incentive salience relative to other subjects. The purpose of the current research was to investigate a more accurate measure of classifying subjects as sign and goal trackers. T-tests were used to determine whether subjects engaged in a significant amount of sign or goal tracking across 125 trials. Thus, classification was based on an absolute criterion (statistical significance). Results showed that of 17 subjects, 4 were classified as STs, 9 as GTs and 4 as ITs (Intermediates; neither ST nor GT). The use of the rank order split would have categorized an estimated 6 STs, 6 GTs and 5 ITs. Detailed examination of the subject's behavior revealed that the t-test classification was more accurate. This approach can easily be adapted to incentive salience models in other species.

Financial support for this research was provided by National Institute of Drug Abuse (NIDA), grant #DA022451 awarded to CKA.

8.

Hegumen Theophan (V.I. Kryukov)

The problem of non-invasive memory erasure and its solution ~ Monfils et al. (2009) found that administering fear-extinction trials in rats performed within a short interval (10 or 60min but not 6h) following a retrieval cue showed no return fear (the ret+ext effect: no spontaneous recovery, renewal, reinstatement, and slower reacquisition). This procedure permanently attenuates the fear memory without the use of drugs. These results were partially replicated in rats, mice, crabs, humans. This procedure has great promise as a therapeutic intervention that significantly reduces relapse in drug dependent clinical populations. However, the big problem is that the mechanism, the brain circuits and conditions for this effect are unclear. In particular: 1) it is unclear why many studies around the world have failed to obtain the ret+ext effect, with some studies even reporting the opposite effect; 2) it is unclear why such an effect has not also been seen in normal extinction training; 3) it is quite unexpected that reversing the order of the retrieval and extinction sessions reduced overall levels of fear similar to that observed if the retrieval trial was given before extinction. We have found that this problem is actually similar to the problem of trace conditioning because both problems are critically dependent on the same neuronal implementation of long delay (minutes and hours) between CS and US in the first case and the retrieval and extinction in the second one. Therefore the "Neurolocator" model which solves the first problem (Kryukov, 2012) can also help us to solve the second one and answer the above questions. In particular we found the boundary condition in analytical form which explains the failures to reproduce the original ret+ext effect. Similarly, the mathematical operation of convolution which involved in trace conditioning can explain not only ret+ext but also the very similar ext+ret effect due to commutativity of the convolution.

9.

Campese VD, & LeDoux, JE

General motivational processes drive aversive Pavlovian-to-instrumental transfer in rodents ~ Studies of Pavlovian-to-instrumental transfer (PIT) are capable of demonstrating control of instrumental behavior by Pavlovian conditioned stimuli (CSs). More work on PIT has involved appetitive reinforcers. Additionally, both general and sensory-specific motivational processes have been revealed using experimental designs that involve multiple Pavlovian and instrumental relationships with distinct unconditioned stimuli (USs: Corbit & Balleine 2005). Although little work has been done on aversive PIT, recent studies in rodents have begun to make progress, starting with a simple design in which a single US (footshock) is used in all phases (Campese et al, 2013; 2014). With this design we have shown that a CS paired with shock, but not an unpaired CS or a novel stimulus, facilitates on-going shock-motivated behavior. We now turn to additional designs that will help better characterize the psychological nature of the aversive PIT effect. Four behavioral studies were conducted. Experiment 1 assessed the efficacy of extinction and safety-training treatments on aversive PIT and found evidence for reduced transfer after both. Experiment 2 evaluated whether a stimulus trained as a conditioned inhibitor for food was capable of modulating aversive instrumental responding, and found that this was not so. Experiment 3 sought to determine whether or not it is critical for the USs to match across phases in order to observe PIT. We compared the PIT effect on shock avoidance when the CS predicted the same shock US versus a different US (klaxon) and found less facilitation when the US was different. Experiment 4 investigated how the motivational nature of the response contributes to the influence exerted by the aversive CS during transfer. This study showed that if the same physical behavior as was used in avoidance (i.e., two-way shuttling) was trained as a means of appetitive reward, conditioned suppression instead of facilitation was obtained when the aversive CS was presented. Together these data suggest that there is a general-motivational foundation to the aversive PIT effect produced using our task (Campese et al, 2013), which treatments such as extinction and safety-training can influence.

10.

Lewon M, & Parrott Hayes LJ

The Effects of Varying Levels of Food Deprivation on Escape and Avoidance Responding in Mice ~ Motivating operations (MOs) are typically held to alter the extent to which specific stimuli are reinforcing or aversive, which is correlated with changes in an organism's behavior with respect to those specific stimuli as consequences. It is likely, however, that any given MO affects the reinforcing/punishing efficacy of a wide range of reinforcers and/or aversive stimuli. The present study examined whether the MO of food deprivation, which establishes food as a more effective reinforcer, also alters the extent to which other stimuli function as aversive. Mice were taught to respond on a nose poke apparatus to escape or avoid the presentation of a loud noise in a signaled avoidance procedure, and the rate of responding to terminate or avoid the noise was taken as a measure of the noise's efficacy as an aversive stimulus. Relative to sessions in which subjects were not deprived of food, substantially more escape/avoidance responses were made during sessions in which subjects were deprived of food for either 16 or 24 hours. These findings suggest that, in addition to altering the value of food as a reinforcer, food deprivation also alters the extent to which noise functions as an aversive stimulus.

11.

Leaderbrand KL, Chen HJ, Corcoran KA, & Radulovic J

Cholinergic but not glutamatergic antagonism in retrosplenial cortex impairs contextual fear acquisition ~ Contextual fear learning is a model of anxiety disorders, such as post-traumatic stress disorder (PTSD), which are characterized by aberrant fear learning and retrieval. The retrosplenial cortex (RSC) is a brain region with reciprocal connections to known regions of the fear circuit, such as hippocampus and prefrontal cortex, and is overactive in PTSD sufferers. Here, we present data suggesting intra-RSC application of the muscarinic acetylcholine receptor (mAChR) antagonist scopolamine prior to fear conditioning prevents the acquisition of contextual fear. Further experiments designed to uncover the mAChR subtype responsible for this effect indicate that the M1 receptor in RSC is critical for contextual fear acquisition. Interestingly, antagonists of AMPA, NMDA, and metabotropic glutamate receptors had no effect. Determining how mAChRs modulate fear memory is critical for developing a complete model of and potential therapies for anxiety disorders.

12.

Eisenreich BR, & Szalda-Petree AD

Effects of Fluoxetine on stimulus control of aggressive responding in B. Splendens ~ Previous research (Lyn et al., 2007) demonstrated that Fluoxetine, a SSRI, reduces the aggressive behavior of B. splendens to a mirror stimulus. However, few studies have examined the impact of Fluoxetine on stimulus features important for associative learning. To examine the impact of Fluoxetine on associative learning, a go or no go task was created consisting of a straight-alleyway maze with a removable mirror on one end. Two discriminative stimuli in the form of checkered patterns were counterbalanced such that one pattern always predicted mirror exposure and the other did not for each subject. Each of the discriminative stimuli was placed on removable doors, inserted in the maze before the mirror to create a goal box. Experimental trials consisted of lifting the door to the start box and exposing the subject to the discriminative stimulus pattern and recording both the latency to swim down the maze as well as the amount of aggressive behavior aimed at the discriminative stimuli before entry into the goal box. Using this task, the impact of Fluoxetine on a conditioned aggressive response and an instrumental swimming response for 8 male B. Splendens exposed to a 10 μMol concentration of Fluoxetine was analyzed. Results indicated that Fluoxetine may exert its anti-aggressive effects in B. splendens by altering the salience of mirror stimuli through a lowered level of arousal.

13.

Fuchs JR, Darlington SW, Morielli AD, & Green JT

Measuring Changes in Surface Kv1.2 Expression in Cerebellar Cortex following Eyeblink Conditioning, Unpaired Stimulus or Context Exposure Controls ~ Eyeblink conditioning (EBC) is a well-studied form of classical conditioning supported by plasticity in the cerebellum. EBC involves trials in which a tone conditioned stimulus (CS) is followed by an eyelid stimulation unconditioned stimulus (US), and eyeblink conditioned responses (CRs) occur to the CS prior to the US. Both Purkinje cells (PCs) and neurons of the interpositus nucleus (IPN) receive CS and US inputs; in order for a CR to be exhibited, the tonic inhibition of the IPN neurons from PCs needs to be lifted. Our model proposes that the regulation of Kv1.2, an α-subunit of Kv1 voltage-gated potassium channels that is densely expressed on basket cell (BC) axon terminals in the cerebellar cortex, is integrally important through a feed-forward inhibitory pathway involving parallel fibers, BCs and PCs. We have previously shown that blocking Kv1.2 through intracerebellar infusions of tityustoxin (TSTX), a selective blocker of Kv1.2, dramatically enhanced acquisition of CRs. Additionally, we showed that intracerebellar infusions of secretin, a retrograde messenger released by PCs that reduces surface Kv1.2 at BC-PC synapses, facilitated conditioning. Finally, we showed that intracerebellar infusions of a secretin receptor antagonist impaired conditioning. These findings led us to hypothesize a reduction in the surface expression of Kv1.2 in BC axon terminals during EBC. In order to test this hypothesis, we developed new techniques to measure global changes of Kv1.2 expression in the lobulus simplex region as well as region-specific changes of Kv1.2 expression on the BC axon terminal region of the cerebellar cortex. Focusing on the region-specific changes, we developed and optimized a technique utilizing multiphoton microscopy to detect changes in surface Kv1.2 expression of rats after EBC, compared to unpaired stimulus presentations or context exposure controls. To measure changes in surface Kv1.2 expression after the last day of training, rats are perfused with 4% paraformaldehyde and 200-400μm parasagittal sections of lobulus simplex are sequentially assigned to one of two experiments. For region-specific changes, sections are stained with ATTO-TSTX-594, a fluorescent conjugate of TSTX that binds specifically to Kv1.2, and imaged using a LSM 7 multiphoton microscope. The specific microzone for EBC is isolated during sectioning and is further isolated by taking images only of the area around the base of the primary fissure. To analyze the images, we create a mask using a z-projection of all z-sections based on the maximum intensity to isolate BC axon terminals in the PC layer and then measure the integrated intensity of objects ranging from 10-500 pixels in size from the z-projection of the sum of all sections. Preliminary data, using non-paraformaldehyde fixed slices, showed that we were able to detect reductions in surface expression of Kv1.2 at BC-PC synapses, using the LSM 7 microscope system, after treatment with Forskolin, an adenylate cyclase activator. To measure global changes in Kv1.2 surface expression, the other half of the sections are biotinylated and analyze via western blot, a method developed specifically to measure global

changes in Kv1.2 surface expression. Between these two techniques, we expect to be able to observed region-specific and global changes in Kv1.2 surface expression in cerebellar cortex after EBC.

