

2013

Annual Pavlovian Society Meeting

Hilton Garden Inn • Austin, TX



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Special thanks to Michael Drew, PhD and the University of Texas
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Annual Pavlovian Society Meeting • Sept 26-29, 2013

Hilton Garden Inn Downtown/Convention • Austin, TX



Thursday (Sept 26) • *Eighteenth Over Austin (18th floor)*

6:00-10:00PM Welcome Reception • Cash Bar

Friday (Sept 27) • *Sabine and Rio Grande Rooms*

7:30-8:25AM *Breakfast*

8:25-8:30AM *Welcome -- Steve Maren (TAMU)*

8:30-9:30AM *Address -- Fred Westbrook (UNSW) • "Making safety signals safer"*

9:30-10:00AM *Talk -- Michael Fanselow (UCLA) • "Aberrant generalization of auditory fear results from an interaction between mature and immature dentate granule cells"*

10:00-10:20AM *Coffee Break*

10:20-12:00PM *Symposium • Remote Memory (Paul Frankland)*

10:20-10:45 **Paul Frankland** (Toronto) • "Identification of a functional connectome for long-term fear memory"

10:45-11:10 **Brian Wiltgen** (UC Davis) • "Reactivation of hippocampal and neocortical networks during consolidation"

11:10-11:35 **Dave Bucci** (Dartmouth) • "Learning, memory, and plasticity in retro-hippocampal circuits"

11:35-12:00 **Kevin Corcoran** (NWU) • "How do memories of remote stressful events become less distressful: A role of cortical molecular mechanisms"

12:00-1:30PM *Lunch [For EC members: Lunch in Creekside room, 1st floor executive center]*

1:30-3:10PM *Symposium • Prediction Error (Gavan McNally)*

1:30-1:55 **Josh Johansen** (RIKEN) • "Teaching the amygdala to fear: a role for multiple learning rules"

1:55-2:20 **Mihela Iordanova** (NIDA) • "Amygdala central nucleus activity during extinction of reward"

2:20-2:45 **Vincent Laurent** (Sydney) • "Pavlovian predictive learning induces translocation of striatal delta-opioid receptors to guide future choice between actions"

2:45-3:10 **Ki Goosens** (MIT) • "Not so great expectations: how stress and uncertainty drive fear"

3:10-3:30PM *Coffee Break*

3:30-4:00PM *Talk -- Greg Quirk (UPR) • Moving from Pavlovian fear conditioning to avoidance: prefrontal-striatal-amygdala circuits*

4:00-5:00PM *Address -- Shona Chattarji (Bangalore) • "Neuronal encoding of the transition from specific to generalized fear in the amygdala"*

5:00-5:30PM *Coffee Break*

5:30-7:30PM *Poster Session (Snacks and Cash Bar) • Travis and Shoal Creek Rooms*

Saturday (Sept 28) • Sabine and Rio Grande Rooms

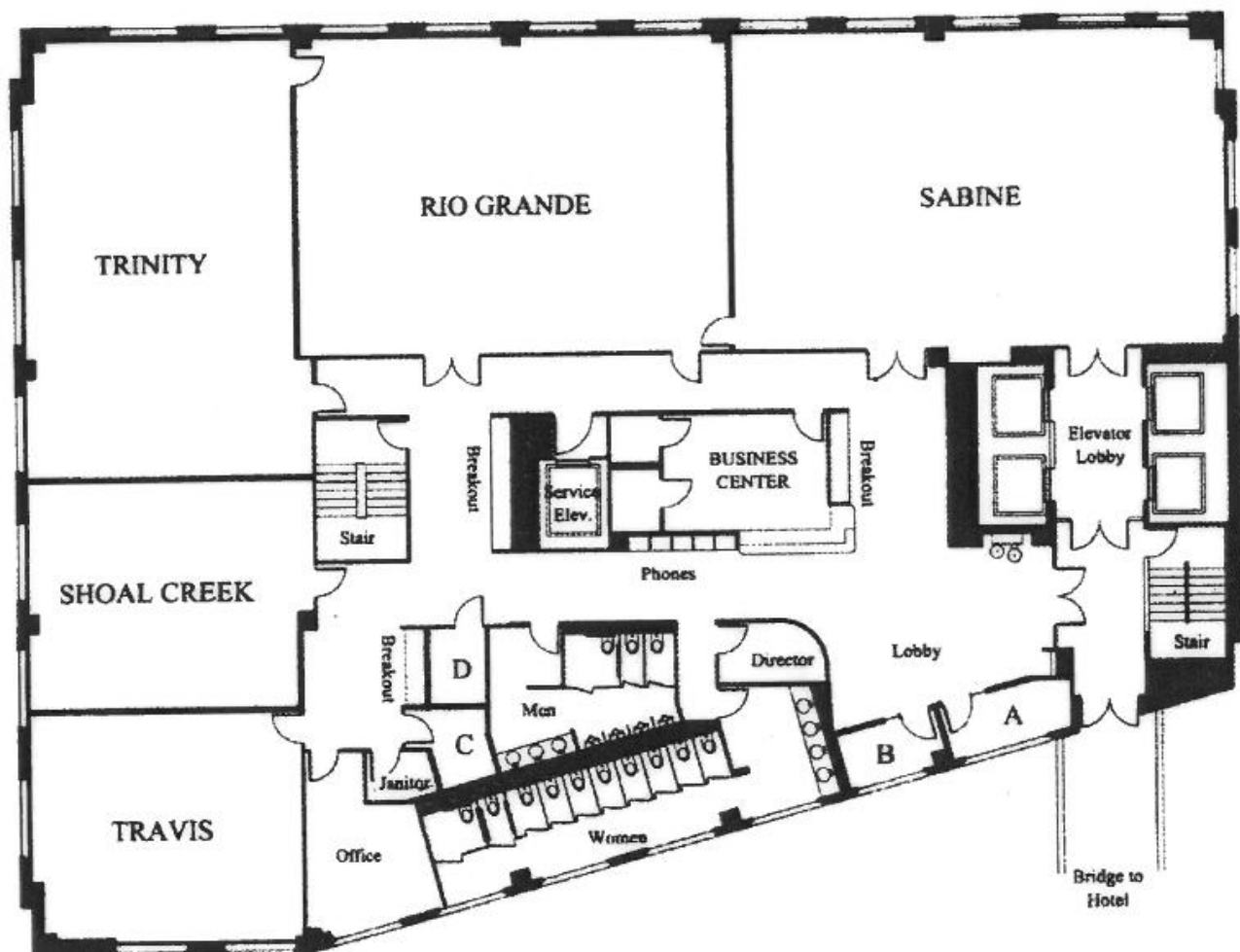
- 7:30-8:25AM *Breakfast*
- 8:25-8:30AM *Welcome -- Steve Maren -- TAMU*
- 8:30-9:30AM *Address -- Jack Byrne (UTHSC-Houston) • “Computational design of enhanced learning protocols”*
- 9:30-10:00AM *Talk -- Jim Grau (TAMU) • “Learning within the spinal cord: A sense of time and memory”*
- 10:00-10:20AM *Coffee break*
- 10:20-12:00PM *Symposium • Learning and Feeding (Gorica Petrovich)*
- 10:20-10:45 **Gorica Petrovich** (BC) • “Forebrain networks and the control of feeding by learned cues”
- 10:45-11:10 **Joanne Lee** (U Texas) • “Conditioned orienting to an appetitive cue: Behavioral phenotypes in males and females”
- 11:10-11:35 **Alexander Johnson** (MSU) • “Neuropeptide modulation of cue-potentiated feeding”
- 11:35-12:00 **John O’Doherty** (Cal Tech) • “The role of the human amygdala in model-based learning and Pavlovian to instrumental transfer: evidence from high resolution fMRI”
- 12:00-1:30PM *Lunch [Satellite activity: Women in Learning Lunch]*
- 1:30-3:10PM *Symposium • Memory Modulation (Tom Gould)*
- 1:30-1:55 **Tom Gould** (Temple) • “Novel cellular mechanisms of nicotine’s effects on learning and memory”
- 1:55-2:20 **Martin Sarter** (Michigan) • “Modes and models of forebrain cholinergic mediation in cognition”
- 2:20-2:45 **Matt Lattal** (OHSU) • “Modulation of extinction by dopamine D1 receptors”
- 2:45-3:10 **Melissa Mahgoub** (UTSW) • “HDACs in learning and memory”
- 3:10-3:30PM *Coffee Break*
- 3:30-4:00PM *Talk -- Tracey Shors (Rutgers) • “Mind does matter: mental and physical (MAP) skill training keeps new neurons alive”*
- 4:00-5:00PM *Women in Learning Address • Cathy Rankin (UBC) • “Insights into the complexity of the simplest form of learning”*
- 5:00-5:30PM *Coffee Break*
- 5:30-7:30PM *Poster Session (Snacks and Cash Bar) • Travis and Shoal Creek Rooms*
- 7:30-9:00PM *Banquet • Eighteenth over Austin (18th floor)*
- Terry Robinson** (Michigan) • “Resisting temptation: Individual variation in the motivational properties of Pavlovian reward cues”

Hilton Garden Inn Austin, Executive Center Meeting Rooms:

Talks will be held in *Sabine-Rio Grande*

Posters will be in *Travis* (Posters 1-35) and *Shoal Creek* (Posters 36-70)

Welcome reception and Saturday banquet will be in the *Eighteenth Over Austin* (18th floor restaurant)



Speaker Abstracts:

Westbrook, R. F. (University of New South Wales) • **How to make safe stimuli safer: the role of error correction mechanisms in extinction (and latent inhibition) of conditioned fear** • Theories of associative learning that rely on error correction mechanisms have been successful in explaining and predicting a range of Pavlovian acquisition phenomena. Such theories hold that these mechanisms regulate associative change in extinction and predict conditions that enhance this change. For example, they predict that additional extinction of a conditioned stimulus (CS) exhibiting recovery will enhance associative change and deepen response loss. Several experiments restored fear responding to an extinguished CS by allowing time to lapse (spontaneous recovery), presenting an unconditioned stimulus (US), shifting contexts (renewal), and compounding the extinguished target with an extinguished or a non-extinguished CS. These experiments showed that additional extinction of a CS exhibiting restoration enhanced associative change, that this change was regulated by both common and individual error terms, and that the change could be a simple increase or a conversion of the extinguished CS into a net inhibitor. Moreover, additional extinction of a CS exhibiting any one type of restoration (e.g., reinstatement) was indexed by a reduction in another (e.g., renewal). We have recently shown similar results with latent inhibition of conditioned fear and provided evidence that the endogenous opioid system and its receptors codes for the error that regulates associative change across CS pre-exposure. Extinction of fear is a goal of exposure-based treatments of anxiety disorders and latent inhibition is attenuated in schizophrenics. The present results suggest ways in which this treatment can be improved and raise the possibility that error correction mechanisms may be impaired in schizophrenics.

Fanselow, M. S. (UCLA) • **Aberrant generalization of auditory fear results from an interaction between mature and immature dentate granule cells** • Expression of fear in safe situations is a hallmark of anxiety disorders. Overgeneralization of learned fear to untrained stimuli is one source of maladaptive fear. Rats and mice that received pairing of one auditory stimulus (either white noise, a low, or a high frequency tone) with shock and then tested with one of these three auditory stimuli show patterns of generalization that vary considerably with the particular combination of trained and tested stimuli. These patterns do not conform to any linear dimension (e.g., frequency) nor do they fit current associative models of generalization processes. While hippocampal manipulations typically do not affect fear of a trained auditory stimulus they do influence auditory generalization. For example, lesions of the dorsal hippocampus reduce generalization but do not affect fear to the trained stimulus. Additionally, elimination of all post-natal neurogenesis in the dentate gyrus increases generalization. To clarify the mechanisms of this aberrant auditory generalization we combined a triple transgenic mouse that allows us to temporarily block neurotransmitter release from mature granule cells, with focal irradiation that allowed us to eliminate immature neurons. Under conditions of severe generalization, loss of immature neurons eliminated generalization. However, generalization could be partially restored by simultaneously silencing mature neurons. Under conditions of little generalization, silencing either mature or immature neurons, but not both, increased generalization. The data suggest that rather than acting independently, immature and mature granule cells regulate auditory fear generalization by interacting, presumably as a local microcircuit, within the dentate gyrus.

Frankland, P. (Toronto) • **Identification of a functional connectome for long-term fear memory** • While the hippocampus may play an essential role in the expression of memories soon after encoding, expression of the same (or at least equivalent) memory may become independent of the hippocampus at later time points. One predominant view is that the transition of the memory from a hippocampus-dependent to hippocampus-independent form reflects a time-dependent process of *reorganization*, leading to the permanent storage of the memory in cortical networks. Our lab uses molecular, behavioral and graph theoretical approaches to understand this consolidation process, and, in my talk, I will highlight our new studies aimed at 1) identifying the broad network of cortical regions supporting remote contextual fear memories, and 2) understanding how the structure of this distributed network impacts its function.

Wiltgen, B. (UC-Davis) • **Reactivation of hippocampal and neocortical networks during consolidation** • The hippocampus is thought to reactivate neocortical circuits during memory retrieval. This idea is widely accepted in psychology and neuroscience, although there is little direct evidence to support it. To address this issue, we used transgenic mice to tag cortical neurons that were active during context fear conditioning. We then examined reactivation of these same neurons when the hippocampus was intact or inactivated during memory retrieval. We found that hippocampus inactivation reduced the degree of reactivation in the anterior cingulate cortex and the retrosplenial cortex. Overall activity levels in these regions were not affected, which suggests that the role of the hippocampus is to activate specific cells during memory retrieval. In control mice, we observed strongly correlated activity in the neocortex during memory retrieval. These correlations were completely eliminated by hippocampus inactivation. These results provide the first direct evidence that reactivation of neocortical neurons during memory retrieval requires the hippocampus.

Corcoran, K.A., Leaderbrand, K., Radulovic, J. (Northwestern University) • **Extinction of remotely acquired fear depends on an inhibitory NR2B/PKA pathway in the retrosplenial cortex** • As memories age, their processing increasingly relies upon cortical rather than hippocampal circuits, but the adaptive significance and mechanisms of this shift are not fully understood. Here we investigated the behavioral features and cortical mechanisms underlying extinction of remotely acquired context fear. N-methyl-D-aspartate receptors (NMDAR) in the retrosplenial cortex (RSC) are required for the retrieval of fear-provoking context memory, which in turn is necessary for fear extinction. We therefore focused on the role of these receptors and their associated signaling pathways in the extinction of remotely versus recently acquired fear. Behavioral experiments consisted of contextual fear conditioning followed by extinction, reinstatement, and re-extinction. We demonstrated that remote versus recent fear extinction was more effective, as indicated by increased savings of the extinction memory after reinstatement. At a molecular level, antagonism of NR2B subunit-containing NMDAR selectively blocked remote extinction and prevented the extinction-related downregulation of cyclic AMP response element binding protein phosphorylation (pCREB). Pharmacological manipulations of cyclic AMP-dependent protein kinase (PKA), demonstrated that activity of this kinase supports retrieval and prevents extinction of remote, but not recent, fear. Interestingly, NR2B/PKA interactions weakened independently of the age of the memory, however the functional significance of this molecular change was evident only as retrieval became PKA-dependent with memory age. Thus, cortical PKA signaling may provide a molecular signature of when a memory has become “remote,” and inhibition of this pathway may open the door for modulation of remote memories. This work was supported by National Institute of Mental Health Grants MH073669, MH078064, and Dunbar Funds to JR and K12GM088020 to KAC.

Laurent, V. (University of Sydney) • **Pavlovian learning induces translocation of striatal delta-opioid receptors to guide future choice between actions** • Pavlovian conditioning imbues a stimulus with the fundamental ability to guide future actions. For instance, a stimulus conditioned to predict a particular outcome biases choice towards an action trained to earn that same outcome. It is well established that this outcome-specific Pavlovian-to-instrumental transfer (PIT) requires activity in the nucleus accumbens shell (Nac-Sh), and recent evidence suggests that local δ -opioid receptors (DOR) play an essential role. Using knock-in mice expressing functional fluorescent DOR in place of native receptors, the present experiments aimed to investigate the relationships between DOR-related processes in the Nac-Sh and the influence of predictive learning on choice between actions. These experiments revealed that learning specific stimulus-outcome associations, which are necessary for PIT, increased DOR expression on the somatic plasma membrane of cholinergic interneurons (CINs) in the Nac-Sh. This DOR accumulation positively correlated with the conditioned responses elicited by the Pavlovian stimuli and the degree to which these stimuli subsequently biased choice during the transfer test. Further, the change in DOR expression altered the firing pattern of CINs, increasing the variance of action potential activity, an effect that was potentiated by a DOR agonist. Finally, systemic blockade of DOR or specific lesion of Nac-Sh CINs was found to abolish specific PIT. Thus, these findings reveal a long-term and Pavlovian learning-dependent plasticity in opioid receptor expression on striatal modulatory interneurons that plays a critical role in decision-making processes.

Johansen, J. (RIKEN) • **Teaching the amygdala to fear: a role for multiple learning rules** • 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 memory formation, aversive experiences activate neural 'teaching signal' circuits which engage plasticity in brain regions involved in learning and memory and these signals may regulate memory strength. 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 (LA). Using a combined optogenetic, behavioral and physiological approach, we examined the learning rules and neural mechanisms which control the strength of fear memories. We found that prediction error coding in LA neurons sets the maximal strength of fear memories at a certain aversive US intensity (i.e. learning asymptote). However, we also found that fear memory strength can be reduced (contingency degradation) even after learning has occurred and show that Bayesian models explain this phenomenon better than cue competition (such as prediction error) based algorithms. Interestingly, while different learning rules can be utilized for setting learning asymptotes and degrading contingency, aversive US activation of LA neurons can either increase (learning asymptote) or decrease (contingency degradation) fear memory strength depending on the timing of the aversive US relative to predictive cues in the environment. Together these data suggest that multiple learning rules are employed to dynamically control fear memory strength and reveal that temporally specific aversive activation of LA neurons can bidirectionally regulate fear learning.

Iordanova, M., Deroche, M.L.D., Esber, G. R., Sadacca, B. & Schoenbaum, G. (NIH) • **Neuronal responses of central nucleus of the amygdala during extinction and overexpectation** • The firing pattern of neurons in the central nucleus of the amygdala (CeA) was examined during learning when lower than expected outcomes are delivered in extinction and overexpectation. Rats were trained to expect a reward following the presentation of each of three auditory cues and one visual cue. Subsequently, one of the auditory cues was paired with the visual cue to generate the expectation of double reward, yet only a single reward was delivered, yielding the overexpectation condition. In addition, the other two cues were presented in compound with a second visual cue, which was never reinforced. Each one of those compounds resulted in the delivery of either a single or no reward, yielding the control and extinction conditions. Neural firing in the CeA was found to track the expectation of reward such that the overexpectation compound yielded a higher neural response compared to the control. In addition, CeA neurons tracked the changes in reinforcement contingencies: the greater level of neural responding to the overexpectation compound at the start of compound training reached that of the control compound across training. Similarly, neural firing to the Extinction compound was similar at the start of extinction training but declined across training. These results suggest a role for the CeA in regulating learning under conditions when actual rewards are lower than expected rewards.

Byrne, J. (UTHSCH) • **Computational design of enhanced learning protocols** • Learning and memory are influenced by the temporal pattern of training stimuli. Multiple learning trials spaced over time generally produce long-term memory (LTM) more effectively than a single trial or multiple trials massed together. However, virtually all of the learning protocols and their neuronal analogues used in animal and human studies have been developed on an *ad hoc* basis. The optimal procedure or spacing of trials is not predicted by any learning theory. The hypothesis that the efficacy of a protocol is determined, in part, by interactions among biochemical cascades that underlie learning and memory was examined. Previous studies suggest that the PKA and ERK cascades are necessary to induce long-term synaptic facilitation (LTF) in *Aplysia*, a neuronal correlate of (LTM). A computational model of the PKA and ERK cascades was developed, and used to identify a training protocol that maximized PKA/ERK interactions. The predicted protocol had non-uniform interstimulus intervals (ISIs), which is in marked contrast to the fixed intervals that are generally used in experimental psychology and in previous studies of LTF and LTM in *Aplysia*. The novel training protocol induced significantly greater LTF in sensorimotor cultures; significantly increased long-term excitability (LTE), a second correlate of LTM; significantly increased the levels of phosphorylation of CREB1, a transcription factor essential for LTF; and significantly improved LTM following behavioral training. Similar computational approaches were used to design a training protocol to rescue a deficit in LTF produced by a siRNA-induced knockdown of CREB-binding protein (CBP) in a cellular model of Rubinstein-Taybi syndrome. The results

demonstrate the feasibility of using computational models of biochemical signaling to design training protocols that enhance normal LTM as well as deficits in LTM associated with cognitive disorders.

Petrovich, G. (Boston College) • **Forebrain networks and the control of feeding by learned cues** •

Learning plays an important role in the control of food motivation and consumption. Associative learning and related anticipatory mechanisms facilitate homeostatic regulation of food intake and body weight. Associative processes can also enable cues from the environment to control appetite and eating independent of hunger. Such cues effectively rival physiological signals to stimulate eating despite satiety, or to inhibit eating despite hunger, and if persistent could lead to long-term dysregulation and ultimately to eating disorders. Nevertheless, how learned environmental cues are integrated with physiological signals and the underlying brain substrates are not well known. This talk will provide an overview in recent advances in deciphering the critical brain systems in two rodent models that use Pavlovian conditioning to control feeding. In one preparation conditioned cues for food stimulate feeding in sated rats (cue-induced feeding), while in another preparation conditioned cues that signal footshocks (fear-cues) inhibit feeding in hungry rats. Four key components of the forebrain network that supports cue-induced feeding have been identified thus far. These are telencephalic areas critical for associative learning, memory encoding, and decision making, the basolateral amygdala, hippocampus and medial prefrontal cortex and the lateral hypothalamus, which functions to integrate feeding, reward, and motivation. This network also involves orexigenic peptides, ghrelin and orexin. A parallel circuitry that includes the central nucleus of the amygdala supports fear-cue mediated cessation of feeding. These findings in animals are relevant to human appetite and eating, particularly regarding maladaptive environmental mechanisms that contribute to overeating and anorexia.

Johnson, A.W. (Michigan State University) • **Neuropeptide modulation of cue-potentiated feeding** •

Initially meaningless stimuli such as sights, sounds and smells can acquire new cognitive, affective and behavioral functions when they occur in predictive relations with food. The influence of food-paired cues on food preference and overeating has been studied in laboratory rodents using cue-potentiated feeding. Due to its expression in central circuitry critical for cue-potentiated feeding (i.e., lateral hypothalamus, basolateral amygdala, prefrontal cortex) we investigated the orexigenic neuropeptide, Melanin Concentrating Hormone (MCH). Mice were trained to associate one cue (CS+) with delivery of a sucrose solution and a second cue with no reward (CS-). On completion of training, mice were placed on free access to food for several days, which resulted in significant weight gain. Mice were then tested for sucrose consumption during CS+ and CS- under these sated conditions, where it was noted that the overeating to the CS+ in control mice was due in part to an increase in the hedonic taste features of the sucrose reward. Notably, deletion of the MCH receptor, or pharmacological MCH receptor inactivation targeted at the basolateral amygdala, selectively disrupted overeating and the increase in taste hedonics of sucrose during the CS+. Furthermore, diet-induced obese mice tested for cue-potentiated feeding showed an augmented feeding response to the CS+ cue, which was disrupted following pharmacological inactivation. These results suggest a key role for MCH in cue-potentiated feeding.

O'Doherty, J. P. (Caltech) • **The role of the human amygdala in model-based inference during Pavlovian conditioning** •

Contemporary computational accounts of instrumental conditioning have emphasized a role for a "model-based" system in which values are computed with reference to a rich model of the structure of the world, and a model-free system in which values are updated without encoding such structure. Much less studied is the possibility of a similar distinction operating at the level of Pavlovian conditioning. Here I will present findings from a high resolution fMRI protocol during which participants were scanned as they participated in a Pavlovian conditioning task with a simple structure, while measuring activity in the human amygdala. After fitting a model-based algorithm and a variety of model-free algorithms to the fMRI data, we found evidence for the superiority of a model-based algorithm in accounting for activity in the amygdala compared to the model-free counterparts. These findings support an important role for model-based algorithms in describing the processes underpinning Pavlovian conditioning, as well as providing evidence of a role for the human amygdala in model-based inference.

Gould, T. (Temple University) • **Novel cellular mechanisms of nicotine's effects on learning and memory** • Nicotine enhances hippocampus-dependent learning. This presentation examines whether nicotine enhances learning through modulating signaling cascades normally involved in learning or through recruitment of additional cell signaling cascades. Data will be presented that suggests that nicotine enhances hippocampus-dependent learning by modulating cell signaling cascades in the dorsal hippocampus normally involved in learning but changes the temporal pattern of activation of these cascades resulting in the activation of additional cell signaling cascades. Specifically, during contextual fear conditioning nicotine shifts the activation of PKA and ERK1/2 and activates JNK1. JNK1 is not essential for contextual fear conditioning but is essential for nicotine enhancement of learning. Data will also be presented that suggests that while JNK1 is not required for contextual fear conditioning, it is involved in strengthening learning when overtraining occurs. Together these data aid in understanding learning and nicotine effects on learning by suggesting that the strength of training may influence the cell signaling cascades involved in learning and that nicotine may enhance learning by activating cell signaling cascades that are activated during training that results in stronger long-term memories.

Sarter, M. (University of Michigan) • **Modes and models of forebrain cholinergic mediation of cognition** • Attentional processes influence the efficacy of learning and the former are subject to top-down control based on memory. The cortical cholinergic input system mediates two separate aspects of attention. First, a neuromodulatory component, fluctuating on a scale of tens of seconds to minutes, influences the efficacy of cortical circuitry mediating the detection of cues (detection is defined as a cognitive process yielding a response indicating the presence of a cue). Levels of cholinergic neuromodulatory are regulated top-down by cortico-mesolimbic-basal forebrain circuitry and they modulate cortical circuitry primarily via stimulation of nAChRs expressed on the terminals of thalamic afferents. The level of this cholinergic neuromodulation is a function of the top-down demands on attention but not of levels of attentional performance. Second, cue-evoked, second-based cholinergic release events ("transients") in the cortex are generated by cortical circuitry and via heteroreceptors expressed on cholinergic terminals. These transients are necessary for the detection of cues in trials that also involve a shift from a state of monitoring for cues to cue-directed behavior ("up-shift"). Converging evidence from electrochemical studies in animals and fMRI studies in humans supported the significance of the cognitive operations attributed to these transients. Results from optogenetic studies confirmed that these cholinergic transients control cue detection, in part by demonstrating that generating cholinergic transients during non-cued trials evokes false alarms. Finally, our circuitry model describing cholinergic neuromodulation of cortical glutamatergic-cholinergic transient interactions predicted the finding that stimulation of $\alpha 4\beta 2^*$ nAChR enhances the detection of cues specifically in cued trials involving attentional up-shifts.

Lattal, M., Abraham, A. • **Modulation of extinction by dopamine D1 receptors** • Research on dopamine lies at the intersection of sophisticated theoretical and neurobiological approaches to learning and memory. Dopamine has been shown to be critical for many processes that drive learning and memory, including motivation, prediction error, incentive salience, memory consolidation, and response output. Theories of dopamine's function in these processes have, for the most part, been developed from behavioral approaches that examine learning mechanisms in appetitive tasks. A parallel and growing literature indicates that dopaminergic signaling is involved in consolidation of memories into stable representations in tasks such as fear conditioning. Relatively little is known about how dopamine may modulate memories that form during extinction, when organisms learn that the relation between previously associated events is severed. I will review studies demonstrating that targeting dopamine receptors with SKF 81297 (a D1/D5 receptor agonist) during extinction of learned fear leads to an enhancement in extinction. These effects also occur in extinction of cocaine-induced conditioned place preferences, suggesting that the effects on extinction are not specific to a particular type of procedure (aversive or appetitive). Genetically modified mice with a selective deletion of the D1 receptor have deficits in extinction. I will describe different ways of thinking about these results in terms of effects on appetitive-aversive interactions and memory consolidation.

Mahgoub, M., Adachi, M., Monteggia, L.M. (UT Southwestern Medical Center) • **HDACs in learning and memory** • Histone deacetylases (HDACs) compress the chromatin structure, restricting access of transcription factors to the DNA and consequentially repressing gene expression. While individual HDAC genes are widely expressed throughout the body and possess differing deacetylase activity, their specific function in various tissues is only now starting to be examined. Recent work has shown that broad acting pharmacological HDAC inhibitors improve performance in learning tasks in rodents and may have therapeutic potential for ameliorating memory deficits in neurodegenerative disorders. Two of the individual HDAC genes, HDAC1 and HDAC2, are class I HDACs that are ubiquitously expressed, localized to the nucleus, and generally found together in corepressor complexes. While recent work has shown that conditional brain-specific HDAC2 knockout mice have enhanced learning and memory, with no measurable effects observed following manipulation of HDAC1 expression, it is unclear how the brain specific loss of both HDAC1 and HDAC2 impact complex behavior. We generated conditional brain-specific HDAC1 and 2 double knockout (DKO) mice and found they die at approximately 9 weeks of age, although survival is not impacted in the single knockouts. The DKO mice have behavioral abnormalities including hypoactivity and heightened anxiety shortly after the deletion of HDAC1 and 2. Hematoxylin and Eosin (H&E) staining reveals abnormal neuronal morphology as represented by disruptions in cortical lamination and cell layering in the hippocampus. These studies suggest that the combined loss of HDAC1 and 2 in the brain results in detrimental effects and suggests redundant functions of these HDACs in postmitotic neurons.