14.

Ardiel EL, Giles AC, & Rankin CH

Dual Process Theory Revisited: Habituating and Sensitizing components of an optogenetically triggered response ~ Failure to avoid stimuli detected by the ASH polymodal nociceptors could be fatal for the microscopic roundworm, C. elegans. Why then does the avoidance response habituate? Our data indicate that habituation is part of a strategy to promote dispersal. To investigate habituation of ASH-mediated reversal responses we developed a high-throughput learning assay using real-time computer vision software for behavioral tracking and optogenetics for ASH stimulation. Two response metrics (latency and reversal duration) displayed a decrement following repeated or persistent ASH activation. The decrement in reversal duration could be readily reversed using a Petri plate tap as a dishabituating stimulus. Repeated ASH activation also suppressed spontaneous reversals and accelerated forward movement. Food and dopamine signaling promoted responding to persistent ASH activation and we identified the D1-like dopamine receptor, DOP-4, as the key mediator. Neuropeptide synthesis mutants displayed impaired habituation for a variety of metrics, prompting us to perform an RNAi screen of neuropeptide receptors and precursor genes. Evaluating both spontaneous behavior and ASH-mediated responses and plasticity we identified known and novel loss-of-function phenotypes for posture, locomotion, and learning. Thus, we are genetically dissecting behavioral components of habituation and sensitization to understand how a persistent aversive stimulus elicits the optimal escape strategy – minimizing non-essential backward movement and accelerating forward movement.

NSERC Discovery Grant to CHR

15.

Hong W, Kim DW, & Anderson DJ

Antagonistic Control of Social Behaviors by Inhibitory and Excitatory Neurons in the Medial Amygdala ~ Animals display a range of innate social behaviors that play essential roles in survival and reproduction. While the medial amygdala (MeA) has been implicated in prototypic social behaviors such as aggression, the circuit-level mechanisms controlling such behaviors are not well understood. Using cell-type specific functional manipulations, we find that distinct neuronal populations in the MeA control different social and asocial behaviors. A GABAergic subpopulation promotes aggression and two other social behaviors, while neighboring glutamatergic neurons promote repetitive self-grooming, an asocial behavior. Moreover, this glutamatergic subpopulation inhibits social interactions independently of its effect to promote self-grooming, while the GABAergic subpopulation inhibits self-grooming, even in a non-social context. These data suggest that social vs. repetitive asocial behaviors are controlled in an antagonistic manner by inhibitory vs. excitatory amygdala subpopulations, respectively. These findings provide a framework for understanding circuit-level mechanisms underlying opponency between innate behaviors, with implications for their perturbation in psychiatric disorders.

16.

Trask S, & Bouton ME

Discriminative Role of the Reinforcer in the Inhibition of Operant Behavior ~ Extinguished instrumental responding can recover when the behavior is tested following removal from the physical context of extinction (renewal) and following removal of alternative reinforcement (resurgence). Both renewal and resurgence can be viewed as illustrations of the fact that the context of extinction controls inhibition of the response. In renewal, the context is the physical conditioning chamber, whereas in resurgence, the “context” of extinction is theoretically created by reinforcer presentations during the response elimination (extinction) phase. The latter view gives a discriminative role to reinforcer presentations. Two experiments therefore tested whether reinforcer presentations can indeed control operant extinction. The first experiment studied renewal. It used an ABA renewal design in which all animals learned to lever press for a reinforcer, O1, in Context A. Following acquisition of this behavior, all animals were switched to Context B, where responses no longer produced pellets, but a second reinforcer, O2, was presented noncontingently. Animals were then tested for responding back in A,

under both typical extinction conditions and with O2 pellets delivered noncontingently. Consistent with a discriminative role for reinforcer presentations, animals showed renewed responding when tested back in Context A, but this effect was attenuated by the presentation of O2 pellets. A second experiment examined the discriminative role of reinforcers in resurgence. Initially, all animals were taught to perform a response, R1, for a reinforcer, O1. In a second phase, R1 was placed on extinction, while a newly available response, R2, produced a second reinforcer, O2. Following extinction of R1 and acquisition of R2, both responses were placed on extinction and tested with either no reinforcer presentations, noncontingent O2 presentations, or noncontingent O1 presentations. Animals tested with no pellets or O1 pellets showed robust resurgence, but this effect was abolished in the group that received O2 pellets during the test. Together, the results confirm that reinforcer presentations can control the inhibition of instrumental behavior.

This research was supported by Grant RO1 DA033123 from the National Institute on Drug Abuse to MEB.

17.

Schepers ST, & Bouton ME

Reducing the resurgence of an instrumental behavior after extinction by altering the temporal distribution of reinforcers during the response elimination phase ~ Resurgence is the relapse of an extinguished behavior that occurs when an alternative behavior introduced to replace it is also placed on extinction. Resurgence experiments involve three phases. During Phase 1, a response (R1) is trained; in Phase 2, it is then extinguished while a new behavior (R2) is introduced and reinforced. Then, in a third and final phase (the resurgence test), R2 is also placed on extinction, and responding on R1 resurges despite remaining on extinction. Several theories have attempted to explain the resurgence effect. To distinguish between them, the current experiments examined how the temporal distribution of reinforcers delivered over Phase 2 sessions affect resurgence when they are removed during the resurgence test. Experiment 1 demonstrated weaker resurgence when the reinforcement rate for R2 gradually became leaner over Phase 2 (i.e., forward thinning) and in the reverse condition when the reinforcement rate was first lean and then became richer (i.e., reverse thinning). However, only the forward thinning procedure completely eliminated resurgence. In Experiment 2, resurgence was also eliminated when reinforcement for R2 was only available in alternating Phase-2 sessions. In contrast, groups that received either the same reinforcement rate during the final Phase-2 session or the same average reinforcement rate over the phase displayed similarly robust resurgence effects. The results suggest that resurgence may be a special type of renewal effect in which extinguished R1 responding recovers when the context provided by reinforcer presentations is removed. Resurgence can be weakened when R1 is extinguished in a lean pellet context that generalizes better to the extinction context that prevails during a resurgence test.

18.

Pisansky MT, & Gewirtz JC

Social fear learning in mice using a novel social conditioned place aversion paradigm ~ Fear can be acquired vicariously through observation of another's distress. In rodents, this phenomenon of social fear learning has been replicated using modified Pavlovian fear conditioning paradigms. However, in mice, these paradigms produce small levels of conditioned responses (freezing), and do not adequately measure other forms of fear (e.g., escape/aversion). To address these limitations, our lab has been investigating social fear learning in mice using a novel social conditioned place aversion (sCPA) paradigm. In this paradigm, a mouse (observer) observes another (demonstrator) being exposed to foot shocks (US) on one side of a standard conditioned place chamber (CS). In addition to measuring freezing behavior of the observer mouse during conditioning, this paradigm allows for measurement of escape/aversion between pre- and post-test days, as well as the collection of ultrasonic vocalizations (USVs) emitted from demonstrators during conditioning. In sibling observer mice, repeated foot shocks elicited freezing behavior (conditioning day 1) and avoidance of the demonstrator-containing chamber (conditioning day 2), as well as learned avoidance during a post-test conducted 24hrs after conditioning. Moreover, freezing behavior of observer mice correlated with the total duration of USVs emitted by demonstrator mice, suggesting a contribution of vocal-auditory cues to conditioning. These effects were not present for a control condition in which the demonstrator was not shocked. Interestingly, non-sibling observers demonstrated a reduced level of freezing and conditioned aversion consistent with other reports of familiarity effects on social fear learning. Ultimately, the sCPA paradigm may provide a means for investigating the effects

of pharmacological, genetic, or optogenetic manipulations on social fear learning or empathy-like behavior in rodent models.

This research is supported by the Autism Initiative (University of Minnesota), the Gralnick Prize for Research on Severe Mental Disorders (American Psychological Foundation), Lieber Prize for Outstanding Schizophrenia Research (NARSAD), and Center for Cog

19.

Ferrara NC, Gilmartin MR, Reis DS, Lee JL, & Helmstetter FJ

ERK-mTOR interactions in the lateral, basolateral, and central amygdala during fear memory consolidation ~ The amygdala receives projections from the thalamus and hippocampus and is generally considered a critical locus of neural plasticity following fear conditioning. The different subnuclei within the amygdala interact through intrinsic connections and have different roles during the consolidation and expression of aversive learning. The details about how the lateral, basolateral, and central nuclei interact during long term memory (LTM) formation are not well understood. ERK and mTOR are two major signaling pathways that regulate synaptic plasticity supporting memory consolidation in the amygdala. mTOR complex 1 regulates downstream translational machinery, and the phosphorylation of ERK regulates transcriptional and translational processes. There is some evidence supporting an interaction between ERK- and mTOR-mediated translation during activity dependent synaptic plasticity (e.g.,Tsokas et al., 2007). The current study focused on amygdala-subnuclei specific interactions between ERK and mTOR during LTM consolidation after fear conditioning. Consistent with our prior work, results suggest that bilateral microinfusion of pharmacological inhibitors of ERK (U0126) or mTOR (rapmycin) phosphorylation into the lateral amygdala, leaving the central nucleus of the amygdala unaffected, is sufficient to prevent memory formation when assessed 24-hours after training. Conversely, blocking ERK phosphorylation within the central nucleus of the amygdala did not impact fear memory. Immunohistochemistry results revealed that mTOR inhibition reduced phosphorylated ERK in the lateral amygdala, phosphorylated p70s6k in the lateral and basolateral amygdala, and increased phospho-ERK immunopositive cells in the central amygdala. Additionally, inhibition of ERK resulted in a significant reduction of both phosphorylated p70s6k in the basolateral and ERK in the lateral amygdala. This effect suggests a bi-directional interaction between the ERK and mTOR pathways that is dependent on the specific population of cells within the amygdala.

NIMH R01 MH069558

20.

Oliver CF, Kutlu MG, & Gould TJ

The Effects of Chronic and Withdrawal from Chronic Nicotine on Extinction Learning ~ Anxiety disorders such as Post-Traumatic Stress Disorder (PTSD) are attributed to deficits in extinction learning with several studies showing that fear extinction is delayed in these patients. Given the high rate of cigarette smoking in anxiety and PTSD patients and the recent finding that an acute dose of nicotine impairs contextual fear extinction, the current study was conducted to examine the effects of chronic nicotine and chronic nicotine withdrawal on extinction learning in mice. Animals were first trained in a background contextual fear conditioning paradigm using white noise as a conditioned stimulus (CS), which co-terminated with a 2 s 0.57 mA unconditioned foot-shock stimulus (US). During initial testing and extinction sessions, animals were placed in the same context with no CS or US presentations, and freezing was measured as an indication of fear. Chronic animals were administered nicotine (12.6 mg/kg/d) for 10 days and throughout training (Day 11), initial testing (Day 12), and extinction sessions (Days 13-17) whereas withdrawal animals were given nicotine during training and initial testing but not during extinction sessions. No significant difference was found between Saline and Nicotine groups during initial testing. However, both chronic nicotine and withdrawal from chronic nicotine impaired contextual fear extinction (Drug X Test Trial interaction $p<0.05$). Interestingly, the way these two nicotine administration regimens affected extinction was different between conditions. While the difference between saline and chronic nicotine animals persisted throughout extinction trials, withdrawal from nicotine resulted in impaired extinction only during the first 2 extinction trials. Based on previous studies examining the effects of chronic and withdrawal from chronic nicotine on hippocampus-dependent learning and memory, these results may be due to upregulation of nicotinic acetylcholine receptors, a hypothesis that will be tested in future studies that examine the effect of nicotine on extinction learning.

21.

Eddy MC, Todd TP, Bouton ME, & Green JT

Exercise in adolescent rats reduces renewal of extinguished instrumental behavior ~ Voluntary exercise, particularly during development, may have wide-ranging benefits, from improved executive function to enhanced cognitive flexibility. Previous work in our lab suggests that exercise facilitates prefrontal-dependent set-shifting in adolescent but not adult rats. Here we have examined the effects of exercise during adolescence (i.e., postnatal days 30-44) on renewal of responding after extinction of instrumental lever-pressing. Male Wistar rats were given access to running wheels for two weeks during acquisition, extinction and testing. Non-exercise controls were given locked running wheels. Following magazine training, acquisition of lever-pressing for sucrose pellets on a VI-30 schedule occurred over six daily 30-minute sessions in context A. Extinction took place in context B over four 30-minute sessions. At test, all rats were tested in context A and B in 10-minute sessions during which no reinforcer was available. Groups were counterbalanced for acquisition context and order of test sequence. There were no differences between exercising and non-exercising rats in rates of acquisition or extinction. Both exercising ($p= 0.001$) and non-exercising ($p< 0.001$) groups showed ABA renewal of responding when returned to context A, though exercising rats showed significantly less responding in the renewal context than non-exercising rats ($p= 0.03$). Using the same experimental timeline, a separate group of rats was tested for AAB renewal. In this experiment, there was evidence (marginally significant, $p = 0.057$) of renewal in non-exercising rats only. The AAB renewal effect has been demonstrated to be much subtler than ABA, due to a decrement in responding when instrumental lever pressing is tested in a new context (see Bouton et al., 2011). Because of this relatively subtle effect, there may be a floor effect in observable changes of AAB renewal in exercising rats. The ventro-medial prefrontal cortex may mediate exercise effects on ABA and AAB renewal. Current experiments are examining the role of the infralimbic and prelimbic cortices in renewal of extinguished instrumental lever-pressing for sucrose pellets.

22.

Pina MM, & Cunningham, CL

Chemogenetic inactivation of the bed nucleus of the stria terminalis disrupts ethanol conditioned place preference ~ Pavlovian conditioned associations between external stimuli and drugs of abuse are believed to contribute substantially to drug dependence and relapse to drug use. The conditioned place preference paradigm (CPP) is an animal model used to reproduce this phenomenon in the laboratory in order to identify its underlying neural mechanisms. The bed nucleus of the stria terminalis (BNST) of the extended amygdala has been identified as an important neural substrate involved in the expression of psychostimulant- and opiate-induced CPP. However, it is less clear whether the BNST is involved in the expression of a place preference induced using ethanol. Therefore, the purpose of the present experiment was to evaluate BNST signaling in ethanol CPP expression in adult male DBA/2J mice ($n = 45$). Under stereotactic guidance, we bilaterally infused a viral vector carrying an inhibitory designer receptor exclusively activated by designer drug (Gi-DREADD) into the BNST (from bregma, AP 0.26, ML ± 2.33, DV - 4.32) at a 20° angle avoiding the lateral ventricles. After viral infusions, 4-6 weeks were given before the start of behavioral procedures to allow for recovery and transgene expression. A two-compartment unbiased place conditioning procedure was used, where ethanol (2 g/kg) was paired with a distinct tactile cue. Conditioning sessions were run twice daily, with saline (CS-) trials occurring in the morning and ethanol (CS+) trials in the afternoon. Trials were conducted across 2 days for a total of two sessions of each type (2 CS+ and 2 CS-) and the place preference test was administered 24 h after the final conditioning session. Thirty min before the preference test, mice were given an IP injection of clozapine-N-oxide (CNO; 10 mg/kg). Inactivation of the BNST via CNO stimulation of Gi-DREADDs before the expression test disrupted ethanol CPP compared to DREADD-expressing control mice that received vehicle injections ($p = .002$), but there was no effect on activity. Control experiments showed that this finding was not due to a direct effect of CNO, as injections of CNO (10-20 mg/kg) before an ethanol CPP expression test did not affect CPP or locomotor activity in control mice ($n = 48$) not expressing Gi-DREADDs. These data indicate that the BNST is involved in cue-induced ethanol seeking, as measured by CPP. Combined with the broader literature, our findings also suggest that the BNST may be a promising target for therapies aimed at reducing craving and preventing relapse. Follow-up studies are needed to determine the distinct circuits and neurochemical systems that are responsible for the BNST's role in drug seeking induced by conditioned cues.

Supported by NIH-NIAAA grant R01AA007702 and a Vertex Scholar Award.

23.

Seo D, Shue F, Nguyen M, & Drew MR

Role of adult neurogenesis in trace conditioning: Associative and nonassociative contributions ~ Adult hippocampal neurogenesis is widely believed to contribute significantly to learning and memory. However, studies examining the requirement of adult hippocampal neurogenesis in hippocampus-dependent learning and memory tasks have often yielded conflicting results. The literature on trace conditioning, a hippocampus-dependent form of classical conditioning, is a representative example. Trace conditioning rescues newborn hippocampal neurons from death (e.g., Gould et al., 1999; Shors et al., 2004), but the arrest of neurogenesis has been reported to impair, enhance, or have no effect on trace conditioning. The conflicts among studies raise the possibility that the contribution of adult neurogenesis to behavior is subject to modulation by unidentified factors. We revisited the role of adult neurogenesis in trace conditioning using two methods to arrest adult hippocampal neurogenesis: targeted x-irradiation and a novel, inducible transgenic DCX-TK mouse line. Neurogenesis-arrested mice displayed similar levels of tone fear in both delay and trace procedures. However, an unexpected difference in context-elicited fear emerged. In the trace but not the delay procedure mice lacking neurogenesis exhibited significantly more context fear than controls. This unexpected phenotype was present with both methods of arresting neurogenesis and suggests that although the arrest of neurogenesis failed to prevent acquisition of trace conditioned fear, the mechanisms of learning may differ between neurogenesis-arrested and control mice. Follow-up experiments showed that in the trace procedures, tone-elicited freezing was largely nonassociative. Exposure to footshock by itself, without explicit tone-shock pairings, produced tone-elicited freezing and increased anxiety-like behavior in the open field. The nonassociative fear and anxiety induced by footshock were stronger in mice lacking neurogenesis than their WT littermates, consistent with previous research showing that the arrest of neurogenesis potentiates the response to acute stressors (Snyder et al., 2011). The data led us to hypothesize that the arrest of neurogenesis impaired conditioning to the trace CS, but this impairment was masked by nonassociative fear. Consistent with this hypothesis, when the trace conditioning procedure was reworked to minimize the contribution of nonassociative plasticity, neurogenesis-arrested DCX-TK displayed an impairment in trace conditioning relative to controls. The data are consistent with the idea that neurogenesis contributes to both cognitive function and emotional regulation. Mice lacking neurogenesis display an exaggerated emotional response to footshock, and this response can mask cognitive deficits in fear conditioning.

NIH5R00MH083943 and NIHR01MH102595

24.

Bacharach SZ, Kawa AB, & Calu DJ

Sign- and goal-tracking rats learn differently in the face of changing reward value ~ During a simple autoshaping procedure, where the extension of a lever precedes the delivery of food pellets, rats show individual differences in conditioned responding; some rats approach and contact the lever (sign-tracking (ST)), whereas other rats approach and contact the food cup (goal-tracking (GT)). Here we use an unblocking procedure to examine whether ST and GT rats show differences in learning about changing reward value. After characterizing rats as ST or GT, we trained them in an unblocking task that uses odor cues as predictors of sucrose reward. During initial conditioning, rats learned to nose poke into an odor port, where they sampled odor cue (A), after which responding in a fluid well below resulted in the delivery a fixed quantity of sucrose. During compound conditioning, the previously learned odor cue (A) was followed by one of three novel odor cues (X, Y, or Z), which was predictive of the same (X), more (Y), or less (Z) reward than expected from (A) alone. During a probe test, rats received X, Y, and Z in extinction conditions and time spent in the sucrose well was an indicator of reward expectancy. ST rats learned about increases, but not decreases in reward value, whereas the opposite was true for GT rats. Individual differences in conditioned responding during autoshaping correlated with the rats' ability to learn about changing reward value. Presently, we use in vivo electrophysiology in rats performing in this unblocking task to examine the neural correlates underlying these individual differences in learning.

This work was supported by NIDA/NIH

25.

Kaganovsky KK, Ahmed S, & Marchant NJ

A critical role of Nucleus Accumbens dopamine D1-like receptors in renewal of punished alcohol seeking ~ In humans, places or contexts previously associated with alcohol use often provoke relapse during abstinence. The ABA renewal procedure, in which extinction is utilized to suppress operant responding, is used as an animal model of context-induced relapse to alcohol seeking. However, human alcoholics typically abstain from alcohol use due to the negative consequences of excessive drinking. We recently developed a procedure to study renewal in laboratory rats after abstinence is imposed by negative consequences (footshock punishment). The mechanisms of renewed alcohol seeking after punishment are largely unknown. Previous studies have shown a critical role for dopamine D1-like receptors in renewal of alcohol seeking after extinction. Here we examined the role of dopaminergic signaling, via D1-like receptors, in nucleus accumbens (NAc) core and shell in renewal of alcohol seeking after punishment. We trained alcohol preferring "P rats" to self-administer 20% alcohol in context A and then suppressed their alcohol taking with response-contingent footshock punishment in context B. We then tested for renewal of alcohol seeking in contexts A and B (counter-balanced order) without alcohol or footshock. In Exp. 1 we tested the effect of systemic injections of the D1-like receptor antagonist SCH 23390 on renewal of alcohol seeking after punishment-imposed abstinence. In Exp. 2, we tested the effects of NAc core and shell infusions of SCH 23390 on this renewal. We found that both systemic and NAc core and shell injections of SCH 23390 decreased renewal of alcohol seeking. Results demonstrate a critical role of dopamine D1-family receptors and dopamine signaling in accumbens core and shell in renewal of alcohol seeking after punishment-imposed abstinence.