Shors, T. J. (Rutgers) • **Mind does matter: mental and physical (MAP) skill training keeps new neurons alive** • The adult brain continues to produce new neurons throughout life. Once produced, many of these new cells die. However, many can survive if new learning occurs (Gould, Beylin, Tanapat, Reeves & Shors, 1999). Therefore, new learning enhances the presence of neuronal matter within the adult brain. Most of these new cells are produced in the hippocampus, a brain region necessary for select processes of learning. However, training alone is not sufficient to rescue new neurons from death; learning itself must occur. The types of learning that increase cell survival within the hippocampus include those that require many trials to learn, either as a function of task difficulty or natural ability (Waddell & Shors, 2008; Waddell, Anderson & Shors, 2011; Curlik & Shors, 2011). These training procedures are similar in concept to those used for mental training in humans because they are effortful and challenging to the individual. How can these laboratory findings be translated into skills learned by humans? We recently determined that new neurons can be rescued by physical skill learning, during which animals are challenged to acquire a gross motor skill (Curlik, Maeng, Agarwal & Shors, 2013; Curlik and Shors, 2013). This effect is not due to exercise alone. The type of physical skill training presumably mimics that which occurs while humans learn a new sport. Together, these findings indicate that acquiring mental and physical skills throughout life retains neurons in the brain that would have otherwise died. These types of activities are being translated into a clinical intervention known as MAP training, which is yielding positive consequences for brain function and mental health in humans.

Rankin, C. (UCB) • **Insights into the complexity of the simplest form of learning** • Habituation is a fundamental form of learning highly conserved phylogeny. Because selective attention has limited capacity, most learning protocols begin with habituation to the experimenter and the experimental environment. Animals habituated to aspects of an experimental paradigm prior to testing perform better than animals that have not been habituated. Although habituation is considered the simplest form of learning, until recently almost nothing was known about the cellular mechanisms of habituation. In the years that my lab has studied habituation in *C. elegans* we have developed an understanding of tap habituation and the neural circuit mediating this behavior. We are now focusing on the genes underlying this learning using a novel high-throughput behavioral analysis system, the multi worm tracker. With this system we examined 508 known nervous system gene mutations and identified a large number of habituation mutants. Detailed analyses of habituation phenotypes revealed four genetically independent features of habituation: rate of habituation and final habituated level for response probability, rate of habituation and final level of habituation for response magnitude. Analysis of genes for these features led to the hypotheses that final level of response probability involves a ubiquitin mediated process, and final level of response magnitude is mediated by a kinase pathway that includes PKC and MAP kinases. We have also been genetically analyzing habituation of behaviors elicited

by photoactivation of two sensory neurons in the head of the worm. We have found that this habituation is also made up of different subcomponents that show different patterns/kinetics of habituation.

Robinson, T.E. (University of Michigan) • **If you want new ideas read the old literature** • Despite no background in the field, I recently found myself dragged into the realm of Pavlovian conditioning, when we found that there is considerable individual variation in the degree to which Pavlovian reward cues acquire incentive motivational properties. In this talk I will recount how the rediscovery (and exploitation) of a phenomenon first described in 1937 in dogs led to a series of studies on variation in the motivational control of behavior by Pavlovian cues associated with either food or drug rewards. The studies have implications for understanding susceptibility to impulse control disorders, including addiction, and also provide a way to parse learning vs. motivational processes in studies concerned with the neurobiological basis of each.

Poster Abstracts:

Posters 1-35 in TRAVIS; Posters 36-70 in SHOAL CREEK

(1) **Partial reinforcement with US-alone trials rather than CS-alone trials produces enhanced acquisition of conditioned eyeblinks in behaviorally inhibited individuals** • Cordero, C.A., Allen, M.T. (University of Northern Colorado), Myers, C.E. (Rutgers University-Newark), Caulfield, M.D., Servatius, R.J. (University of Medicine and Dentistry, New Jersey) • Facilitated associative learning in individuals self-reporting behavioral inhibition has been found to be most evident in partial reinforcement situations in which the predictive relationship between the conditioned stimulus (CS) and unconditional stimulus (US) is less than optimal. In the current study, we extended our work with partial reinforcement to include US alone trials rather than CS alone trials. The occurrence of an unpredicted US may be a more natural situation than the occurrence of a CS without the subsequent US. Forty one college-aged undergraduates participated in the study. All participants completed the Adult Measure of Behavioral Inhibition (i.e., AMBI). Eyeblink conditioning consisted of 3 US alone trials, 60 acquisition trials, and 20 CS-alone extinction trials presented in one session. Conditioning stimuli were a 500 ms tone conditioned stimulus (CS) and a 50-ms air puff unconditional stimulus (US). A median split was done for the AMBI scores to group participants as behaviorally inhibited and non-inhibited. Consistent with our previous findings, those scoring high on the AMBI scale acquired eyeblink CRs to a higher degree than those scoring lower on the scale. Comparison of 50% partial reinforcement with US alone trials produced greater facilitation than prior findings with CS alone trials. Partial reinforcement with US alone trials produced less of a partial reinforcement extinction effect as compared to prior findings with CS alone trials. Future work will extend these findings with heart rate monitoring to investigate possible physiological differences underlying the observed learning differences in individuals with self-reported behavioral inhibition.

(2) **Extremely non-optimal parameters of only 25% cs-us paired trials produce enhanced eyeblink conditioning in behaviorally inhibited individuals** • Chamberlain, C.M., Allen, M.T. (University of Northern Colorado), Myers, C.E. (Rutgers University-Newark), Caulfield, M.D., Servatius, R.J. (University of Medicine and Dentistry, New Jersey) • The manner in which individuals learn associations between stimuli and extinguish these associations may represent a diathesis for anxiety disorders. Facilitated associative learning has been most evident when the predictive relationship between the conditioned stimulus (CS) and unconditional stimulus (US) is less than optimal. We previously demonstrated facilitated acquisition of conditioned eyeblink responses in an explicitly 50% partial reinforcement paradigm with CS alone trials. In the current study, we extended our work with partial reinforcement to extremely non-optimal parameters of only 25% CS-US paired trials. Thirty nine college-aged undergraduates participated in the study. All participants completed the Adult Measure of Behavioral Inhibition (i.e., AMBI). Eyeblink conditioning consisted of 3 US alone trials, 60 acquisition trials, and 20 CS-alone extinction trials presented in one session. Conditioning stimuli were a 500 ms tone conditioned stimulus (CS) and a 50-ms air puff unconditional stimulus (US). Acquisition trials consisted of 75 CS alone trials and 15 CS-US paired trials pseudo-randomly presented. A median split was done for the AMBI scores to group participants as behaviorally inhibited and non-inhibited individuals. Consistent with our previous findings, those scoring high on the AMBI scale acquired eyeblink CRs to a higher degree and exhibited slower extinction than those scoring lower on the scale. Surprisingly, acquisition to 25% paired trials produced learning curves that were not significantly different than those produced by 50% paired trials. This current finding indicates that individuals self-reporting behavioral inhibition overlearn and fail to extinguish conditioned responses even at extremely non-optimal parameters.

(3) **Amygdalar Arc expression following trace-fear conditioning** • Galvez, R., Chau, L.S. (Psychology Department, Neuroscience Program, University of Illinois at Urbana-Champaign) • It has been well documented that the amygdala plays a key role in fear-related memories. Studies utilizing auditory fear conditioning have established that the amygdala is important for both acquisition and consolidation of

Pavlovian fear learning (Kim and Jung, 2006; Johansen et al., 2011). However, trace-fear-conditioning, has not been extensively investigated. In trace-conditioning, there is a separation in time between the CS and US. The following analysis utilized amygdala expression of activity-regulated cytoskeleton-associated protein (Arc/Arg 3.1), an immediate early gene believed to be important for synaptic plasticity (Steward et al., 1998; Peebles et al., 2010), to determine the amygdala's role in consolidating trace-cued-fear-conditioning. Adult C57BL/6 mice were randomly assigned to one of four groups: trace-conditioned, delay-conditioned, backward-conditioned and cage-controlled. Trace-conditioned mice were presented with a tone (30s; 68db), followed by a stimulus-free interval (45s; trace) and a mild foot-shock (2s; 0.6mA). Delay-conditioned received a tone (30 s; 68db) that co-terminated with a mild foot-shock (2s; 0.6mA). Backward-conditioned mice were presented with a mild foot shock (2s; 0.6mA), followed by a stimulus-free interval (45s; trace) and a tone (30s; 68db). This group served as a control for neuronal stimulation. Cage-controlled mice did not receive any conditioning and served as a learning and stimulation control. Our findings demonstrated that trace-conditioning increases Arc protein expression in the BLA 1h following training, compared to delay-conditioned, backward-conditioned and cage-controlled mice. These findings offer important insight into the underlying pathway and possible neuronal mechanisms for acquisition and subsequent consolidation for trace- compared to contextual- and delay-fear-associations.

(4) Neocortical synaptic modifications following forebrain-dependent trace-associative learning

• Chau, L.S., Prakapenka, A.V., Zendeli, L., Galvez, R. (University of Illinois at Urbana-Champaign) • Learning-induced neocortical synaptic modifications have been demonstrated with general learning and memory paradigms, such as enriched rearing and motor skill learning. However, few have explored neocortical synaptic modifications following more specific learning and memory paradigms, such as associative learning. One suitable associative learning task is trace-eyeblick conditioning. During eyeblick conditioning, subjects are presented with a neutral, conditioned stimulus (CS) paired with a salient, unconditioned stimulus (US) to elicit an unconditioned response (UR). With multiple CS-US pairings, subjects learn the CS-US association and exhibit a conditioned response (CR) when presented with the CS. In trace conditioning, there is a stimulus free interval between the CS and the US. Using whisker stimulation as the CS during trace-eyeblick conditioning (whisker-trace-eyeblick: WTEB), previous findings have demonstrated that acquisition and retention of this task requires S1. Additionally, expansion of cytochrome oxidase representation for conditioned barrels in layer IV of S1 has been reported following WTEB conditioning. Though the underlying cause of this increased metabolic representation is not known, one possible explanation is synaptic modification. We have recently shown increased synapsin I expression, a presynaptic maker, in conditioned barrels following WTEB conditioning, further suggesting the increased metabolic activity observed following WTEB acquisition is due to synaptic modification. To further explore this hypothesis, the present study examines dendritic properties of Golgi-Cox-stained neurons in layer IV of S1 following WTEB conditioning. Consistent with previous findings demonstrating increased metabolic activity and synapsin I expression in conditioned barrels, preliminary findings demonstrate that forebrain-dependent trace-associative learning increases neocortical spine density.

(5) Controllability and predictability modulate the neural response to a threat

• K. H. Wood, K.H. Bowen, J.R. Shumen, M.D. Wheelock (UAB), L.W. Ver Hoef (UAB, Birminham VA Medical Center), D.C. Knight (UAB) • The ability to predict and control stressful events influences our emotional response to future threats. Prior animal research has demonstrated a diminished emotional response to predictable and controllable stressors, whereas unpredictable and uncontrollable stressors result in an enhanced emotional response. The present study was designed to better understand the effect of predictability and controllability on threat-related brain activation. Two groups of healthy volunteers participated in a Pavlovian fear conditioning study during functional magnetic resonance imaging (fMRI). Similar to prior animal research, the groups consisted of yoked pairs of which one group (Controllable Condition; CC) was able to terminate the unconditioned stimulus (UCS), and the other group (Uncontrollable Condition; UC) was not able to terminate the UCS. The threat-related fMRI signal response was diminished on predictable compared to unpredictable trials within the dorsolateral prefrontal cortex (PFC), dorsomedial PFC, ventromedial PFC, ventrolateral PFC, and posterior cingulate for both CC and UC groups. A predictability x controllability interaction was observed within ventromedial PFC and left hippocampus. Specifically, the threat-related response within these brain

regions was diminished on predictable vs. unpredictable trials for the CC group. The current findings suggest the PFC plays a key role in regulating the emotional response to a threat, and provide a better understanding of the neural circuitry that mediates the modulatory effects of predictability and controllability on the emotional response to a threat.

(6) Epigenetic mechanisms can transform transient sensory experience into specific and enduring memory • Bieszczad, K.M., Bechay, K. (UC Irvine), Rusche, J.R. (Repligen Corp.), Weinberger, N.M., McGaugh, J.L., Wood, M.A. (UC Irvine) • Chromatin modifications, e.g., via histone acetylation by transferases (HATs) and deacetylases (HDACs), can control transcription in adult neurons to modify neuronal function, and ultimately, animal behavior. For example, HDAC3 is an essential determinant of long-term memory formation: it can transform what otherwise would be forgotten into a robust memory that persists beyond the point at which normal long-term memory fails. Unknown is whether HDAC3 also regulates the specificity of memory formation. Here, specificity is defined as the particular multi-dimensional features of a sensory experience, such as the specific acoustic frequency heard during an auditory learning experience. Rats injected with an HDAC3-inhibitor (RGFP966; 10 mg/kg) were trained to associate specific sound-frequencies with reward (vs. vehicle-treated rats). A behavioral frequency-specificity test after training revealed enhanced responses to the trained frequencies over other non-signal sounds only in RGFP966-treated rats – they had developed specific memory for sounds associated with reward. Furthermore, only RGFP966-treated rats had cortical remodeling of acoustic frequency-representation in primary auditory cortex (A1) (measured electrophysiologically) that linked to frequency-specific memory. Thus, RGFP966 mediated highly specific memory and A1 representational expansion of trained frequencies. Combined with reports that A1 plasticity underlies formation of auditory memory, these results support that HDAC3 regulates the transformation of experience into strong and specific memory by engaging neural plasticity that encodes highly specific features of transient experiences. This reveals an epigenetic molecular mechanism for the specificity of memory formation and introduces A1 as a model for studying specific neural plasticity that links epigenetic mechanisms to behavior.

(7) Renewal after the extinction of discriminated operant behavior: Role of context-specific response inhibition • Travis P. Todd, Drina Vurbic, Mark E. Bouton (University of Vermont) • Three experiments demonstrated, and examined the mechanisms that underlie, the renewal of extinguished discriminated operant behavior. In Experiment 1, rats were trained to perform one response (lever press or chain pull) in the presence of one discriminative stimulus (S; light or tone) in Context A, and to perform the other response in the presence of the other S in Context B. Next, each of the original S/response combinations was extinguished in the alternate context. When the S/response combinations were tested back in the context in which they had been originally trained, responding in the presence of S returned (an ABA renewal effect was observed). The experimental design ruled out differential context-reinforcer associations as the only contributing mechanism of renewal. In Experiment 2, ABA renewal was reduced when the renewing context was previously associated with extinction of the same response. In Experiment 3, previous extinction of the response in the renewing context (occasioned by a different S) eliminated AAB renewal more than did extinction of the different response. Taken together, the results indicate that ABA and AAB renewal of a discriminated operant occurs when context-reinforcer associations are controlled. They are also consistent with the idea that extinction performance is at least partly controlled by a direct inhibitory association that is formed between the context and the response. Renewal of discriminated operant behavior is produced by a release from that inhibition, and can be reduced if the response is first extinguished in the renewing context in the presence of another discriminative stimulus. This research was supported by Grant RO1 DA033123 from the National Institute on Drug Abuse to MEB.

(8) Effects of medial amygdala lesions on Pavlovian conditioning, Sidman avoidance and aversive Pavlovian-instrumental transfer • McCue, M.M. (Nathan Kline Institute), LeDoux, J.E. (Nathan Kline Institute & NYU), Cain, C.K. (Nathan Kline Institute & NYU) • Pavlovian conditioned stimuli (CSs) play an important role in the reinforcement and motivation of instrumental active avoidance (AA). Threatening Pavlovian CSs can also invigorate ongoing AA responding (Pavlovian-instrumental transfer or PIT). The neural

circuits mediating Pavlovian-instrumental interactions are poorly understood, although lesion studies suggest that lateral, basal and central amygdala nuclei, as well as infralimbic prefrontal cortex, make key contributions. We recently completed an extensive analysis of brain c-Fos expression in good vs. poor avoiders following an AA test. This analysis identified medial amygdala (ME) as a potentially important region for mediating competition between Pavlovian reactions and instrumental actions. ME is known to contribute to innate defensive responding and social behaviors, but its contribution to aversive Pavlovian-instrumental interactions is unknown. We evaluated the effect of electrolytic ME lesions on Pavlovian conditioning, Sidman two-way AA conditioning (shuttling) and aversive PIT in rats. Mild footshocks served as the unconditioned stimulus in all conditioning phases. Pretraining ME lesions had no effect on AA but impaired Pavlovian responses in the AA context. ME lesions also blocked the expression of aversive PIT. Interestingly, ME lesions failed to affect Pavlovian context- or cue-elicited freezing when assessed outside of the AA chamber. This latter finding differentiates ME from central amygdala, as central amygdala lesions abolish Pavlovian freezing inside and outside of the AA context. Taken together, these results suggest that ME plays a selective role in mediating aversive Pavlovian-instrumental interactions. [This work supported by grants to CKC (NIMH, MH097125) and JEL (NIMH, MH038774; NIDA, DA029053; NSF, 0920153)]

(9) Higher-order instrumental performance reveals spatiotemporal heterogeneity in dopamine signaling within the nucleus accumbens • Sadoris, M.P., Sugam, J.A., Wang, X., Carelli, R.M. (UNC Chapel Hill) • Animals use contingently predictive cues and actions to guide behavior towards optimal goals. Well-established models of animal learning typically employ an error prediction mechanism, whereby deviations between the prediction and outcome generate error signals that accrue to predictive stimuli with learning. However, incentive salience theory suggests that associative stimuli themselves become attractive and rewarding with learning. Both theories demonstrate that dopamine (DA) signaling in the nucleus accumbens (NAc) acts as an important neural mechanism for these associations, though in importantly different ways. However, previous experimental tasks have either employed relatively simple learning paradigms or recorded DA signaling in anatomically diffuse regions, making disambiguation of these theories difficult. Here, we adapted a seeking/taking chained instrumental task, allowing us to explore differences in (1) DA signaling between task initiation (seeking) versus consummatory actions (taking), and (2) real-time DA signaling between discrete subregions of the NAc. Using voltammetry, rapid DA release was recorded with acute electrodes placed in either the NAc core (n=16) or shell (n=14) while rats performed the task. DA signaling in the core peaked rapidly at seeking-cue onset, and quickly returned to baseline, most consistent with error prediction models; in contrast, shell DA signaling was marked by multiple release events for seeking and taking cues as well as and reward consumption, more consistent with incentive salience. These results confirm the heterogeneity of DAergic learning and attention signals at the level of the NAc, and suggest multiple and parallel estimations of value and prediction in the service of goal-directed behavior.

(10) Basolateral amygdala-medial prefrontal cortex pathway recruitment across Pavlovian appetitive conditioning • Keefer, S., Reppucci, C.J., Petrovich, G.D. (Boston College) • The Pavlovian appetitive conditioning paradigm using tone-food associations can condition rats to eat following the tone, independent of physiological hunger. Using this model we have identified three nodes of an essential forebrain network: the basolateral area of the amygdala (BLA), the medial prefrontal cortex (mPFC) and the lateral hypothalamus. Recent work found that distinct BLA nuclei were differentially recruited during learning acquisition and expression. The current goal is to determine whether pathways to the mPFC from different BLA nuclei are differentially recruited during early and late training. Male Long-Evans rats were iontophoretically injected with the retrograde tracer Fluoro-Gold (FG) into the mPFC. For training half of the rats received tone presentations followed with immediate delivery of food pellets (paired group), and the other half received tone only in the conditioning chamber followed by later delivery of the food pellets in their home cage (control group). To examine the acquisition of appetitive conditioning, half of the rats were perfused after one training session (D1), and half were perfused after ten training sessions to examine the expression of appetitive conditioning (D10). Preliminary results found the D10 paired group had significantly higher food cup behavior during the tone, evidence of learning, compared to the D10 control group ($p < 0.05$), and the D1 paired group increased food cup behavior during the tone compared to the D1 control group ($p = 0.09$). Brains

were processed with double-labeled fluorescent immunohistochemistry for detection of FG and Fos. We anticipate selective recruitment of different BLA-mPFC pathways during learning acquisition and expression. Support: NIH Grant R01DK085721

(11) Male and female rats differ in context-dependent renewal of appetitive conditioned responses • Anderson, L.C., Petrovich, G.D. (Boston College) • Little is known about sex differences in contextual processing and associative learning. Here, we used a preparation that depends on contextual processing for renewal of extinguished conditioned responses. Conditioning and extinction were conducted in different contexts, and the renewal of responding was induced by return to the conditioning context (“ABA renewal”). During conditioning, food-deprived male and female rats were presented with a tone (conditioned stimulus, CS) paired with food pellets (unconditioned stimulus, US). The conditioning was conducted within one of two distinct contexts (A or B, respectively; counterbalanced) that varied in olfactory, visual, and tactile features. The primary measure of learning was an increase in time spent at the food receptacle (“food-cup behavior”) during the CS. After training (5 sessions, each with 8 CS-US pairings) all rats showed similar amounts of food-cup behavior. Rats then received extinction training with CS tone presentations without USs in either the conditioning context (control N=7; AAA, BBB) or in a different context (experimental N=8; ABA, BAB). After 2 extinction sessions (each with 8 CSs) all groups showed decreased food cup behavior. All rats were then tested for renewal with CS presentations in the conditioning context. An ANOVA revealed a significant group difference with experimental groups showing higher responding (Group: $F(1,30)=10.01$, $p<.01$). However, a post hoc analysis revealed that only males showed renewal. Experimental males (that received extinction training in a different context) showed significantly more food cup behavior compared to control males ($t(1,13)=4.91$, $p<.01$), while females in both groups showed similar responding rates ($p>.05$). Support: NIH Grant R01DK085721

(12) Variants of Trace and Delay Fear Conditioning in the C57/Bl6 mouse • Simmons, C.A., Lei, L., Burman, M.A. (University of New England) • Classical fear conditioning paradigms are commonly used to study the brain structures involved in fear and anxiety. Previous research has found that delay conditioning requires a neural circuit involving the amygdala, but not usually the hippocampus. Trace and contextual fear conditioning typically require both the amygdala and hippocampus. While these paradigms were developed primarily using rat models, they are increasingly being used in mice to study genetically modified subjects. A review of the literature produced 90 articles using trace conditioning in mice. However, few of those articles used any kind of behavioral control group, which is required to rule out non-associative factors causing fearful behavior. Even fewer used unpaired groups involving tones and shocks in the same session, which is the optimal control group. The current studies develop fear conditioning and control paradigms to allow for the assessment of trace and delay fear conditioning in C57/Bl6 mice. Our initial protocol yielded clear delay conditioning. However, trace conditioning failed to differentiate from an unpaired group and was not hippocampus dependent. These results suggested that the protocol needed to be modified to specifically accommodate the C57/Bl6 mice. In order to reduce unconditioned freezing and increase learning, the final protocol was developed by decreasing the intensity of the tone and increasing the inter-trial interval. This protocol produced trace conditioned freezing that was significantly greater than that following unpaired stimulus exposure and was disrupted by hippocampus lesions. This protocol can be used in future studies examining genetically modified C57/Bl6 mice.

(13) Lmo4 in the Basolateral Amygdala Modulates Selective Aspects of Cue-Reward Learning • Maiya, R (The University of Texas at Austin), Heberlein, U (Howard Hughes Medical Institute, Janelia Farm Research Campus), Messing, R.O. (The University of Texas at Austin) • We have previously demonstrated a role for the transcriptional regulator Lmo4, which is highly expressed in the basolateral amygdala (BLA) in fear learning. The BLA has also been shown to modulate selective aspects of cue–reward learning. Hence, in this study we sought to examine the role of Lmo4 in the formation of cue-reward associations and the subsequent ability of reward-paired cues to control goal-directed behavior. We first examined cue-reward learning in mice heterozygous for a genetrapp insertion at the Lmo4 locus (Lmo4gt/+). Both wild type (WT) and Lmo4gt/+ mice readily learned to associate the presentation of stimuli with the delivery of an appetitive reinforcer. Further,

cues were able to elicit approach behavior in the absence of the primary reinforcer (goal tracking) equivalently in both genotypes. We next examined conditioned reinforcement, which is a measure of the motivational properties of the reward-paired cue, in both genotypes. Interestingly, our results indicate that reward-paired cues served as effective conditioned reinforcers in WT but not *Lmo4gt/+* mice. Since the BLA along with the nucleus accumbens (NAc) and several forebrain structures plays a critical role in imbuing reward-paired cues with motivational value, we examined the effects of knockdown of *Lmo4* in the BLA and NAc on conditioned reinforcement. shRNA-mediated knockdown of *Lmo4* expression in the BLA, but not the NAc, recapitulated the conditioned reinforcement deficit observed in *Lmo4gt/+* mice. In summary, these results suggest a novel and selective role for the transcriptional regulator *Lmo4* in the BLA in attributing motivational significance to conditioned cues.

(14) Alcohol withdrawal and learning: A genetic model of acute withdrawal mediates

withdrawal-induced enhancement of fear conditioned learning and memory • Tipps, M.E., Raybuck, J.D., Lattal, K.M., Buck, K.J. (Oregon Health and Science University) • The involvement of normal learning and memory-related processes in the development of addiction has led to the hypothesis that addiction is a maladaptive form of learning. While alcohol intoxication has been shown to alter several aspects of learning and memory, less is known about the effects of alcohol withdrawal on these processes. *Kenj9*, which codes for a G protein-coupled inwardly rectifying potassium channel subunit (*GIRK3*), is a quantitative trait gene (QTG) candidate for physiological dependence to alcohol and the associated withdrawal in mice. The *GIRK* channel family is a direct target of alcohol and is involved in the formation of long-term potentiation, a molecular mechanism of learning and memory, suggesting that these channels may play a role in the interaction between alcohol and learning and memory. We trained *GIRK3* knock-out (KO) and wild-type (WT) littermates for fear-based learning and memory under both alcohol naïve and acute withdrawal conditions using trace fear conditioning and delay fear conditioning. We found that WT mice trained under acute alcohol withdrawal showed increased cue responses but decreased contextual learning, suggesting that acute withdrawal is sufficient to alter long-term learning and memory. In addition, this increase was not observed in the KO mice. Our results implicate *GIRK3* in the alcohol withdrawal-induced increase in fear-based learning and specifically highlight the role of *GIRK* signaling in the hippocampus and amygdala. Preliminary work using region-specific shRNA-mediated *GIRK3* knock-down to verify these effects are underway. [Supported by AA10760, DA005228, and DA07262 (to KJB); 1F32AA022011 (to MET)]

(15) Learning history and hippocampal involvement in the use of an image under ambiguous

situations • Fast, C.D., Flesher, M.M., Nocera, N.A., Fanselow, M.S., Blaisdell, A.P. (UCLA) • Fast & Blaisdell (2011) reported that prior learning modulates the use of imagery in ambiguous situations. Rats that learned negative patterning (NP; A+/B+/AB-) responded significantly less during probe trials with A while B was covered (ambiguously absent) compared to when B was explicitly absent. Rats that learned the computationally simpler positive patterning (PP; A-/B-/AB+), however, were unaffected by the cover. Rats that had learned both PP and NP did treat the tests differently, suggesting that NP is necessary for the use of imagery. Presently, we investigated the features of prior learning that promote the use of imagery. Experiments 1-3 ruled out ratio of reinforcement, cue modality, and task difficulty as factors. Experiment 4 revealed an important role of the dorsal hippocampus (DH) in modulating this ability. Micro-infusions of the cholinergic antagonist, scopolamine, into the DH eliminated the influence of the cover on performance. Experiment 5 supported this result using *cFos* expression to compare DH activity between PP and NP covered and uncovered conditions. Finally, Experiment 6 challenged the necessity of NP to promote reasoning by training Pavlovian conditioned inhibition (A+/AX-) before testing on A-alone trials with X either covered or uncovered. Test sessions followed micro-infusions of either saline or scopolamine (within-subject). Sensitivity to the cover was found following saline but not scopolamine, replicating the results of Experiment 4 and suggesting that neither NP nor DH involvement during initial training are critical. Collectively, the results offer insight into the mechanisms that mediate the flexible use of an image during ambiguous situations.