This work was supported by NIDA/NIH.

27.

Cai D, Chen S, Pearce K, & Glanzman DL

Reinstatement of long-term sensitization memory in Aplysia after reconsolidation blockade or inhibition of PKM ~ As in mammals, reconsolidation blockade (Nader & Hardt 2009) and inhibition of PKM (Sacktor 2011) in Aplysia can disrupt consolidated long-term memory (Cai et al 2011, Cai et al 2012). An important question in both mammals and Aplysia is whether the amnesia that results from these two antimnemonic manipulations represents elimination of the stored memory or, instead, disruption of its retrieval. We have addressed this question in cellular and behavioral experiments in Aplysia. Previously, we showed that reconsolidation blockade and inhibition of the Aplysia homolog of PKMζ, PKM Apl III, disrupt the long-term memory (LTM) for behavioral sensitization, and also eliminate long-term facilitation (LTF), the synaptic mechanism of LTM. Here, we tested the effect of reconsolidation blockade and inhibition of PKM Apl III on the growth of presynaptic varicosities produced by LTF. Sensorimotor cocultures were trained with spaced pulses of serotonin (5-HT), which induces LTF; 24 hr later the cocultures were subjected to a reconsolidation blockade protocol or treatment with chelerythrine, a drug that inhibits PKM Apl III. Training with 5-HT produced approximately a doubling of the number of varicosities on the processes of presynaptic sensory neurons, as determined by confocal imaging. Blockade of reconsolidation of the memory for LTF, or treatment with chelerythrine, reversed the 5-HT-induced synaptic growth. (In control trained cocultures the increase in varicosity number persisted for ≥ 48 hr.) In subsequent behavioral experiments, however, we found that the LTM for sensitization could be reinstated after the two amnesia-inducing manipulations through modest additional sensitization training. Importantly, this modest training does not produce LTM in naïve animals. Thus, LTM can be reinstated following treatments that eliminate both the behavioral and synaptic expression of the memory. Taken together, our results indicate that reconsolidation blockade and inhibition of PKM cause amnesia by disrupting the synaptic expression of sensitization memory, but that LTM nonetheless persists.

NIH/NINDS, NIH/NIMH, NSF

28.

Rose JK, Knauft, S, Hall B, & Spearman B

Introduction of a Classical Conditioning Assay to Study Mechanisms of Learning in Caenorhabditis elegans ~ The simple nervous system of Caenorhabditis elegans is comprised of 302 neurons and the neuronal circuitry of specific behaviors has been identified facilitating the mapping of putative sites that change as a result of experience. Although previous studies in C. elegans have paired various environmental conditions with the

absence of food (e.g., NaCl, temperature), these protocols are typically conducted with a long conditioning period (> 60 minutes) making it difficult to separate mechanisms of learning from memory. In an effort to take advantage of this well-characterized model to further understand mechanisms of learning, a Classical Conditioning assay, with rapid acquisition of learning, was tested. This Classical Conditioning assay introduced pairing of a mild vibrational mechanosensory stimulus (a 100 Hz auditory tone; NS/CS) with a naturally aversive stimulus; ultraviolet light (a known mutagen; US). Following pairing, C. elegans responded on average with larger avoidance reversals following CS tone-alone presentation compared to responses prior to pairing. This increase in responsiveness was likely not due to sensitization as US-alone presentation during conditioning trials did not result in an increase in responsiveness. As well, delayed pairing where the CS onset preceded US onset resulted in the strongest effect as demonstrated in other Classical Conditioning studies. Using this method, we have begun to test mutant C. elegans strains and have found that a strain carrying a deletion mutation for an isoform of the worm CaMKII gene (unc-43; orthologous to human CaMKIIγ) does not show increased responsiveness following pairing compared to wild-type worms. Future research will include a behavioral screen of several mutant strains with the goal of identifying novel mechanisms involved in Classical Conditioning.

29.

Hitchcock LN, Raybuck JD, Wood MA, & Lattal KM

A histone deacetylase 3 inhibitor enhances extinction and attenuates reinstatement of self-administration in rats ~ Addiction is a chronic, often relapsing disease that causes compulsive drug seeking. The neurobiological basis of relapse in humans is often studied with an animal model of reinstatement. In the acquisition phase of this paradigm, animals press a lever to receive reinforcing intravenous cocaine infusions. During extinction, reinforcers are then withheld and the animal eventually inhibits this lever-pressing behavior. But like humans, rats will relapse, or renew this drug-seeking behavior once they are removed from the extinction context or exposed to drug-associated cues. Given that there are no long-term therapies for cocaine addiction to date, I investigated whether a novel and selective epigenetic drug (RGFP966) could promote extinction and weaken reinstatement. After rats were trained to self-administer cocaine to stable and high rates, rats were given a subcutaneous injection of RGFP966 (histone deacetylase (HDAC) 3 inhibitor) prior to the first extinction day (no cocaine reinforcers). As a result, RGFP966-injected rats responded significantly less during extinction and reinstatement tests than vehicle-injected rats. These effects were not likely due to a performance deficit or a change in motivation to self-administer cocaine, as injections of RGFP966 had no effect on stable responding during a fixed or progressive ratio schedule of cocaine self-administration in subsequent studies. Results suggest that a systemic injection of RGFP966 enhanced extinction and suppressed reinstatement after cocaine self-administration by inhibiting HDAC3 activity. Future studies will determine whether brain region specific decreases in HDAC3 activity further suppress drug seeking.

30.

Winiecki PA, Lynch III JF, Ortiz S, Riccio DC, & Jasnow AM

GABAB(1a) Receptors May Be Necessary For The Consolidation and Retrieval of Precise Contextual Fear Memories ~ Anxiety disorders, such as PSTD, are characterized by a generalization of fear responses to neutral stimuli. To assess fear generalization, animals are trained in context fear conditioning, which involves pairing a context (conditioned stimulus) with several foot shocks (unconditioned stimulus) and then tested in the training context or a neutral context. Through this procedure, we have identified GABAB(1a) receptors as playing a role in the generalization of context fear memory. These receptors are involved in presynaptic inhibition at glutamatergic synapses, and GABAB(1a) knockout mice show generalized fear to a neutral context 24 hours after training, but not at 2 or 6 hours. The same pattern is observed with object location and recognition, suggesting that this receptor subtype is required for the maintenance, but not the consolidation, of a precise memory. To determine if GABAB(1a) receptors are involved in the consolidation and retrieval of precise context fear memories, the GABAB(1a) antagonist, CGP 36216, was infused at different time points. Pre-training infusions of CGP 36216 (2mM and 3mM) elicited fear generalization to the neutral context at a 24 hour retention test but not at 6 hours. Interestingly, pre-testing infusions (3mM) also elicited fear generalization to the neutral context. These data suggest that GABA-mediated presynaptic inhibition may be necessary for both the

consolidation and retrieval of precise context memories. Current experiments are examining the duration of the fear generalization produced by pre-training infusions.

Farris Family Foundation Award and a Whitehall Foundation Grant to AMJ

31.

Li X, Zeric T, Kambhampati S, Bossert JM & Shaham Y

A critical role of the central amygdala nucleus in incubation of methamphetamine craving ~ Background: Cue-induced methamphetamine seeking progressively increases after withdrawal. The mechanisms underlying this "incubation of methamphetamine craving" are unknown. Here we studied the role of central amygdala (CeA), ventral medial prefrontal cortex (vmPFC), and orbitofrontal cortex (OFC), brain regions previously implicated in incubation of cocaine and opiate (morphine or heroin) craving, in incubation of methamphetamine craving. We also assessed the role of basolateral amygdala (BLA) and dorsal medial prefrontal cortex (dmPFC). Methods: We trained rats to self-administer methamphetamine for 10 days (9 hr/day, 0.1 mg/kg/infusion) and tested them for cue-induced methamphetamine seeking under extinction conditions during early (2 days) or late withdrawal (4-5 weeks). We first confirmed that 'incubation of methamphetamine craving' occurs under our experimental conditions. Next, we assessed the effect of reversible inactivation of CeA or BLA by GABAA+GABAB receptor agonists (muscimol+baclofen, 0.03+0.3 nmol) on cue-induced methamphetamine seeking during early and late withdrawal. We also assessed the effect of muscimol+baclofen reversible inactivation of vmPFC, dmPFC, and OFC on 'incubated' cue-induced methamphetamine seeking during late withdrawal. Results: Lever-presses in the cue-induced methamphetamine extinction tests were higher during late withdrawal than during early withdrawal (incubation of methamphetamine craving). Muscimol+baclofen injections into CeA but not BLA decreased cue-induced methamphetamine seeking during late but not early withdrawal. Muscimol + baclofen injections into dmPFC, vmPFC or OFC during late withdrawal had no effect on incubated cue-induced methamphetamine seeking. Conclusions: Together with previous studies, our results suggest that the CeA plays a critical and unique role in incubation of both psychostimulant and opiate craving.

Acknowledgments: The work was supported by the Intramural Research Program of the National Institute on Drug Abuse.

32.

Ulmen AR, Burbules D, Vesia W, Jasnow AM, & Riccio DC

MK-801 in the Dorsal Hippocampus Causes State-Dependent Memory Reconsolidation for Passive-Avoidance ~ Memory reconsolidation refers to the concept that when a memory is reactivated or retrieved, that information becomes malleable and must undergo re-storage in order to be used again in the future. State-dependent memory is evident when there is a mismatch in the cues associated with a memory between learning or storage and retrieval. Previous work had found state-dependent memory for reconsolidation of passive-avoidance in adolescent rats using peripheral injection of the NMDA receptor antagonist MK-801 (Flint, Noble, & Ulmen, 2013). Here, we began to address whether the dorsal hippocampus mediates state-dependent reconsolidation of passive-avoidance using central administration of MK-801. In Experiment 1, rats received intracerebroventricular (icv) MK-801 into the left lateral ventricle. In Experiment 2, rats received bilateral dorsal hippocampal infusions of MK-801. All rats were trained in passive-avoidance and, 48 hours later, given a reactivation of the training via exposure to the passive avoidance chamber. Immediately following reactivation, animals were infused with either MK-801 or saline. Then, 48 hours after reactivation and 10 minutes prior to test, animals were infused with MK-801 or saline and tested for passive avoidance memory. Experiment 1 demonstrated that icv infusion of MK-801 produced state-dependent reconsolidation for passive-avoidance, suggesting central administration of MK-801 produces state-dependent memory deficits similar to what is observed with peripheral injections. Preliminary data for Experiment 2 suggest that the dorsal hippocampus may be playing a key role in the state-dependent reconsolidation of passive-avoidance.