(16) Specific deletion of NMDA NR1 subunits within corticotropin-releasing factor (CRF)-expressing neurons alters behavioral responses to social defeat • Meduri, J.D., DaMert, J.P.,

Jasnow, A.M. (Kent State University) • Corticotropin-releasing factor (CRF) plays an important role in the regulation of physiological and behavioral responses to stress. CRF-containing neurons are populated in a variety of neuroanatomical sites associated with learning and memory, stress responses, and emotional behavior. Site-specific infusions of CRF receptor antagonists significantly blunt the negative behavioral responses to social defeat. Thus, activation of the CRF system plays an important role in regulating behavioral responses to social defeat. However, determining how CRF-expressing neurons themselves are regulated during stress has been challenging. Therefore, we crossed mice expressing Cre-recombinase driven by the CRF promoter with mice containing floxed NR1 subunits to generate CRF-specific NR1 knockouts, eliminating NMDA receptor function specifically within these neurons. CRF-NR1 knockouts (KO) display normal anxiety-like behavior, but display enhanced fear expression and deficits in fear extinction. Previously, we have demonstrated that acute social defeat in mice produces susceptible and resilient phenotypes based on social interaction, but that resilient mice display enhanced fear expression and deficits in fear extinction. Interestingly, social defeat in CRF-NR1-KO's results in bidirectional effects in that KO susceptible mice display greater avoidance than WT susceptible mice and KO resilient mice display more social interaction than WT resilient mice. In addition, it appears that social defeat in CRF-NR1 KO's shifts the behavioral response in favor of resilience, with no apparent changes in anxiety-like behavior. Taken together, these data suggest that NMDA receptor-dependent regulation of CRF neurons plays a significant role in regulating responses to social defeat and emotional learning. Moreover, modulation of NMDA receptor function in CRF-expressing neurons may underlie directional shifts in behavioral responses to stress.

(17) Dissociation of dorsal prefrontal cortex connectivity during UCR diminution • Wheelock, M.D. (University of Alabama at Birmingham), Sreenivasan, K.R. (Auburn University), Wood, K.H. (University of Alabama at Birmingham), Ver Hoef, L. (University of Alabama at Birmingham), Deshpande, G. (Auburn University), Knight, D.C. (University of Alabama at Birmingham) • Prior work from our laboratory has demonstrated that many brain regions show a diminished response to predictable aversive events (100db white noise). The present study examined the connectivity underlying the UCR diminution to predictable versus unpredictable unconditioned stimulus (UCS) presentations. Twenty-four healthy volunteers participated in this fear conditioning study. One tone coterminated with a white noise (CS+UCS) and the UCS was also presented alone (UCS alone) to assess brain connectivity during the unconditioned response. Functional MRI data were processed using AFNI. The mean time series was extracted from fifteen activated regions of interest (ROI). Connectivity values for ROI were assessed using a deconvolved Granger causality analysis. Additionally, CS+UCS and UCS alone connectivity path weights were correlated with trait anxiety to assess the relationship between anxiety and connectivity. A larger number of connections were observed between dorsomedial prefrontal cortex (dmPFC) and other brain regions when the UCS was unpredictable. In contrast, a larger number of connections were observed between dorsolateral PFC (dlPFC) and other brain regions when the UCS was predictable. This is consistent with the view that the dmPFC coordinates the brain to take action during an unpredicted adverse event in a reactive manner and the dlPFC maintains cues in working memory in a proactive manner to prepare for imminent threats. Further investigation revealed individuals with higher trait anxiety had greater connectivity from the dlPFC during predictable (CS+UCS) trials, suggesting that individuals with higher anxiety have greater connectivity that may be associated with vigilance towards threat related stimuli.

(18) Controllability and predictability modulate the neural response to a threat • Wood, K.H., Bowen, K.H., Shumen, J.R., Wheelock, M.D., Ver Hoef, L.W., Knight, D.C. (UAB) • The ability to predict and control stressful events influences our emotional response to future threats. Prior animal research has demonstrated a diminished emotional response to predictable and controllable stressors, whereas unpredictable and uncontrollable stressors result in an enhanced emotional response. The present study was designed to better understand the effect of predictability and controllability on threat-related brain activation. Two groups of healthy volunteers participated in a Pavlovian fear conditioning study during functional magnetic resonance imaging (fMRI). Similar to prior animal research, the groups consisted of yoked pairs of which one group (Controllable Condition; CC) was able to terminate the unconditioned stimulus (UCS), and the other group (Uncontrollable Condition; UC) was not able to terminate the UCS. The threat-related fMRI

signal response was diminished on predictable compared to unpredictable trials within the dorsolateral prefrontal cortex (PFC), dorsomedial PFC, ventromedial PFC, ventrolateral PFC, and posterior cingulate for both CC and UC groups. A predictability x controllability interaction was observed within ventromedial PFC and left hippocampus. Specifically, the threat-related response within these brain regions was diminished on predictable vs. unpredictable trials for the CC group. The current findings suggest the PFC plays a key role in regulating the emotional response to a threat, and provide a better understanding of the neural circuitry that mediates the modulatory effects of predictability and controllability on the emotional response to a threat.

(19) Amygdala nuclei critical for Pavlovian fear conditioning exhibit unique gene expression patterns • Hosek, M.P., Partin, A.P., Luong, J.A., Lella, S.K. Sharma, S.A.R., Ploski, J.E. (University of Texas at Dallas) • The amygdala is a heterogeneous, medial temporal lobe structure that has been implicated in the formation, expression and extinction of emotional memories. This structure is composed of numerous nuclei that vary in cytoarchitectonics and neural connections. In particular the Lateral nucleus of the Amygdala (LA), Central nucleus of the Amygdala (CeA), and the Basal (B) nucleus contribute an essential role to emotional learning. However, it is still unclear to what extent these nuclei differ at the molecular level. We have performed whole genome gene expression analysis on these nuclei to gain a better understanding of the molecular differences and similarities among these nuclei. Specifically the LA, CeA and B nuclei were laser microdissected from the rat brain, and total RNA was isolated from these nuclei and subjected to RNA amplification. Amplified RNA was analyzed by whole genome microarray analysis which revealed that 129 genes are differentially expressed among these nuclei. Notably gene expression patterns differed between the CeA nuclei and the LA and B nuclei. However gene expression differences were not considerably different between the LA and B nuclei. Secondary confirmation was performed by in situ hybridization to validate the microarray findings, which also revealed that for many genes, expression differences among these nuclei were consistent with the embryological origins of these nuclei. Knowing the stable gene expression differences amongst these nuclei will provide novel avenues of investigation into how these nuclei contribute to emotional arousal and learning, and potentially offer new genetic targets to manipulate emotional learning and memory.

(20) Adeno-associated viral serotypes produce differing titers and differentially transduce glutamatergic excitatory neurons within the rat basolateral amygdala • Holehonnur, R., Luong, J.A., Chaturvedi, D., Ho, A., Lella, S.K., Hosek, M.P & Ploski, J.E. (University Of Texas At Dallas) • In recent years, there has been an increased interest in using recombinant adeno-associated viruses (AAV) to make localized genetic manipulations within the rodent brain. For AAV, differences in the capsid proteins of the AAV serotypes greatly influence their transduction efficiency of particular cell types / brain regions. Therefore we aimed to determine the AAV serotype that is optimal for targeting excitatory glutamatergic neurons within the Basal and Lateral Amygdala (BLA) since the transduction efficiency of AAV has not been previously examined within the BLA and notably this region is desirable to genetically manipulate due to its role in Pavlovian fear conditioning. We accomplished this by screening 9 different AAV serotypes (AAV1, AAV2, AAV5, AAV7, AAV8, AAV9, AAVrh-10, AAVDJ and AAVDJ8) designed to express red fluorescent protein (RFP) under the regulation of a alpha Ca²⁺/calmodulin-dependent protein kinase II promoter (☒CamKII). We determined that these serotypes appear to naturally produce differing amounts of virus under standard laboratory production. Notably AAV2 consistently produced the lowest titers compared to the other serotypes examined. Next we bilaterally infused these nine serotypes into the rat BLA at the highest titers achieved for each serotype and at a normalized titer of 7.8E+11 GC/ml and 21 days following viral infusion the rats were sacrificed and the degree of transduction was quantitated throughout the amygdala. These viruses exhibited differential transduction of excitatory neurons within the BLA. AAV7 possessed the highest efficiency of transduction. However taking into the consideration the ability of certain serotypes to achieve high titers and transduce glutamatergic excitatory neurons, in our hands AAVDJ8 and AAV9 appear to be the optimal serotypes to use when targeting glutamatergic excitatory neurons within the BLA.C

(21) Cerebellar secretin and eyeblink conditioning • Fuchs, J. R., Robinson, G. R., Morielli, A. D., & Green, J. T. (University of Vermont) • Eyeblink conditioning (EBC) is a form of classical conditioning supported by plasticity in the cerebellum. Both Purkinje cells (PCs) in cerebellar cortex and interpositus

nucleus (IPN) neurons receive CS and US inputs. PCs are regulated by basket cells (BC), inhibitory interneurons whose axon terminals have the highest concentration in the brain of the voltage-gated K⁺ channel α -subunit, Kv1.2. Depolarized PCs release secretin, surface Kv1.2 in BC terminals is reduced by secretin, and secretin or a Kv1.2 blocker increases inhibitory postsynaptic currents in PCs. We predicted that disinhibition of IPN neurons through inhibition of PCs, would facilitate EBC, which we confirmed by infusing either a Kv1.2 blocker or secretin into cerebellar cortex prior to conditioning. The current experiments expanded on these initial findings. In Experiment 1, rats received infusions of a secretin receptor antagonist, 5-27 secretin, or vehicle into cortex immediately prior to Sessions 1-3 of EBC. Rats that received 5-27 secretin showed slower acquisition. In Experiment 2, rats received infusions of secretin or vehicle into cortex immediately prior to Sessions 1 or 2 of extinction. Rats that received secretin prior to Session 1 of extinction showed slower extinction. In Experiment 3, rats received infusions of 5-27 secretin or vehicle into cortex prior to Sessions 1 or 2 of extinction. No differences between groups were observed. Our working model is that cerebellar cortical secretin modulates expression of CRs by reducing surface levels of Kv1.2 at BC terminals, thereby increasing inhibition of PCs. Support for this research came from the University of Vermont Neuroscience, Behavior and Health Initiative.

(22) Amygdala lesions disrupted signaled instrumental avoidance acquisition in Wistar Kyoto and Sprague Dawley rats • Moench, K.M., Wilson, J.J., Resch, Z.J. (Carthage College, Kenosha, WI), Miller, D.P. (Carthage College, Kenosha, WI; Stress and Motivated Behavior Institute, NJMS-UMDNJ, Newark, NJ), Miller, K.A., Pang, K.C.H., Servatius, R.J. (Stress and Motivated Behavior Institute, NJMS-UMDNJ, Newark, NJ; Neurobehavioral Research Lab, DVA Medical Center, NJHCS, East Orange, NJ) • Servatius et al. (Behav. Brain Res., 2008) demonstrated that inbred Wistar Kyoto (WKY) rats acquired signaled avoidance responding more rapidly and were resistant to extinction compared to Sprague Dawley (SD) rats. Coupled with the observed behavioral inhibition of the WKY strain, we have suggested that these characteristics model significant vulnerabilities seen in humans with anxiety and stress disorders. Here we examined the role of the amygdala in acquisition of signaled instrumental avoidance learning. Male rats of both strains received electrolytic lesions of the amygdala similar to the procedure used by Lee and Kim (2004). Two weeks later both lesioned and sham lesioned rats began nine days of avoidance training (3 sessions/week for 3 weeks). Combined lesions of the central and basolateral amygdala disrupted acquisition of avoidance learning, reduced asymptote, and lengthened trial response latency. Perseverative barpressing immediately following escape responding has been suggested to represent an anxiety-like response state. As expected, sham lesioned WKY rats showed elevated levels of barpressing following escape responding, while lesioned rats of both strains showed a significant reduction in barpressing following escape across sessions. Thus, our data demonstrated the importance of the amygdala both early in training for acquisition of signaled instrumental avoidance learning and in barpressing following escape. This suggests that WKY rats in particular have perseverative emotional processing and/or expectancy following removal of the aversive stimulus. Our results also support the WKY strain as a model for understanding the role of emotional processing and avoidance learning in humans with anxiety or stress disorders.

(23) Allopregnanolone in the bed nucleus of the stria terminalis impairs acquisition and expression of contextual fear in male rats • Acca, G., Maren, S., Nagaya, N. (Texas A&M University) • Sex differences in anxiety disorders are evident in humans, suggesting an important role for gonadal hormones and their metabolites in the regulation of fear. Previous work in our laboratory has revealed sex differences in Pavlovian fear conditioning in rats; male rats exhibit more contextual fear (indexed by freezing behavior) than females (Maren et al., 1994). The bed nucleus of the stria terminalis (BNST) is not only sexually dimorphic, but also implicated in contextual fear memory, suggesting it may be a locus for hormonal regulation of fear underlying these sex differences. The progesterone metabolite allopregnanolone (ALLO, a modulator of GABAA receptors) has anxiolytic properties and thus may drive the sex differences in freezing behavior. First, we explored whether the anxiolytic properties of ALLO are mediated by the BNST. Adult male Long-Evans rats were bilaterally implanted with stainless steel guide cannulae directed at the BNST. After recovery from surgery, rats received five fear conditioning trials consisting of tone (80 dB, 10 s)-footshock (1 mA, 2 s,) pairings with a 1-min inter-trial interval. Twenty-four hours later the rats were infused with ALLO (8 μ g/ μ l,

0.25 μ l, 0.25 μ l/min) or vehicle (30% β -cyclodextrin) and then tested 10 min later in the conditioning context without the tone (10 min). Forty-eight hours after conditioning, the rats were infused with the same doses of ALLO or vehicle and presented with the conditioned stimulus (CS, 80 dB, 10 s, 1-min intertrial interval) in a different context over four trials. ALLO infusions produced a significant impairment in the expression of freezing in the conditioning context, but not to the CS alone. To determine the effects of ALLO on the acquisition of fear memory, rats were infused with the same doses of ALLO or vehicle 10 min prior to fear conditioning. Subjects were then tested in the conditioning context and a different context with the conditioned stimulus at 24 and 48 hours post infusion, respectively. ALLO infusions to the BNST prior to fear conditioning produced impaired freezing in response to the context, but not to the CS. These results suggest that neurosteroid modulation of BNST GABAA receptors is involved in the acquisition and expression of fear memory and contributes to sex differences in fear and anxiety. Supported by NIH R01MH065961 to SM

(24) Differential neutral stimulus processing in prefrontal regions in an animal model for anxiety vulnerability • Ko, N., Pang, K.C.H., Myers, C.E., & Servatius, R.J. (RBHS - NJMS) • Studies have long shown differential processing of threat-related stimuli in anxious and anxiety-vulnerable individuals. It is becoming increasingly clear, however, that these processing abnormalities extend to neutral stimuli as well. Vulnerable populations may be unable to adequately modulate attention to even neutral environmental events, potentially exacerbating anxiety symptom pathogenesis. This project investigated this by assessing the effects of simple auditory stimulus presentations on behavior and neural activation in an animal model for anxiety vulnerability, the Wistar-Kyoto (WKY) rat. Sprague-Dawley (SD) controls and WKY rats were exposed to zero, one, or thirty presentations of an auditory conditioned stimulus (CS) and then subjected to delay eyeblink conditioning (EBC), pairing that CS with periorbital stimulation, or processed for c-Fos-related immunoreactivity, a marker of neuronal activation. Strain-dependent effects of CS exposure were found in both behavior and region-specific neural activation. Thirty CS exposures impaired acquisition of the EBC conditioned response ($F(2,32)=3.283$, $p=0.05$) and suppressed activation in the anterior cingulate cortex ($F(2,13)=4.226$, $p=0.039$) of SD controls, but not anxiety-vulnerable WKY rats. These results suggest that suppression of EBC by CS pre-exposure, a normal learning effect known as latent inhibition, may be contingent on the capacity to down-regulate neural activation following repeated stimulus presentations. Moreover, the region implicated by the immunohistochemical results points to the behavioral effect being driven in part by top-down attention modulation which may be impaired in anxiety-vulnerable WKY rats. These findings support the hypothesis that anxiety-vulnerable populations exhibit abnormal processing of neutral, in addition to threatening, environmental stimuli.

(25) Exposure to a dangerous context results in the relapse of extinguished fear • Goode, T.D., Kim, J.J., Maren, S. (Texas A&M University) • Stress is thought to be a major factor in the relapse of fear after interventions for anxiety, including exposure therapy. Extinction of fear in rats has proven to be an important model of fear suppression in humans. Fear (i.e., freezing) to an extinguished conditioned stimulus (CS) has been shown to relapse following an exposure to a dangerous context in rodents (Morris et al., 2005). We sought to replicate this finding as a prelude to studies exploring brain mechanisms of relapse. 24 hours prior to conditioning (consisting of 5 tone-shock pairings), male Long-Evans rats were either habituated to a novel context ('neutral') or presented with an unsignaled shock in the same context ('dangerous'). Extinction (45 tone-alone trials per day for 2-3 days) occurred in either the conditioning context ('ambiguous') or in a separate novel context ('safe'). 20 minutes prior to a retention test in either the ambiguous or safe context, rats were briefly exposed to the dangerous or neutral context. Rats exposed to the dangerous context expressed high fear in that context and subsequently relapsed to the CS in the ambiguous and safe contexts. Relapse was shown to continue 24 hours later. Additionally, removal of the short-term retention test after the dangerous exposure did not eliminate relapse 24 hours later. Substituting the conditioning context for the dangerous context did not induce relapse, however freezing during the exposure remained lower than in the unsignaled shock context. These data indicate that exposure to a dangerous context can result in relapse to the CS.

(26) Over-expectation using an exteroceptive context and an interoceptive drug stimulus • Barrett, S.B., Pudiak, C.M., Falco, A.M, Bevins, R.A. (University of Nebraska - Lincoln) • The over-expectation

effect is produced by presenting the compound of two or more separately established conditioned stimuli paired with the previously employed unconditioned stimulus. The result is a decrease in magnitude of the conditioned response controlled by each stimulus when subsequently tested alone. Previous demonstrations of the over-expectation effect have largely used discrete, exteroceptive stimuli. The present experiment sought to extend the over-expectation effect to a compound stimulus that was composed of an exteroceptive contextual stimulus and an interoceptive drug stimulus. Eight male Sprague-Dawley rats were first conditioned with each stimulus element alone. For one element, brief presentations of 26% (w/v) liquid sucrose were paired with a “noisy” oscillating audio-visual contextual stimulus. For the other element, 0.4 mg base/kg nicotine, injected SC, was paired with the same liquid sucrose. Conditioned responding was recorded as head entries in the dipper receptacle during each session before the first sucrose delivery (i.e., goal-tracking). Following separate conditioning of the stimulus elements, the “noisy” and nicotine contextual stimuli were presented in compound and paired with the same schedule of liquid sucrose delivery over 80 sessions. The strength of conditioned responding evoked by the nicotine and the “noisy” stimulus were tested separately before and after compound conditioning. Both stimuli evoked less conditioned responding following compound conditioning, demonstrating that the over-expectation effect generalized to combining an exteroceptive and interoceptive stimulus.

(27) Can generalization of extinction with nicotine substitutes be enhanced through successive extinction training? • Barrett, S.T., Pudiak, C.M., Falco, A.M., Bevins, R.A. (University of Nebraska - Lincoln) • Nicotine and other drugs are complex stimuli that may be better characterized as the compound of many interoceptive stimulus elements. For example, following excitatory conditioning between nicotine and an appetitive stimulus, many nicotinic acetylcholine receptor agonists substitute for nicotine during brief 4-min stimulus substitution tests. The same ligands reveal a different substitution pattern when tested across repeated 20-min extinction sessions. Previous work has demonstrated that extinction with bupropion, varenicline, nornicotine, and other “nicotine-like” stimuli will generalize back to the original nicotine training stimulus, weakening its control of conditioned responding. The present experiment investigated if generalization of extinction learning could be improved by successive extinction training with two different nicotine-like ligands. Eighty four male Sprague-Dawley rats received excitatory conditioning in which 0.4 mg/kg nicotine was paired with 26% (w/v) liquid sucrose. Following conditioning, over the first three extinction sessions, rats were administered nicotine, 20 mg/kg bupropion, or saline. In the subsequent three extinction sessions, rats that previously received bupropion were divided into five groups and either continued to receive bupropion or to receive nornicotine (3.0 or 5.6 mg/kg) or varenicline (0.1 or 1.0 mg/kg). Rats that received nicotine or saline previously continued to receive the same over these sessions. Finally, all groups were tested over two sessions for conditioned responding evoked by nicotine or saline. Bupropion substituted for the nicotine stimulus in extinction. There was no evidence of enhanced generalization of extinction learning in groups that were switched to nornicotine or varenicline in the second half of extinction.

(28) Acute administration of nicotine interferes with the expression of safety cue memory • Connor, D.A., Gould, T.J. (Temple University) • Acute treatment with nicotine, prior to conditioning and testing, has been shown to enhance contextual fear conditioning. Additionally, altered fear learning may result in deficits in learning safety cues. We hypothesized that nicotine enhancement of contextual learning, leading to an increased fear response to the conditioning context, would result in a deficit in learning or recalling an inhibitory safety cue. In our design, the context becomes associated with aversive footshock and acts as a behavioral exciter (increased fear response). A cue (tone) is explicitly unpaired with footshock and learned as a safety cue, acting as a behavioral inhibitor (decreased fear response). Upon testing, the context elicits a fear response, which is reduced during safety cue (tone) presentation. Using a three-day training protocol, with testing on the fourth day, we demonstrated that all mice learned the safety cue, showing reduced freezing to the training context during cue (tone) presentation on testing. Mice administered nicotine on the third day of training and testing demonstrated enhanced contextual learning and a similar response to the safety cue compared to saline. Mice administered nicotine on all three training days and testing resulted in a more dramatic enhancement of contextual memory. Additionally, mice administered nicotine on all three days of training demonstrated an altered response to the safety cue indicating that the enhanced contextual fear

memory interfered with expression of the safety cue memory. 24 hours after testing, in a drug free state, both nicotine treated groups showed increased fear to the training context.

(29) Effect of dorsal hippocampal MK-801 administration on trace and long-delay fear

conditioning in juvenile rats • Schreiber, W. B., Brennan, L. E., Jablonski, S. A., (University of Delaware), Hunt, P. S., (College of William & Mary), Stanton, M. E. (University of Delaware) • Short-delay fear conditioning develops earlier in ontogeny than trace or long-delay conditioning (Barnet & Hunt, 2005; Moye & Rudy, 1987). The hypothesis that this reflects hippocampal development has been advanced (Stanton, 2000) but not tested. The present study asked whether blocking dorsal hippocampal (dHPC) NMDA receptors would impair trace, but not long-delay conditioning in juvenile rats, as has been reported in adult rats (Quinn, Loya, Ma, & Fanselow, 2005). Thirty-day-old (± 1 day) rats received 10-trials of either trace (10-sec CS, 10-sec trace), or Long-Delay (20-sec CS) conditioning with a visual CS (25 W) and a 1-sec, 2 mA footshock US. Prior to training, rats in each group received a bilateral 5 μ g infusion of either dizoclipine (MK-801) or saline into the dHPC. A single unpaired group was formed from subsets of rats that were unpaired counterparts of the 4 paired groups. Animals were tested 24-hrs later for cued freezing in an alternate context. A 2 (drug) x 2 (task) ANOVA on the paired groups revealed higher freezing in long-delay vs. trace groups and in saline vs. MK-801 groups, regardless of task. Follow-up comparisons revealed that all groups except the MK-801-trace group showed significantly elevated freezing over unpaired controls. Implications for the role of dHPC in trace and delay fear conditioning during development are discussed. Support provided by 1-R21-HD070662-01.

(30) Chronic preexposure to cocaine facilitates sexual conditioning and increases resistance to

extinction • Rice, B.A., Akins, C.K. (University of Kentucky) • There is widespread acceptance that cocaine and other drugs of abuse alter sexual behavior (see Frohmader et al., 2009 for review). Further, there is evidence that chronic preexposure to cocaine may facilitate a conditioned sexual response (Levens and Akins, 2004). The purpose of the current research was to test dose-dependent effects of cocaine on sexual conditioning and to determine whether cocaine would alter the rate of extinction of the sexually conditioned response. The results indicated that quail that received COC demonstrated greater approach to the CS across conditioning trials that was dose dependent, $F(9, 252) = 2.04, p < 0.05$. Furthermore, COC paired subjects were slower to extinguish sexual conditioned responding compared with saline paired subjects, $F(9, 126) = 2.53, p < 0.05$. The results indicate that chronic preexposure to COC may facilitate sexual motivation and that this drug-induced facilitation of conditioned responding may persist under extinction conditions. Furthermore, the persistence of responding in the absence of the reinforcer may indicate that the association between the cue and sexual reinforcement may have been strengthened by cocaine. Similar mechanisms may mediate sexual motivation in human drug users and potentially play a role in risky sexual behaviors in the presence of a drug.

(31) Posttraining peripheral administration or intra-dorsolateral striatum injection of the cannabinoid receptor agonist WIN 55, 212-2 impairs the consolidation of habit memory

• Goodman, J., Packard, M.G. (Texas A&M University) • The cannabinergic system plays a role in modulating memory processes. Previously, we reported that peripheral administration of a cannabinoid agonist impairs the consolidation of habit memory in the water plus-maze. In the present study, we examined whether this impairing effect of peripheral cannabinoids can be replicated using a visible-platform water maze and whether posttraining infusions into the dorsolateral striatum (DLS; a region responsible for consolidation of habit memory) also produces a habit memory impairment. Adult male Long-Evans rats were trained for 1 day (8 trials) in a cued-platform water maze wherein rats were released from different start points and in order to escape had to find a cued platform that was moved to various spatial locations across trials. Immediately following training, rats received an i.p. injection of CB1/CB2 receptor agonist WIN 55,212-2 (1mg/kg or 3mg/kg) or vehicle solution. In another study using the same task, rats received bilateral intra-DLS injections of WIN 55,212-2 (100ng/0.5 μ L or 200ng/0.5 μ L) or vehicle. 24 hours later rats were given two probe trials, and the latency to reach the platform on these two trials served as an index of memory. Relative to the vehicle-treated controls, peripheral WIN 55,212-2 at the 3mg/kg dose significantly impaired memory ($p < .05$), and intra-DLS WIN 55,212-2 at 200ng/0.5 μ L also produced a memory impairment ($p < .05$). Our results indicate that peripheral or intra-DLS injection with a CB1/CB2 receptor agonist impairs consolidation of DLS-

dependent habit memory. We speculate drugs targeting the cannabinergic system could help alleviate habit-like symptoms of some psychopathologies.

(32) Acute estradiol injections has a genomic effect on fear generalization • Lynch III, J.F. Dejanovic, D. Mulvany, J. Winiecki, P. Vanderhoof, T. Jasnow, A.M. Riccio, D.C. (Kent State University) • 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 equivalent levels of fear when tested in the training context or in a neutral context (Jasnow, Cullen, & Riccio, 2012). Therefore, over time, animals will generalize their fear to neutral stimuli. Our lab has previously shown that females demonstrate faster rates of generalization than males, a process driven, in part, by estrogens (Lynch III, Cullen, Jasnow, & Riccio, in press). The current set of experiments attempts to elucidate a more complete understanding of what role estrogens play in the enhancement of fear generalization in female rats. In the first set of experiments, rats were ovariectomized and given acute injections of estradiol benzoate (15µg) at distinct time points during passive avoidance training and testing. Our results suggest that estradiol has a genomic effect on retrieval: injections 24 hours prior to testing result in fear generalization to a neutral context, whereas injections 1 hour before testing do not elicit fear generalization. These results provide evidence of when estrogens need to be present in order to influence fear generalization to a neutral context. The second set of experiments looked at which estrogen receptor subtype (ER α or ER β) needed to be activated in order to see enhanced fear generalization. In these experiments, different doses of Estrogen Receptor agonists were given separately and in combination, 24 hours before testing. These results provide evidence of which estrogen receptor subtype is driving the enhanced fear generalization seen with estradiol injections 24 hours prior to testing.

(33) Modulation of single neuron firing in medial prefrontal cortex by footshock stress in freely moving rats • Fitzgerald, P.J., Maren, S. (Texas A&M University) • The neurophysiological effects of psychological stress or mild trauma remain poorly understood, and gaining greater knowledge of them would foster understanding of stress-induced neuropsychiatric conditions such as post-traumatic stress disorder. Previous work suggests that medial prefrontal cortex (mPFC), including infralimbic cortex (IL) and prelimbic cortex (PL), regulates conditioned responses to footshock stress in rodents. Studies by Quirk and colleagues suggest that ongoing firing in PL is involved in fear expression whereas IL regulates inhibition of fear. In contrast, Neugebauer and colleagues have found that induction of inflammatory pain deactivates PL. Our previous work has shown that neuronal responses in PL are reduced soon after conditioning so we expect, consistent with Neugebauer's work, suppressed firing in PL (and possibly IL) within minutes after footshock. Here we test this hypothesis in freely moving rats that were implanted with microelectrode arrays which spanned both IL and PL. In a first recording session, rats received five fear conditioning trials, consisting of auditory tone-footshock (0.5 sec, 1 mA) pairings, followed immediately by a 60 minute no-tone extinction period. The next day, rats received an additional extinction session. Ongoing results suggest that both IL and PL single neuron responses tend to be suppressed in the minutes following footshock stress. Further, in both recording sessions and in both subregions of mPFC, we find cells that change their firing patterns across extinction, with some returning to their pre-tone firing rate, whereas others exceed or suppress their firing. Supported by NIH Ro1MH065961 to SM.