33.

Ramsaran AI, Westbrook SR, & Stanton ME

Differential Ontogeny of Object, Object Location, Object-in-Context, and Object-Place-Context Recognition in the Rat ~ The novelty-preference paradigm (Ennaceur & Delacour, 1988) has become valuable for the neurobiological study of memory. The paradigm offers task variants that assess different combinations of incidental object, spatial, context, and temporal memory; variants that depend on the separate or combined

functions of the perirhinal cortex, hippocampus, and medial prefrontal cortex (e.g., Mumby et al., 2002; Barker et al., 2007; Langston & Wood, 2010). Not much is known about the ontogeny of memory system functions in this paradigm; therefore, we investigated neurobehavioral development using a group of novelty recognition task variants. In this series of experiments, we examined the ontogenetic profile of four incidental recognition tasks: the object recognition (OR) task, object location recognition (OL) task, the object-in-context (OiC) task, and object-place-context (OPC) task. We report that task performance in Long-Evans rats emerges before postnatal day (PD) 17 for the OR and OiC tasks, between PD17 and 21 for the OL task, and between PD26 and 31 for the OPC task. These findings raise the possibility that PD17 rats can learn and remember conjunctions of nonspatial cues whereas spatial variants are not learned until PD21-31. The findings also suggest that the separate roles of the perirhinal cortex, hippocampus, and medial prefrontal cortex in these tasks emerge earlier in ontogeny, whereas an interactive role of these structures appears to emerge later in ontogeny. This work highlights the versatility of novelty recognition tasks in studying the neural correlates of memory during development.
NIH grant R21-HD070662-01 to MES, UD Undergraduate Research Program, UD McNair Scholars Program

34.

Lynch III JF, Winiecki PA, Vanderhoof T, Riccio DC, & Jasnow AM

Estradiol increases fear generalization through activation of nuclear estrogen receptors and a genomic effect on fear memory retrieval ~ A learned fear response is context dependent in that the fear response is disrupted when testing occurs in a neutral context after a short interval (e.g. 24 hours). However, at a long delay, rodents display generalized fear when tested in the training context or in a neutral context. Generalization is a common symptom of many anxiety disorders and females are 60% more likely to suffer from an anxiety disorder than males. Therefore, one hypothesis for the large sex difference in anxiety disorder rates is that females exhibit higher rates of fear generalization than males. Indeed, our previous data demonstrated that female rats generalize learned fear to neutral contextual cues at a faster rate than males and this effect was mediated by estradiol interactions with memory retrieval mechanisms through activation of ERB. To determine what type of receptors —cytosolic versus membrane—mediated this effect, female rats were given infusions of ICI 182,780, an estrogen receptor antagonist, into the lateral ventricle simultaneously with estradiol treatment. Infusions of ICI blocked the generalization produced by estradiol, suggesting that estradiol acts through cytosolic receptors. In a complementary 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 did not result in generalized responding. These data suggest that estradiol-induced enhancements in fear generalization are mediated through activation of nuclear receptors. Future experiments are aimed to discover the specific mechanisms of estradiol action on fear generalization.

35.

Reis DS, Sehgal M, & Helmstetter FJ

Activity dependent proteolysis in the amygdala modulates protein synthesis in the amygdala and dorsal hippocampus during consolidation of fear conditioning ~ The requirement for protein synthesis in the consolidation of fear memories in the amygdala is well documented (Parsons, et al., 2006; Kwapis et al., 2011). Post-training infusions of the translation inhibitor anisomycin into the amygdala impair the formation of both auditory and contextual fear memory (Kwapis et al., 2011). Recent work from our lab has shown that protein degradation mediated by the ubiquitin-proteasome system (UPS) is also critically involved in the consolidation of auditory fear memories in the amygdala (Jarome et al., 2011). Some evidence suggests that UPS-mediated protein degradation may drive the requirement for de novo protein synthesis during the consolidation period (Ghosh et al., 2008; Jarome & Helmstetter, 2013). However, the specific relationship between synthesis and degradation remains unclear. Here, animals were trained with auditory fear conditioning and given immediate post-training intra-amygdala infusions of vehicle, the protein synthesis inhibitor anisomycin, or the proteasome inhibitor clasto-lactacystin-β-lactone. Using a modified version of the surface sensing of translation (SUnSET) assay (Schmidt et al., 2009), we measured the level of protein synthesis in the amygdala and dorsal hippocampus of each rat at 60 min after fear conditioning. Our results indicate that inhibition of UPS-mediated protein degradation in the amygdala significantly reduces the amount of protein synthesis. In addition, we found that inhibition of protein synthesis in the amygdala immediately after fear conditioning dramatically reduces the amount of global protein synthesis in the dorsal hippocampus as compared to the vehicle infused group. In fact,

this manipulation seems to reduce protein synthesis in the dorsal hippocampus back to basal levels, as seen in the untrained vehicle group. These results provide in vivo evidence of an interaction between UPS-mediated protein degradation and de novo protein synthesis in memory and support the idea that UPS-mediated protein degradation may be a primary regulatory mechanisms critical to the initial formation and consolidation of auditory fear memory. Finally, the finding that inhibition of protein synthesis in the amygdala impairs global protein synthesis in the dorsal hippocampus lends further support to the idea that the amygdala is a primary site of synaptic plasticity during fear conditioning and may regulate specific mechanisms of memory consolidation, like protein synthesis, in other supporting neural structures.

36.

Cullen PL, Ferrara, NC, & Helmstetter FJ

Using optogenetics to alter fear and molecular signaling within the amygdala ~ It is well accepted that the amygdala is required for the acquisition, consolidation, and retrieval of conditional fear memory. However, our present understanding of amygdala function comes primarily from pharmacological manipulations or lesions that either permanently damage the amygdala or render the amygdala inactive/impaired for an imprecise duration of time following drug administration. To understand the role of amygdala neurons during memory retrieval, amygdala activity was inhibited specifically during auditory CS presentations. We used virally-mediated expression of the light-driven proton pump ArchT (AAV9-CAG-ArchT-GFP), which when activated by light (523nm) inhibits neuronal firing. At 24-hr after delay fear conditioning animals expressing ArchT exhibited impaired CS freezing during the retrieval trial compared to animals expressing the control virus. This finding suggests that inhibiting activity of amygdala neurons only during brief CS presentations is sufficient to impair fear expression during the retrieval session. We also used the light-gated ion channel channelrhodopsin (AAV9-CaMKIIα-hChR2(H134R)-EYFP) to drive amygdala excitation. When placed in a novel context, excitation of amygdala neurons mirrors biochemical changes that occur during the memory consolidation period following fear conditioning. These results suggest that we are able to manipulate amygdala neurons with precise temporal control to mimic behavioral impairments associated with many pharmacological agents as well as protein changes that are associated with memory consolidation in the absence of fear conditioning itself.

NIMH R01 MH069558

37.

Pierson JL, Pullins SE, & Quinn JJ

Dorsal Hippocampus Infusions of CNQX into the Dentate Gyrus Disrupts Expression of Trace Fear Conditioning ~ The hippocampus is essential for the consolidation of some explicit long-term memories, including trace conditioning. Lesions and pharmacological manipulations of the dorsal hippocampus (DH) have provided strong evidence for its involvement in the acquisition and expression of trace fear memories. However, no studies have specifically targeted DH subregions (CA1 and dentate gyrus) to determine their involvement in trace fear conditioning. In the present study, rats received bilateral cannulation targeting either the CA1 or dentate gyrus of the DH. Following surgery, animals were trace fear conditioned. The next day, rats were tested for fear of the training context. Forty-eight hours following training, rats received bilateral infusions of the AMPA/kainate glutamate receptor antagonist, CNQX, or vehicle. Ten minutes following the start of the infusion, rats were placed in a novel context for the tone test. Rats that received CNQX into the dentate gyrus froze significantly less to the tone and during the trace interval compared to their vehicle counterparts. Rats that received CNQX into the CA1 subregion of the DH showed no difference in freezing during the tone or trace interval compared to their vehicle infused controls. These data support a role for the dentate gyrus in the expression of trace tone fear conditioning. This is different from the pattern previously observed for contextual fear, suggesting dissociable processes within the DH for trace and contextual fear.

This work was supported by R15 MH100689, and Miami University Summer Scholars Program.

38.

Pellman BA, Kim ES, Kashima J, Motch O, de la Iglesia HO, & Kim JJ

Rats living in a risky environment exhibit threat-entrained anticipatory circadian rhythms ~ Rodents with restricted feeding schedules show food-anticipatory activity organized around expected feeding times, which appears to depend on endogenous circadian oscillators distinct from those involved in the entrainment of light-

dark circadian rhythms. Here we show that male Long-Evans rats living in a risky closed economy environment —wherein the risk of unsignaled footshock is associated with the dark phase of the light cycle (12-h light/12-h dark)—exhibit rhythmic anticipatory activity prior to the onset of the safe light phase. Rats were continuously monitored in a semi-naturalistic live-in chamber comprised of a safe nesting area and a foraging area with a shock-grid floor, which the rats had to enter to procure food pellets. Initially, animals pressed a lever to procure food pellets (CRF) in the foraging area and entrained to the environmental conditions for at least 7 stable baseline days. Afterwards, animals were subjected to (i) 14 days of 'Unsignaled' footshocks (0.8 mA; pseudo-random 2 shocks/hr) which were delivered only during the dark phase of the LD cycle, and (ii) 14 days of 'Signaled' footshocks (a 9-sec light cue preceding the footshock) also during the dark cycle (counterbalanced). By the 14th day of unsignaled fooshock, rats showed robust anticipatory feeding and locomotor activity before the transition to the light phase. These effects persisted during the unsignaled-to-signaled fooshock transition. In contrast, during the baseline-to-signaled footshock days, rats quickly learned to avoid the fooshock and did not show anticipatory locomotor activity before the transition to the light phase. Lesioning the amygdala or the SCN prevented the anticipatory behavioral changes under the unsignaled shock conditions. Additionally, a separate group of rats exposed to the same baseline and unsignaled shock conditions, and then exposed to 10 days of constant dark lighting without the risk of shock, exhibited a free-running circadian rhythm of anticipatory activity. These results suggest a fear-entrainable oscillator, comprised of the amygdala and SCN, is necessary to anticipate the termination of danger.