(34) Inhibition of histone deacetylase with sodium butyrate or suberoylanilide hydroxamic acid (SAHA) ameliorates cocaine-induced deficits in temporally dissociated trace fear conditioning in C57BL6 mice • Raybuck, J. D., Lattal, K.M. (OHSU) • Chronic, binge, and repeated psychostimulant exposure produces long-lasting disruption of reward circuitry. Since deficits in executive function result from drug use and predispose individuals to initial and continued substance abuse, a better understanding of the neural and pharmacological targets underlying these deficits may reveal novel approaches to facilitate cessation, as well as mechanisms that may be exploited to prevent long-term effects of drug-exposure. We used trace fear conditioning, a model of working memory dependent associative learning to investigate the effects of short-term binge-cocaine treatment on cognitive function in C57BL6 mice. Binge-cocaine administration produced robust, long-lasting, exposure-dependent deficits in trace conditioning, without affecting delay or

contextual conditioning, and similar deficits were present in rats following cocaine self-administration. Examination of epigenetic histone acetylation (HA) revealed deficits in learning induced HA in the prelimbic cortex (PrL) of cocaine-treated mice following trace conditioning. Further, systemic (1.2 g/kg NaBut, ip) or local (1 ug/site, SAHA, PrL) histone deacetylase (HDAC) inhibition fully rescued cognitive function in cocaine-treated mice. These results suggest (1) that robust deficits in cognitive function follow binge/repeated cocaine exposure, (2) that deficits are in part mediated by decreased HA in the PrL, and (3) that cognitive deficits can be prevented by either systemic or local administration of HDAC inhibitors. Thus, modulation of HA may serve as a useful target for the treatment of cognitive deficits induced by exposure to cocaine or other psychostimulants. Additionally, HDAC inhibitors may prove to be useful small molecule pharmacotherapies for the treatment of drug-exposure induced cognitive deficits.

(35) Involvement of the dorsal hippocampus in cocaine conditioned place preference • Hitchcock, L.N. and Lattal, K.M. (Oregon Health & Science University) • A key aspect of substance abuse is that drug taking often occurs in a specific context. As a consequence, exposure to drug-associated contexts can trigger cravings and relapse, even after long periods of abstinence. Although many studies have demonstrated that the hippocampus is critical for developing and retrieving contextual and spatial memories, very little is known about the role of the hippocampus in the contextual control of drug seeking. We examined the effects of hippocampal inactivation on expression and extinction of cocaine-induced conditioned place preference (CPP) in mice. During acquisition of CPP, distinct tactile cues were paired with cocaine (20 mg/kg, intraperitoneal, CS+) and different tactile cues were paired with saline (CS-) on alternate days. Groups differed in whether the CS+ and CS- cues were presented in the same large space (one-compartment procedure) or distinct small spaces (two-compartment procedure). Acquisition of CPP was promoted by the two-compartment procedure. Extinction, when mice were exposed to the CS+ cues in the absence of cocaine, was promoted by the one-compartment procedure. These findings suggest that a two-compartment configuration facilitated acquisition and attenuated extinction of a cocaine-induced CPP. Inactivation of the dorsal hippocampus (DH) with a microinjection of the GABAA agonist, muscimol, decreased expression of CPP after acquisition and increased expression of CPP after extinction. These effects differed depending on the spatial configuration, suggesting that the hippocampus may differentially modulate drug seeking following acquisition and extinction of CPP.

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(36) Fear renewal increases Fos expression in ventral hippocampal neurons projecting to both the medial prefrontal cortex and basal amygdala • Jin, J. and Maren, S. (Institute for Neuroscience; Department of Psychology, Texas A&M University) • Knowing when and where to express fear is essential to survival. Previous work in our laboratory has revealed that disconnections of the ventral hippocampus (VH) from either the amygdaloid basal nuclei (BA) or prelimbic (PL) prefrontal cortex eliminated renewal of contextual fear memory. Interestingly, Ishikawa and colleagues suggested that individual VH neurons project to both the PL and BA and were predominantly located in CA1 and subiculum. Here we examine whether there are VH neurons projecting to both PL and BA, and if so, whether they are active during the renewal of the contextual fear memory. Adult male Long-Evans rats were unilaterally infused two different CTB retrograde tracers into PL and BA respectively to label PL- and BA-projecting neurons in VH. Rats then underwent fear conditioning (5 tone-shock trials) and extinction (45 tone alone trials) and were tested their fear to the extinguished stimulus (CS) either in the extinction context or in a new context. Fear level was determined by the freezing behavior. Then c-fos immunohistochemistry was used together with the retrograde labeling in VH to examine the activity of the VH neurons during the context-dependent retrieval of extinguished fear memories. Presentation of the CS in the new context renewed conditioned freezing and were associated with more c-fos expression in PL- and BA-projecting neurons than that induced by CS presentation in the extinction context. C-fos expression was also detected in VH neurons projecting simultaneously to PL and BA and was higher when introduced outside the extinction context. These results indicate that simultaneous inputs from the same neurons in VH to PL and BA may play a crucial role in contextual regulation of fear after extinction.

(37) Egr-1 increases in the prefrontal cortex following training in the context preexposure facilitation effect (CPFE) paradigm • Asok, A., Schreiber, W.B., Jablonski, S.A., Rosen, J.B., Stanton, M.E. (University of Delaware) • The context pre-exposure facilitation effect (CPFE) is a modified form of standard contextual fear conditioning that dissociates learning about the context during a preexposure phase from learning the context-shock association during an immediate shock training phase conducted on separate days. Fear conditioning in the CPFE is an associative process in which only animals that are preexposed to the same context they are later given an immediate shock in demonstrate freezing when tested for conditioned fear memory. The hippocampus and amygdala are necessary for different phases of the CPFE, but whether other brain regions are also involved is unknown. The present study examined expression of the immediate-early gene early growth response gene 1 (Egr-1) in the dorsal hippocampus, lateral nucleus of the amygdala, retrosplenial cortex, and several prefrontal cortex regions (infralimbic and prelimbic medial prefrontal cortex, anterior cingulate, and orbitofrontal cortex) following each phase of the CPFE in juvenile rats. Only animals preexposed to the conditioning context displayed fear conditioned freezing during a retention test. Following context preexposure, Egr-1 mRNA was elevated in context and alternate context exposed animals compared to homecaged control rats in almost all regions analyzed. Following context-shock training, fear conditioned rats displayed significantly more Egr-1 mRNA expression in the infralimbic, prelimbic, and orbitofrontal cortices compared to the alternate context preexposed rats. These differences were not found in amygdala between the preexposed context and alternate context rats. The findings suggest that increased Egr-1 within the prefrontal cortex is associated with contextual fear conditioning in the CPFE paradigm. Acknowledgements This research was supported by NIH grant 1-R21-HD070662-01 to Jeffrey B. Rosen and Mark E. Stanton.

(38) Corticosterone is necessary but not sufficient to drive AMPA receptor changes in the amygdala that support Stress-Enhanced Fear Learning • Perusini, J.N. (UCLA), Meyer, E.M. (UCLA), Long, V.A. (Gallo Institute), Nocera, N. (UCLA), Rau, V. (UCLA), Maksymetz, J. (UCLA), Spigelman, I. (UCLA), and Fanselow, M.S. (UCLA) • Severe stress strongly potentiates future learning. These experiments assessed the contributions of corticosterone (CORT) on basolateral amygdala (BLA) glutamatergic receptor changes in stress-enhanced fear learning (SEFL). First, the CORT synthesis inhibitor metyrapone was administered before 15 shocks in context A. Animals were given 1 shock in a novel context and tested for freezing. Results show that prior stress with 15 shocks enhanced conditioning to the single shock context, indicating SEFL, and metyrapone administration before this stress blocked SEFL. CORT was then co-administered with metyrapone prior to stress to determine if metyrapone specifically acts via CORT. Pre-stress CORT injections rescued the freezing response from the drug; however, CORT without stress did not produce SEFL. Next, the BLA was inactivated with muscimol before or after stress to determine its role in SEFL. Inactivation before but not after stress eliminated SEFL. Then, western blots of BLA tissue were done to analyze glutamatergic receptor subunit changes in shocked- and metyrapone-treated rats. BLA expression of the AMPA receptor subunit, glutamate receptor 1 (GluA1), was significantly increased in shocked rats versus controls, but this increase was attenuated with metyrapone. Lastly, intra-BLA infusions of the AMPA receptor antagonist, NBQX, were administered after the stress to determine if AMPA receptor blockade prevented the sensitized fear response. NBQX attenuated freezing, which was rescued 24 hours later when the drug was no longer on board. These data indicate that both CORT and BLA activity are necessary for SEFL initiation. Moreover, SEFL expression requires CORT-dependent increased GluA1 expression in the BLA. Supported by NIMH grant MH62122 to MSF and T32 grant DA024635-03 to JNP.

(39) Delta-opioid receptor translocation in the nucleus accumbens shell: Necessary but not sufficient for specific Pavlovian-instrumental transfer • Morse, A.K., Laurent, V., Bertran-Gonzales, J., Balleine, B. W. (Brain and Mind Research Institute, University of Sydney) • Animals extract outcome-predictive information from their environment, and can use this information to guide their actions. In the laboratory, this critical ability can be studied through outcome-specific Pavlovian-instrumental transfer (PIT), in which a stimulus associated with a particular outcome biases response choice towards an instrumental action earning that same outcome. Recent evidence from our laboratory indicates that expression of this bias requires the translocation of delta-opioid receptors (DOR) to the plasma membrane of cholinergic

interneurons (mCINs) within the nucleus accumbens shell. Interestingly, this plastic change in receptor expression occurs during Pavlovian training, in which specific and contingent stimulus-outcome associations are established. As specific PIT is sensitive to manipulations of Pavlovian contingencies, but relatively resistant to instrumental manipulations, the present experiments aimed to investigate the effect of post-acquisition manipulations of Pavlovian associations on DOR expression and specific transfer. Consistent with the literature, specific PIT was not affected by extinction, but was abolished by procedures that disrupted Pavlovian contingencies: contingency degradation and non-contingent presentations of the stimuli and outcomes used in training. Surprisingly, none of these procedures affected the change in DOR expression triggered by initial Pavlovian training. Thus, the present results indicate that DOR translocation to mCINs within the shell is necessary but not sufficient for predictive stimuli to influence the choice between actions.

(40) Perturbations of emotional learning in an animal model of environmentally induced autism • Anwasha Banerjee, Jonathan A. Luong, Srihari K. Lella, Bethany L. Sauls, Crystal Engineer, Michael P. Kilgard, Jonathan E Ploski • Autism Spectrum Disorders (ASD) are complex neurodevelopmental disorders characterized by core symptoms including repetitive behavior, impaired social interactions and deficits in social communication. Apart from these core symptoms, a significant number of ASD individuals display maladaptive emotional responses. For example numerous studies indicate that ASD individuals display higher levels of anxiety and some studies indicate that ASD individuals are impaired in their ability to be fear conditioned. Therefore we sought to further examine innate fear and emotional learning utilizing an environmentally induced animal model of ASD. This model focuses on progeny from pregnant rats exposed to the known teratogen, valproic acid (VPA) on day 12.5 of gestation. Specifically we exposed dams to either one of two different doses of VPA (500 and 600 mg/kg) or vehicle on day 12.5 of gestation. Resultant progeny at 60 days of age were examined for innate fear and changes in locomotion using an open field test. We then auditory fear conditioned these rats to a 5 kHz 75 dB tone. Our preliminary data indicates that embryonic exposure to VPA, at both doses enhances anxiety as measured as decreased center entries in the open field test, with no changes in overall locomotion. Animals exposed to 500 mg/kg VPA displayed normal acquisition of fear conditioning, but exhibited reduced extinction of fear memory – data consistent with previously published reports. However we observed that rats exposed to 600 mg/kg of VPA exhibited a significant reduction in acquisition of fear conditioning. To determine if the decrease in fear conditioning was due to a sensory deficit, we examined pain perception and hearing in VPA exposed rats. VPA exposed rats exhibited normal pain sensitivity in a hot plate test and exhibited normal hearing to a 5 kHz, 75 dB tone as determined by normal spike firing within the auditory cortex during exposure to 5kHz tones presented at varying degrees of loudness up to 75 dB. Collectively these data indicated that the reduced acquisition of fear learning is not likely due to sensory deficits, but rather due to deficits in learning. To examine the molecular basis of VPA induced impairment in fear learning in animals exposed to VPA (600 mg/kg), we performed whole genome gene expression analysis using DNA microarrays on amygdala RNA from rats exposed to VPA and vehicle. The microarray data indicated that VPA exposed rats may have dysfunctional glutamatergic signaling within the amygdala. We are currently investigating the hypothesis that deficits in glutamatergic signaling underlie at least in part the impairment in emotional learning in animals exposed to VPA.

(41) The neural pattern of activation underlying memory precision • Cullen, P.K., Mulvany, J., Dejanovic, D., Dulka, B.N., Riccio, D.C., Jasnow, A.M. (Kent State University) • Contextual fear conditioning involves pairing a novel context with several footshocks that serve to condition fear to that context. At early time points rodents can discriminate between a training and neutral context indicating precise contextual memory. However, as the retention interval increases context specificity is lost. In other words, the fear memory is no longer precise or context-specific, but has generalized to novel contexts. In an attempt to investigate the neural pattern of an imprecise contextual memory trace as a function of time, we used fluorescent in situ hybridization following expression context fear memory. Expression of a contextually precise memory involved increased Arc mRNA expression in both the dorsal and ventral CA1 regions of the hippocampus as well as the anterior cingulate cortex (ACC) and infralimbic cortex (IL). Expression of a imprecise fear memory involved Arc mRNA expression in the ventral CA1, ACC, IL, and the prelimbic cortex (PL) suggesting that both the hippocampus and prefrontal cortex are involved in the expression of a remote

contextually imprecise memory. Further, inactivation of the ACC with lidocaine at remote time points specifically returned the context memory to a precise state, but had no effect on memory for the training context. Taken together, these data suggest that as a context fear memory ages, both the hippocampus and prefrontal cortex interact in the retrieval and/or expression of that memory trace resulting in the loss of precision. Preventing this interaction through inactivation of the ACC allows the hippocampus to express the contextually precise memory.