Supported by R01 MH099073 and the University of Washington Royalty Research Fund (J.J.K.).

39.

Thrailkill EA, Todd TP, & Bouton, ME

Effects of CS duration, intertrial interval, and the I/T ratio on appetitive conditioning ~ Time accumulation accounts of conditioning suggest that acquisition of conditioned responding to a conditioned stimulus (CS) depends on a decision to respond that is based on a comparison of the rate of unconditioned stimulus (US) presentation in the CS compared to the rate of US presentation outside of the CS (i.e., in the intertrial interval; ITI) (e.g., Gallistel & Gibbon, 2000; Ward, Gallistel, Jensen, Richards, Fairhurst, & Balsam, 2012). The trial on which the animal decides to respond is proportional to the ratio of time accumulated in the ITI (US-CS interval; I) over the time accumulated in the CS (CS-US interval; T; or I/T ratio). Results of previous studies that examined the effects of I/T in appetitive conditioning of rat magazine behavior do not unequivocally support the notion that conditioned response acquisition depends on I/T. The present studies further analyzed the effects of I/T in the acquisition of appetitive conditioned responding in rats. In Experiment 1, three groups received an auditory CS, during which a food pellet US was delivered after an unpredictable, but proportional period of time. One group received a 10-s CS conditioned with a 120-s ITI, another a 60-s CS with a 120-s ITI, and a third group a 60-s CS with 720-s ITI. In Experiment 2, two groups received either a 10-s CS or a 60-s CS; within these groups ITI durations were manipulated to arrange I/T ratios of either 12 or 72. In Experiment 3, four groups received training with an I/T ratio of 12, but with CS durations of either 10, 27, 43, or 60 s. Overall, analyses of magazine entries, decision criteria, and change-points indicated that CS duration, and not I/T ratio, is a powerful variable in determining conditioning in appetitive conditioning. Analysis of behavior from videotape further suggests that CSs of different durations may evoke qualitatively different behaviors. The results challenge time-accumulation accounts of conditioning and learning that base predictions on I/T.

40.

Calub CC, Mootz JR, & Furtak SC

Medial Temporal Lobe Involvement in Fear Conditioning to a Complex Stimulus ~ The medial temporal lobes (MTLs), an area necessary for declarative memory, are composed of the hippocampal formation, the entorhinal cortex, the perirhinal cortex (PER), and the parahippocampal cortex (postrhinal cortex in rodents). Previous studies have shown that lesions to the PER impair fear conditioning to complex auditory cues, such as pre-recorded rat ultrasonic vocalizations (Lindquist, Jarrard, & Brown, 2004). In particular, the discontinuous nature of ultrasonic vocalizations was found to depend upon PER processing during fear conditioning to these stimuli (Kholodar-Smith, Allen, & Brown, 2008). The current experiment examined whether MTL lesions induced similar impairment in fear conditioning to complex stimuli in a visual modality, here the conditioned stimulus (CS) was a discontinuous light. In this study, Sprague-Dawley male rats were broken into two groups: Lesion

and Sham. The Lesion group underwent surgery and received intracranial injections of NMDA to produce bilateral excitotoxic lesions targeted at PER, a central MTL region, while the Sham group underwent a similar surgical procedure with the exception that no injection was made. Following recovery, all subjects were trained on a three-day fear conditioning paradigm. Day 1, Fear Acquisition, consisted of 5 presentations of the CS (discontinuous light) paired with an unconditioned stimulus (US), a foot shock. The subsequent two days consisted of a Context Test, the rat was placed back into the conditioning chamber with no CS or US presented, and a Light Test, the rat was placed into a new chamber (a context shift) and presented with the CS alone. The order of tests was counterbalanced across rats. The dependent measure was time spent freezing (no movement except that necessary for breathing). A significant effect of lesions on fear acquisition during conditioning to a discontinuous light was found. These results may lend insight into brain regions involved in how we learn to fear complex objects and stimuli in our environment.

CSUS SSIS/UEI Funding

41.

Pizzimenti CP, & Lattal, KM

The Effects of Prior Aversive Events on Intravenous Self Administration and Reinstatement in Rats ~ Even following long periods of abstinence individuals with anxiety disorders have high rates of relapse to drugs of abuse. Although many current models of relapse demonstrate effects of stress, most of these studies examine stressful experiences that occur in close temporal and physical (i.e., within the same context) proximity to the reinstatement test. Little is know 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 on drug-seeking for psychostimulants. In Experiment 1, presentation of a cue previously paired with shock failed to induce reinstatement of cocaine seeking; however, reinstatement was observed a priming injection of cocaine. In Experiment 2, animals were trained to self-administer intravenous methamphetamine, followed by extinction. They 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 3 the battery of shocks were 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. Taken together, these results suggest that a history of fear conditioning may escalate drug intake during acquisition, as well as induce greater rates of reinstatement to drug-related cues, and confer resistance against extinction following reinstatement.

42.

Sehgal M, Bula TS, Fettinger NB, & Moyer JR

Neural circuitry underlying extinction of trace fear conditioning ~ With an increasing proportion of the world population constituting the elderly, aging-related cognitive decline has an immense socio-economic impact. Such cognitive decline includes cognitive flexibility deficits whereby elderly individuals are impaired on learning that a previously learned rule is no longer valid. One example of cognitive flexibility is extinction learning where animals learn that a previously learned conditioned stimulus (CS) is no longer predictive of the unconditioned stimulus (US). Recently, our lab has demonstrated that aging rodents are impaired on extinction of trace fear conditioning (Kaczorowski et al., 2012). Extensive evidence suggests that extinction of delay fear conditioning relies on opposing activity within sub-regions of the medial prefrontal cortex, infralimbic (IL) and prelimbic cortex (PL). While PL activity promotes fear expression, IL activity promotes successful extinction. However, relatively little is known about the neural circuitry that underlies extinction of trace fear conditioning and how this circuit changes during the course of normal aging. In fact few, if any, studies have investigated how normal aging alters learning-related changes in neuronal activity within a distributed memory circuit. The current experiments aim to understand the neural circuitry that mediates extinction of trace fear conditioning and evaluate how this circuit changes during normal aging. Briefly, rats were randomly divided into four groups; naïve, unpaired, trace-fear conditioned (TRACE) or extinction (EXT) group. On day 1, TRACE and EXT group received 10 paired CS-US presentations (white noise CS, 1.3mA footshock, 30s trace interval). The unpaired group received unpaired presentations of 10 CS and US each. On days 2 and 3, rats in the EXT as well as

unpaired group received 10 CS presentations alone. On day 3, fear memory was tested in the TRACE, EXT, and unpaired group using 2 CS-alone presentations. After the CS-test, brains were removed and processed for immunohistochemistry to quantify the expression of a variety of signaling molecules, including the immediate early gene, Zif-268. Preliminary results from adult rats demonstrate that trace fear conditioning significantly increases the number of Zif-268 labeled neurons within PL. This effect was reversed by extinction. Surprisingly, similar results were obtained within IL –Zif-268 labeling was increased following trace fear conditioning but not extinction. Ongoing experiments (data not yet analyzed) are investigating changes in neuronal activation following trace fear conditioning and extinction in aging animals as well as neuronal activity changes in other brain regions that underlie extinction learning.

NIA, UW-MIlwaukee RGI

43.

Latsko M, Dulka B, Lynch, J, Mulvany J, & Jasnow AM

Resistance to Acute Social Defeat Results in Impaired Cued Fear Discrimination, but not Contextual Fear Discrimination ~ Understanding how individual responses to social stress can impact later cognition and emotional behavior is vital to understanding and treating patients with anxiety disorders, such as PTSD. Here, we use an acute social defeat procedure in order to observe individual differences to social stress, which results in two phenotypic responses based on subsequent social interaction. Mice are characterized as susceptible if they display reduced social interaction after experiencing social defeat or characterized as resistant if their social interaction is unaffected by defeat. Previously, we found that mice resistant to social defeat stress displayed significant impairments in extinction learning and retention. Because extinction learning involves the inhibition of responding to a previously excitatory cue (i.e. CS+), we examined the ability of defeated mice to discriminate between a CS+ and a CS- fear cue. In line with our prediction, resistant mice display increased freezing to the CS- cue following social stress, suggesting impaired cued fear discrimination. Importantly, this deficit is not observed if cue discrimination precedes social stress, suggesting that resistance to social defeat stress may alter coping mechanisms associated with inhibitory responding. Next, we examined the ability of defeated mice to discriminate between contexts. Animals were trained in context fear conditioning and tested in the training context or a neutral context at 1, 5, and 14 days after training. Contrary to expectations, both resistant and susceptible animals discriminated between the training and neutral context at all retention intervals, indicating that cued fear, but not context fear, is affected by social stress. These data suggest that social defeat may preferentially affect circuitry involved in cued fear discrimination.

44.

Kirry AJ, Doncheck EM, & Gilmartin MR

Optogenetic stimulation of prelimbic principal cells impairs the formation of trace fear memory ~ The prelimbic area (PL) of the medial prefrontal cortex is necessary for trace fear conditioning in which the conditional stimulus (CS) and unconditional stimulus (UCS) are separated by a 20-second empty trace interval. Units in PL exhibit sustained firing during the trace interval (Gilmartin & McEchron, 2005; Baeg et al., 2001), and we recently demonstrated that neuronal activity specifically during the CS-UCS interval is critical for linking the two events in memory (Gilmartin et al., 2013). These findings suggest that sustained firing in PL provides a bridging signal to link the CS and UCS and raises the possibility that enhancing this signal may accelerate or strengthen the CS-UCS association. We hypothesized that optogenetically stimulating PL neurons during the trace interval would enhance fear memory. Rats were injected with channelrhodopsin (ChR2; AAV9/Camkiia-ChR2(H134R)-eYFP) or inactive control virus (AAV9/Camkiia-eYFP) in PL principal cells and trained 10-14 days later. We found that optogenetic stimulation of the PL (20 Hz, 6 mW) during the trace interval impaired rather than enhanced fear memory, when tested in the absence of optical stimulation the next day. Following re-training, optical stimulation of PL during each CS of extinction training impaired fear expression but did not affect extinction memory. However, stimulation reduced post-shock freezing during acquisition. Given that the PL area of prefrontal cortex may be important for mediating learning based on shock expectancy, optical stimulation of PL may impair acquisition of fear conditioning by interfering with UCS detection or expectancy. To begin to address this possibility, we administered the opioid receptor antagonist naloxone (5 mg/kg, s.c.) prior to training with optical stimulation. While naloxone treatment enhanced freezing at test, it did not reverse the impairment in fear learning in ChR2 rats. Thus, stimulation of PL likely impairs

memory by disrupting the physiological bridging signal within PL or by non-selectively activating downstream targets.

45.

Keller SM, Schreiber WB, & Knox D

Sex differences in fear extinction retention deficits using an animal model of PTSD ~ Single prolonged stress (SPS) is an established animal model of post traumatic stress disorder (PTSD) in male rats. Male rats that have undergone the SPS procedure demonstrate an upregulation of glucocorticoid receptors (GRs) in the hippocampus as well as deficits in fear extinction retention; two symptoms that are observed in PTSD patients. However, it is unknown whether fear extinction retention deficits or hippocampal GR upregulation will manifest in SPS-exposed female rats. Addressing these questions is important, because females are twice as likely to develop PTSD after trauma exposure and GR functionality has been implicated in PTSD. In order to address these questions, we used the Pavlovian fear conditioned freezing paradigm to assess fear extinction retention capabilities in SPS-exposed male and female rats and assayed GR levels in the dorsal and ventral hippocampus. Behavioral results demonstrate that while male SPS-exposed rats show impaired fear extinction retention, female rats that underwent the SPS procedure show no differences in fear extinction retention as compared to control females. Thus, SPS does not generate fear extinction retention deficits in female rats. However, control female rats showed impaired extinction retention as compared to male controls. Behavioral results raise the possibility that sex differences in fear extinction retention could underlie higher prevalence rates of anxiety disorders in females. Western Blotting to assess hippocampal GR protein expression is ongoing.

46.

Tribble JE, Perusini JN, Zelikowsky M, & Fanselow MS

Effects of single- versus pair-housed rats on fear sensitization and baseline anxiety following acute traumatic stress ~ Post-traumatic stress disorder (PTSD) leads to a variety of anxiety and fear-related alterations in an individual's behavioral responding following exposure to a traumatic event. Many of these phenotypes include enhancement in baseline levels of anxiety, and sensitization to subsequent, mildly stressful conditions. We have developed a model called stress-enhanced fear learning (SEFL) to study these changes in rats. In order to test SEFL, single-housed animals are exposed to a 15-shock stressor, and then subsequently conditioned to a single shock in a novel context. Animals that have previously received the 15-shock stressor show enhanced levels of freezing, caused by nonassociative sensitization, following the single-trial fear conditioning. Recently, Zelikowsky et al reported that SEFL occurs in singly but not multiply housed mice. Here we show that when rats are pair housed during the SEFL protocol their baseline anxiety levels, as measured through pre-shock freezing in a novel context, decrease compared to their single-housed counterparts, while, unlike mice, the sensitization to the single-trial context persists. Analyzing fear sensitization and anxiety measures separately allows us to precisely study the effect of single- versus pair-housed rats before, during, and following exposure to an acute traumatic event. Studying the effect of group-housing on specific aspects of fear sensitization and anxiety separately is important for disentangling the many components of the complex PTSD phenotype.
R01MH062122

47.

Rajbhandari AK, Huang Y, Fanselow MS, & Waschek JA

Fear conditioning increases the number of PACAP neurons expressing cFos within the basolateral amygdala ~ Post-traumatic stress disorder (PTSD) has been conceptualized to involve inappropriate inhibitory control over fear after exposure to life-threatening traumatic experiences. Studies have linked a GPCR signaling cascade system, pituitary adenylate cyclase activating peptide (PACAP) and its receptor PAC1 to PTSD diagnosis and symptom severity, and have found that this system is involved in the neural circuitry that regulates fear responses. Thus, studying the involvement of the PACAP/PAC1 system in regulation of conditioned fear behaviors could help understand the neural mechanisms of PTSD and help develop better treatment strategies. The amygdala with its sub-divisions that include the basolateral (BLA) and the central nuclei (CeA) is a crucial part of the fear circuitry. BLA is especially important for fear acquisition, whereas CeA is important for fear expression. This study was designed to investigate whether acquisition of fear alters the levels of immediate

early gene, c-Fos within the PACAP-containing cells of the BLA after contextual fear. The experiments in this study were conducted in mice expressing EGFP in PACAP neurons. A group of PACAP-EGFP mice were placed in a context where they received 0.65mA, 1-second foot shock 4 minutes after being placed into the context. After 6 consecutive days of this fear acquisition phase during which freezing levels reached an asymptotic level, half of the mice were tested in a novel context to assess generalization of fear while the other half were left in the home cage as controls. Ninety minutes following the test in the novel context or home cage, all mice were perfused and their brains extracted. Using immunofluorescence procedures, positive c-Fos and EGFP immunolabeling were analyzed and quantified in PACAP-EGFP mouse brain sections containing the BLA. Preliminary cell counting analysis revealed an increase in the number of PACAP neurons expressing c-Fos in the test group than in the controls, indicating that contextual fear expression may have altered the activity of PACAP neurons within the BLA. These results indicate that the PACAP expressing neurons in the BLA may be involved in the regulation of fear and that targeting this system may be important for understanding the neural circuitry underlying fear dysregulation in anxiety disorders including PTSD. Future studies are needed to understand the specific role of the PACAP/PAC1 system within fear circuitry.
R21MH098506

48.

Hoffman AN, Rajbhandari AK, Tribble JE, Pennington ZT, Perusini JN, Waschek J, & Fanselow MS

Amygdala AMPA receptor subunit specificity underlying fear sensitization following acute traumatic stress ~ Defensive behavior in the presence of a threat is an adaptive and critical response that promotes organismal survival. Enhanced or inappropriate defensive responding in the absence of a threat may underlie physiological processes in anxiety-related disorders. We have developed a model of nonassociative fear sensitization, in which prior exposure to a 15 shock stressor enhances subsequent conditioning to a single shock in a different context. Recently, evidence from our lab suggests that stress induced persistent upregulation of the GluA1 subunit of the AMPA receptor in the basolateral amygdala (BLA) mediates the long term expression of this stress enhanced fear learning (SEFL). However, it is unknown whether the GluA2 subunit plays a role in SEFL induction. While a single infusion of GluA1 antisense oligonucleotide (ASO) in the BLA following the 15-shock stressor prevented fear sensitization, here were show GluA2 ASO does not affect SEFL induction. These data suggest that GluA1 AMPA receptor subunit specificity in the BLA mediates fear sensitization.

49.

Perusini JN, Meyer EM, Rau V, Avershal JA, Rajbhandari A, Hoffman A, Nocera, N, Condro MC, Waschek J, Spigelman I, & Fanselow MS

Mechanisms underlying the induction and expression of fear sensitization caused by acute traumatic stress ~ A single, emotionally traumatic event can have a lifelong impact on the psychological well-being of an organism, including triggering anxiety-related disorders like post-traumatic stress disorder (PTSD). We have developed a model to study such sensitized responding in rats, in which exposure to a 15-shock stressor nonassociatively enhances subsequent fear conditioning trained with only a single trial. Using this model, we show the necessary conditions for induction and expression of stress-enhanced fear learning (SEFL). Specifically, the collective findings show that corticosterone (CORT) acts on glucocorticoid receptors (GRs) in the basolateral amygdala (BLA) during the stressor to induce sensitized fear. SEFL expression is maintained long-term by CORT-dependent upregulation of the GluA1 subunit of the AMPA receptor (R) within the BLA. Furthermore, a single antisense oligonucleotide treatment directed at GluA1- containing AMPARs within the BLA restores normal fear responding, which is especially relevant for developing novel and potentially more effective treatments for PTSD.

50.

Kochli DE, Thompson EC, Fricke EA, Postle AF, Lash KD, Hagerty SL, & Quinn JJ

The basal and lateral amygdala nuclei are critical for trace and contextual fear conditioning ~ Numerous investigations have demonstrated amygdalar involvement in delay cued and contextual fear conditioning. However, much less is known about amygdala contributions to trace cued fear conditioning. Further, relatively little is known about the contributions of individual amygdalar nuclei to both delay and trace cued fear

conditioning. The present experiments assessed potential contributions of the basal and lateral amygdala nuclei to trace and delay fear conditioning using excitotoxic lesions and blockade of de novo protein synthesis. Rats were trained using a 10-trial trace, delay, or unpaired fear conditioning procedure. Pre-training excitotoxic lesions of the basolateral complex created by infusions of NMDA attenuated subsequent contextual, trace, and delay fear memory expression. In subsequent experiments, infusion of the protein synthesis inhibitor, cycloheximide, into the basal or lateral amygdala immediately following conditioning disrupted subsequent contextual and trace tone fear expression while having no effect on the expression of delay fear memories. These data suggest a clear role for the basal and lateral amygdala in trace fear conditioning. However, this pattern of results is inconsistent with existing evidence that the LA is critical for delay conditioning. Current studies are examining the strength and duration of training as potential explanatory factors for these discrepant results.
R15 MH100689 JJQ

52.

Beeman CL, Pullins SE, Hoogendoorn JJ, & Quinn JJ

Complete hippocampus lesions disrupt recent and remote trace fear memories regardless of the lesion-to-test interval ~ Systems memory consolidation asserts a time limited role for the hippocampus (HPC) in explicit memory retrieval. Lesions to the dorsal hippocampus (DH) or ventral hippocampus (VH) made one day following trace fear conditioning produce a deficit in fear memory expression, while those same lesions made thirty days following training do not. In addition, recent work has shown that lesions of either DH or VH made one day following training do not produce a deficit when testing is delayed for 30 days (i.e. long lesion-to-test interval). In the present experiments, NMDA-induced excitotoxic lesions of the complete HPC were made using three conditions; one day following training with testing upon recovery (1/recent), one day post-training with testing delayed for 30 days (1/remote) or 30 days post-training with testing occurring upon recovery (30/remote). There was a significant deficit in fear memory expression during the tone and trace interval for all lesioned animals compared to sham controls. The lesions produced similar deficits in simultaneously learned contextual fear expression. These results suggest that expression of trace fear memory remains dependent upon an intact HPC even at a remote timepoint.
R15 MH100689 (JJQ)

53.

Mahal A, & Cushman JD

Sex Differences in Hippocampal Dependent Tasks: Female dominance in Fear Conditioning, Novel Object Recognition, and Morris Water Maze ~ The hippocampus contributes to important memory functions such as fear learning, latent learning, and spatial learning. Because sex differences are often ignored in experimental analyses, it is important to investigate these effects on hippocampal dependent learning to study how males and females react differently to stressors and other stimuli. This will provide the scientific community with baseline measures of sex differences that may affect future experimental designs. Furthermore, this will influence translational research in a clinical setting by allowing physicians to better understand disease susceptibility and development, and formulate specific treatment options for each sex. Using equal numbers of male and female C57Bl6 mice, the present study investigated sex differences in learning and memory utilizing three highly defined experimental paradigms: auditory fear conditioning, novel object recognition, and Morris water maze. Previous studies have shown sex differences in all three tasks. Using a simplified three-day experimental paradigm for each respective investigation, we found that females acquire auditory fear faster than males, females explore a more similar novel object more than males, with testing order influencing this observation, and females outperform males in a spatial navigation task when a prominent and proximal landmark cue is available. The implications of these results may affect future experiments, as experimenters and clinicians should be aware of these significant findings in their research.
This work was conducted by undergraduates in Psych 111 Learning Lab using UCLA Behavioral Testing Core equipment and facilities

54.

Quinn JJ, Sugimoto C, Grainger LM, Kraus JR, Couse MR, Skipper RA, Kochli DE, & Oswald BB

Infant stress exposure persistently enhances amygdala-dependent learning ~ Stressful experiences early in life persistently impact subsequent physiological, cognitive and emotional responses. In cases of trauma, these early

experiences can result in anxiety disorders such as phobias and posttraumatic stress disorder (PTSD). We have shown that infant footshock stress exposure at postnatal day (PND)17 enhances subsequent fear conditioning during infancy, preadolescence, or adulthood. Thus the impact of the early life stress persists throughout development. There is some data to suggest that this enhancement in subsequent fear conditioning may result from hyperexcitability of the amygdala produced by the early life stress exposure. If so, early life stress exposure should enhance other types of learning that depend upon the amygdala such as Pavlovian approach. In the present experiments, we exposed infant rats to footshock stress at PND17. In adulthood, rats underwent Pavlovian approach conditioning in which a compound light stimulus was paired with sucrose availability. Rats that received early life footshock stress showed enhanced acquisition of magazine approach behavior that was blocked by pre-stress injections of minocycline, a drug that inhibits microglial activation – an early mediator of central immune signaling. Extinction of Pavlovian approach behavior appeared normal in stressed animals, with no effect of infant minocycline. Minocycline blocked the infant stress enhancement of adult fear learning as well. Together, these data suggest that a single stressful event during infancy persistently enhances both aversive and appetitive amygdala-dependent learning. Further, this stress enhancement of learning depends upon microglial activation during the early life stress event.

55.

Hong E-H, Lee J-H, & Choi J-S

Observational fear conditioning: Emotional contagion or Pavlovian conditioning to a social cue? ~ Social learning refers to a broad range of learning situations where new information is passed onto the observer in various ways. One such example is emotional contagion of fear or distress in which an observer experiences similar emotional state to what the partner animal expresses. Recently, social transmission of fear or empathic pain in mice gained much attention as they provide a model system to investigate human-like capacity such as empathy. However, an alternative account, a simple Pavlovian conditioning to a social cue as the conditioned stimulus (CS), is equally plausible but has not been tested in well-controlled experiments. Therefore, we tested whether the mice could acquire conditioned fear response (CR) without being directly exposed to footshock unconditioned stimulus (US) and whether the acquisition of the CR is due to emotional contagion or Pavlovian conditioning to a social cue. In Exp 1, mice (C57BL/6) were either pair-housed (P, n = 20) or single-housed (S, n = 22) for at least two weeks. Half of the mice in each group were designated as the "demonstrator (D)". They were subjected to fear conditioning (COND: 20 unsignaled footshocks, 1 mA, 2 s) on day 1. The other half, designated as “observer (O)” were placed in the adjoining chamber and allowed to observe D's reaction to the footshock (jumping and freezing). On day 2 (TEST-ALONE), O’s freezing was measured in the same chamber, but without D. On day 3, D and O's freezing was measured in the presence of the familiar partner (TEST-F, same as day 1) and on day 4, with an unfamiliar partner (TEST-UF). The test order was counterbalanced across days. The results showed that O displayed significant non-zero freezing during COND ($p < 0.05$) but no freezing during TEST-ALONE. Among Os, P showed significantly more freezing than S during TEST-F ($p < 0.05$). On the other hand, there was no significant difference between P and S during the TEST-UF. These data indicate that the presence of F is a necessary condition for O's freezing during the memory test, and without F, the context alone is not sufficient to elicit the fear CR. In addition, O's freezing during the test session was modulated by the housing history. In Exp. 2, to test whether the fear CR observed in O's in Exp. 1 was due to emotional contagion or simply fear conditioning to a social cue (partner mouse), we trained D alone on day 1 (COND), and tested D and O mice together on day 2 (TEST). D reliably showed a high level of freezing (mean = 54 %). O did not show any freezing during TEST despite the high level of freezing in D, which proves against emotional contagion. Taken together, the current study demonstrate that the mice could acquire fear CR while observing the other receiving the shock and that the presence of the familiar partner serve as an effective CS and elicit the CR only when the D and O share the housing history. Therefore, observational fear conditioning might be accounted for by a Pavlovian mechanism not by emotional contagion.

56.

Kimm SW, Kim DY, & Choi J-S

Amygdala modulates approach-avoidance but not reflexive withdrawal in a semi-naturalistic conflict situation ~ Studies of approach-avoidance conflict propose that amygdala plays an important role in effort-based

decision making and delayed discounting. However, little is known about the role of the amygdala in value evaluation under naturalistic threat. Using a predator-like robot, Choi and Kim (2010) have demonstrated that amygdala regulates defensive response in a highly threatening, life-or-death situation. The current study employed a modified predatory threat to induce more frequent approach attempts and to promote more dynamic interaction between the subject animal and predator robot. Rats with either bilateral lesions of the amygdala (AMG: n = 10) or sham lesion (SHAM: n = 5) were subjected to a foraging situation where a food pellet was available on sight but guarded by a stationary robot with a set of snapping claws (Lobsterbot). The activation speed of the claws was almost instant (110 ms/60°) and therefore exceeded any rat's reaction time. In fact, none of the rats were able to retrieve the food pellet. Following 5-days of Lobsterbot sessions, fear conditioning with a tone conditioned stimulus (CS: 2 kHz, 80dB, 10 s, 3 trials) and footshock unconditioned stimulus (US: 0.6 mA, 2 s), subsequent extinction (CS-only, 10 s, 30 trials) and retention (CS-only, 120 s) tests were administered. Freezing was measured as the conditioned response (CR). All rats in the lesion group showed significant damage in the central and basolateral nucleus of the amygdala. The mean and maximum durations, and number of approach attempts during Lobsterbot sessions were significantly greater in AMG than those in SHAM (ps < .05). In addition, AMG showed significantly greater dwelling time in the danger zone (3-cm from the claws) than SHAM during the early session (Day1 : +29.5%), which was reversed during the late session (Day5: -12.35%). Freezing during fear conditioning, extinction, and retention tests was significantly lower in AMG (ps < .01). Number of approaches showed significant negative correlations with the level of freezing during the conditioning (r = -.70), extinction (r = -.77) and retention(r=-.72). Taken together, the current results suggest that the amygdala regulates approach-avoidance response in a conflict situation, perhaps by suppressing the approach response without compromising the reflexive withdrawal response. It seems that the regulation of approach-avoidance is mediated by a common brain circuitry that also produces fear CR in Pavlovian conditioning. Interestingly, lesions of the amygdala restored the animal's ability to assess the value outcome in the threatening situation where the sham animals continued to re-visit the same danger zone despite their repeated failure to retrieve the food pellet.

57.

Jie HS, Lee BN, Geiller T, Royer S, & Choi J-S

Modality and spatial configuration of the sensory stimulus modulate avoidance learning in a head-fixed preparation ~ The degree of prepared association between a stimulus and the required response is referred to as the stimulus-response compatibility (S-RC). Although S-RC has been investigated frequently in human behavioral experiments since the discovery by Fitts and Seeger (1953), little is known about the underlying brain mechanisms, due to the lack of adequate animal models for invasive investigation. Only a handful of neurophysiological studies were conducted in monkeys (Sato and Schall, 2003). As a first step to look into the neurophysiological correlates of S-RC in emotional behavior, we developed a head-fixed mouse preparation to precisely manipulate spatial parameters of the auditory and visual stimuli. Using a treadmill apparatus in which a head-fixed mouse could walk freely, we tested whether sensory modality of the stimulus and its projecting direction would modulate avoidance learning. We also tested whether the type of avoidance response (Go vs. Stop) would be differentially modulated by the stimulus condition. In Exp 1, light (blue LED) or tone conditioned stimulus (CS: 95 dB, 1 kHz) was presented to a mouse (C57BL/6N, male), head-fixed and allowed to walk freely on a low-friction treadmill. Following three days of treadmill adaptation, the initial reaction to the CS was tested by presenting three CS-only trials. The training was composed of two sessions of 30 trials. During the training session, the mouse could avoid the air puff unconditioned stimulus (US: 4.6 L/min via 5-mm nozzle) by walking forward or stopping to the CS, depending on the paradigm (Go or Stop, respectively). The CS was presented for 15 s and the US was presented for the last 5 s of the CS or until the avoidance response was made, whichever comes first. Finally, their avoidance response was tested again by presenting three CS-only trials. The latency of Go response to the CS during the pre-training period was shorter for the light group than for the tone group (n = 15, p < .01). Overall, a higher rate of avoidance response (Go) was maintained for the light group during the training sessions (not statistically significant). For the Stop paradigm, again the latency of the Go response to the CS was shorter for the light group (n =14, p < .05). However, overall rate of avoidance response (Stop) was similar for both groups. In Exp 2, we manipulated the spatial location of the visual CS, dividing it into three conditions: TOP, SIDE, FRONT. The visual CS was flashed from above toward the mouse (TOP), from the front toward the side wall (SIDE), or from the front toward the mouse (FRONT). The Go paradigm

used was identical to that used in Exp 1, except for two additional training sessions with different CS locations. The avoidance rate of SIDE was significantly higher than FRONT ($n = 12$, $p < .06$) or than TOP ($n = 12$, $p < .05$). However, the rate of FRONT was not significantly higher than TOP. In Exp 3, we manipulated the spatial location of the tone CS in a similar manner. A small speaker was positioned to play the CS from above (TOP), from the side (SIDE), or from the back (BACK) of the mouse. The training schedule was identical to Exp 2. The avoidance rate of SIDE was significantly higher than TOP ($n = 10$, $p < .06$) or than BACK ($n = 7$, $p < .05$). However, the rate of BACK was not significantly higher than TOP. These results suggest that the visual stimulus might be more compatible with a flight-related response than the auditory stimulus. Furthermore, these modality-specific response patterns differentially contribute to avoidance learning, particularly with Go response. In addition, spatial configuration of stimuli, regardless of modality, modulate avoidance learning in Go paradigm.