(42) State-dependent effects of cycloheximide on reconsolidated memory and the use of LiCl as an amnesic agent • Adam Ulmen, Joseph F. Lynch III, David C. Riccio (Kent State University) •

Cycloheximide, a protein synthesis inhibitor, induces deficits in memory either while a memory is being consolidated or reconsolidated (Flint & Marino, 2007, Hernandez & Abel, 2008). We have demonstrated that these deficits can be alleviated in a state-dependent manner by re-injecting cycloheximide prior to testing. The current experiment extends those findings to reconsolidated memories. Animals were trained in passive avoidance and given a 15 second reactivation session 48 hours afterward. Following reactivation, animals were injected with saline or cycloheximide. Testing occurred 48 hours after reactivation. Thirty minutes before test, animals were again injected with saline or cycloheximide. We found that, when cycloheximide is returned at test, memory impairment is attenuated. In experiment 2, we used a different agent, LiCl, which is not normally used as an amnesic agent. In this experiment, we established a conditioned taste aversion memory for 15% sucrose-water and simultaneously induced memory deficits in passive avoidance learning if LiCl was injected immediately after a reactivation session. These experiments demonstrate a state-dependent effect with cycloheximide for a consolidated or reconsolidated memory and the ability to form a new memory for taste aversion while blocking a memory for passive avoidance learning with a single injection of LiCl.

(43) Predictability and heritability of individual differences in fear conditioning and extinction •

Shumake, J., Furgeson-Moreira, S., Monfils, M.H. (The University of Texas at Austin) • Our objective was to characterize individual differences in fear conditioning and extinction, to test potential behavioral predictors of these individual differences, and to assess their heritability using selective breeding. We fear conditioned 100 Long-Evans rats, attempted to extinguish fear the next day, and tested extinction recall on the third day. Most rats showed substantial freezing after fear conditioning, but there were large individual differences during extinction recall. We bred rats from the top and bottom 20% of the distribution of freezing scores observed during extinction recall and ran their offspring through the same conditioning/extinction procedure, except that we began recording ultrasonic vocalizations throughout training and testing. Only a minority of rats emitted distress vocalizations during fear acquisition, but the incidence was less frequent in the offspring of good extinguishers (4%) than in poor extinguishers (14%) or randomly bred controls (19%). The occurrence of distress vocalizations during acquisition predicted higher levels of freezing during fear recall regardless of breeding line, indicated by a significant effect of vocalization, $p = .002$, with no significant effect of breeding, $p = .38$. The relationship between vocalization and freezing was no longer evident following extinction training, at which point freezing levels were influenced only by breeding, $p < .001$, and not by vocalization, $p = .30$. Heritability of extinction recall was estimated at 37% ($p = .001$). These results suggest the existence of two independent phenotypes: one associated with fear conditionability and emotional expressivity and the other associated with the persistence of fear expression.

(44) Neural and behavioral effects of alcoholism on human eyeblink conditioning • Cheng, D.T.,

McCaul, M.E., Rilee, J.J., Wand, G.S., Hua, J., Qin, Q., & Desmond, J.E. (Johns Hopkins University School of Medicine) • Excessive alcohol consumption produces changes in the brain that often lead to memory impairments. Subjects with alcohol use disorders (AUD) have consistently shown a behavioral deficit in eyeblink conditioning. However, there has been no direct evidence identifying which regions of the brain may be responsible for this impairment. The present study is the first to compare conditioning-related activity in abstinent AUD subjects and healthy controls by presenting conditioning trials during fMRI scanning. AUD subjects (30-60 years old) met DSM-IV criteria for alcohol dependence, had at least a 10 year history of heavy drinking (for men, > 2 drinks/day; for women, > 1 drink/day), and were abstinent from alcohol for at least 4 weeks. Functional images were collected using an echo planar imaging pulse sequence while structural imaging

was acquired using a T1-weighted magnetization-prepared rapid acquisition gradient echo pulse sequence. CSs (750 ms) were 1000 Hz tones (95 dB) delivered binaurally; the US was a 100 ms left corneal airpuff (5 psi). Pseudoconditioning was followed by conditioning sessions (paired CS-US trials). Between-group analyses of neurovascular changes (CBF, CBV, and OEF) are ongoing. Preliminary behavioral findings indicate that AUD subjects showed fewer CRs. Greater cerebellar activations and lower whole-brain oxygen extraction fraction measurements were detected in AUD subjects relative to healthy controls. These preliminary findings suggest that the cerebellar hyperactivation by AUD subjects may be related to their learning impairments, possibly reflecting a compensatory response. Supported by NIAAA K01 AA020873 (DTC) and NIAAA R01 AA018694 (JED).

(45) Spatial navigation in adolescent rats using a land-maze task • Bonath, L.M., Chenoweth, A.M. (Hiram College) • Spatial navigation in rats has been widely studied in adult animals but not in adolescents. The purpose of this study was to extend upon the findings of a study by Faraji et al. (2009) using adolescent rats instead of adults. Male and female adolescents, aged P-28 at the start of the experiment, were placed in a ziggurat land maze and allowed to navigate to a target. The number of errors and latency was recorded as a measure of spatial memory. Eight one minute sessions were completed on odd number days and two test trials were completed on even number days. The two test trials served as a measure of spatial memory from the previous day. Males and females only differed in the number of errors made during the initial eight trials but in terms of latency, females appear to be faster than males at finding the goal. These findings contradict previous research that show that males outperform females in spatial navigation tasks so further trials need to be completed in order to verify the present study's results.

(46) Disruption of model-based behavioral control by cocaine • Jones, J.L., Wied, H.M., Cooch, N.K., (National Institute on Drug Abuse IRP and University of Maryland School of Medicine), Berg, B.A., Schoenbaum, G. (National Institute on Drug Abuse IRP) • Addiction is characterized by maladaptive decision-making, in which individuals seem unable to use adverse outcomes to modify their behavior. Adverse outcomes are often infrequent, delayed, and even rare events, especially when compared to the reliable rewarding drug-associated outcomes. As a result, recognizing and using information about their occurrence puts a premium on the operation of so-called model based systems of behavioral control, which allow one to mentally simulate outcomes of different courses of action based on a knowledge of the underlying associative structure of the environment. This suggests that addiction may reflect, in part, drug-induced dysfunction in these systems. To test whether cocaine causes deficits in model-based behavior and learning independent of requirements for response inhibition or perception of costs or punishment. We trained rats to self-administer sucrose or cocaine for 2 weeks. Four weeks later, the rats began training on a sensory preconditioning and inferred value blocking task. As in devaluation, normal performance on this task requires representations of the underlying task structure, however unlike devaluation, it does not require either response inhibition or adapting behavior to reflect aversive outcomes. Rats trained to self-administer cocaine failed to show conditioned responding or blocking to the preconditioned cue. These deficits were not observed in sucrose-trained rats nor did they reflect any changes in responding to cues paired directly with reward. These results show that cocaine disrupts the operation of neural circuits that mediate model-based behavioral control.

(47) Neural substrates involved in SKF 81297 mediated extinction enhancement • Abraham, A.D., Lattal, K.M. (Oregon Health & Science University) • Fear extinction, the process by which a conditioned fear response is suppressed, involves the formation of new inhibitory memories and dopamine D1 receptors are distributed through regions of the brain that are important to fear extinction, such as the prefrontal cortex, nucleus accumbens, hippocampus, and amygdala. We have previously shown that SKF 81297, a full D1 agonist, administered prior to or following a fear extinction session will enhance extinction retention in C57BL/6J mice. Our current experiments aim to identify the neural substrates involved in these observed extinction enhancements. Mice received contextual fear conditioning on Day 1 (four 0.35mA footshocks in a 12-min session). On Day 2, mice were returned to the context for a 12-min exposure with no footshock and given microinjections of of SKF 81297 or vehicle (phosphate buffered saline; PBS) immediately following the session. On Days 3-5, mice were returned to the context each day for 12-min non-reinforced tests. We examined the

effects of prefrontocortical and nucleus accumbens microinjections on fear extinction. A separate set of experiments examined the effect of systemic administration of SKF 81297 (10 mg/kg) or saline on the immediate early gene c-Fos, following extinction or procedural cue exposure. We found activation of c-Fos in the prefrontal cortex following systemic administration of SKF 81297, but no effect of prefrontocortical microinjections of SKF 81297 on fear extinction. Future studies will extend these findings by investigating the cellular mechanisms that guide the SFK 81297-mediated fear extinction enhancements.

(48) Poor discrimination among reward, fear and safety cues during inactivation of prelimbic cortex in rats • Sangha, S., Robinson, P.D., Davies, D.A., Greba, Q. and Howland J.G. (Department of Physiology, University of Saskatchewan) • Reliably recognizing rewards, danger and safety in the environment is important for survival. Fear and reward conditioning have been well studied independently. Much less is known about the overlapping mechanisms of reward, fear and safety learning since the three are rarely studied concurrently in the same paradigm. The prelimbic cortex (PL) mediates fear expression and guides appropriate reward seeking behavior. We used a task designed by Sangha et al (2013, J Neurosci) to clarify the PL's role in flexibly switching between fear and reward seeking behavior to learned reward, fear and safety cues. Rats (n=10) were infused with muscimol/baclofen or saline bilaterally to inactivate the PL shortly prior to assessing memory for learned reward, fear and safety cues. PL inactivation reduced fear expression (i.e., freezing) during the fear cue when compared to controls and low levels of indiscriminate freezing to all cues. In addition, PL inactivation profoundly affected reward seeking: PL inactivation reduced reward seeking to the reward cue and increased reward seeking during fear and safety cues compared to controls. Overall, PL inactivated rats showed indiscriminate reward seeking whereas controls appropriately limited their reward seeking to reward cue presentation. Our data suggest that the PL is essential in discriminating among cues signifying reward, fear and safety resulting in highly disorganized behavior when compromised. Supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to JGH

(49) Dominance status influences the social acquisition of Pavlovian fear in adult male rats • Jones, C.E., Monfils, M.H. (The University of Texas at Austin) • Most animal models of fear learning focus on creating a CS-US association through direct experience using variations of Pavlovian fear conditioning. We have previously shown that some rats will display a conditioned response (CR; e.g. freezing) to a cue after interacting with a cage-mate previously fear conditioned to a CS while this cage-mate is displaying the CR during the presentation of a CS (Bruchey et al., 2010). In the current study, we sought to further investigate the individual differences seen in this fear conditioning by-proxy (FCbP) paradigm by controlling for dominance status of the male rats. One rat from each cage of a triad was fear conditioned to a tone CS. The next day, the conditioned rat was returned to the chamber accompanied by a second cage-mate while the tone was played in the absence of the foot-shock (FCbP). Socially acquired fear was measured as freezing displayed by this second cage-mate to the CS alone on the following day. Dominance was determined using the methods of Pellis et al. (1993) by observing play behavior amongst a triad of related adult males. We then manipulated which rat of the hierarchy was directly fear conditioned or fear conditioned by-proxy. Our results show that subordinate rats express greater levels of fear when observing and interacting with fear conditioned dominant rats than dominant rats observing and interacting with fear conditioned subordinates.

(50) Acquisition and extinction of delay and trace Pavlovian fear conditioning in humans • Schultz, D.H., Balderston, N.L., Hopkin, L.S., Helmstetter, F.J. (University of Wisconsin-Milwaukee) • Pavlovian fear conditioning has been used as a model for the study of learning, memory, and emotion. Previous studies have found that delay and trace acquisition rely on a similar neural circuit, but the temporal gap between the CS and the UCS in trace conditioning requires the recruitment of additional regions to this network. Few studies have compared the neural circuits that support delay and trace extinction learning. We hypothesized that delay and trace extinction would share a similar circuit, but that extinction of a trace memory would require some additional regions. Subjects underwent fear conditioning while UCS expectancy, SCR, and BOLD were recorded in the fMRI scanner. Subjects were assigned to one of three groups: Unpaired, Delay, or Trace. Seven days later the subjects underwent extinction. During the acquisition phase the Delay and Trace groups both demonstrated learning related changes on UCS expectancy and SCR. We observed a general

conditioning effect common to both the Delay and Trace groups across several brain regions including the amygdala, ACC, and thalamus. The hippocampus and mPFC showed activity that was specific to trace conditioning. During extinction, differential responses on both UCS expectancy and SCR diminished for the Delay and Trace groups. Activity in the ACC was increased for the Delay and Trace groups. Activity in the vmPFC was specific to the Delay group. These results suggest that delay and trace acquisition and extinction rely on common brain regions, but that some regions are specifically involved in either delay or trace.

(51) Neural sources of delay and trace fear conditioning recorded with magnetoencephalography • Balderston N. L. (University of Wisconsin-Milwaukee, École Normale Supérieure, Paris, France), Tallon-Baudry (École Normale Supérieure, Paris, France), Helmstetter, F. J. (University of Wisconsin-Milwaukee, Medical College of Wisconsin) • Awareness is required for trace but not delay fear conditioning in humans. One possible explanation for this difference is that trace conditioning relies on additional neural processes. For instance, research with non-human animals suggests that both the hippocampus and the prefrontal cortex are needed for trace but not delay fear conditioning, and that communication between these areas may help to maintain the CS during the trace interval. To test this hypothesis we exposed subjects to differential delay and trace fear conditioning while recording brain activity with magnetoencephalography. According to the communication hypothesis we should see increases in theta coupling between the hippocampus and prefrontal cortex for trace but not delay conditioning. Subjects underwent 6 training blocks each of which contained 10 trials each of differential delay and trace conditioning while we recorded their brain activity with magnetoencephalography (MEG). Delay CSs were presented for 2.5 sec, while trace CSs were presented for 0.5 sec with a 2 sec trace interval. Faces and houses served as CSs and an aversive electrical stimulation served as the UCS. In order to assess implicit and explicit learning we measured pupil dilation and UCS expectancy. Subjects expect the shock on reinforced (CS+ trials, and expect no shock on unreinforced (CS-) trials. Subjects also showed increases in pupil diameter for the CS+ relative for the CS- for both delay and trace conditioning. Preliminary results from the MEG analysis suggest that there is a learning related increase in theta coherence between the right hippocampus and the inferior frontal gyrus for trace but not delay conditioning. These results suggest that communication between the hippocampus and prefrontal cortex may be important for trace fear

(52) Persistent attenuation of fear memories using reconsolidation update mechanisms and post-extinction administration of methylene blue • Auchter, A., Gonzalez-Lima, F., Monfils, M.H. (University of Texas at Austin) • Fear extinction training after a retrieval trial makes the CS less threatening during reconsolidation. While the exact mechanism remains somewhat unclear, this phenomenon essentially enhances the effectiveness with which subjects persistently diminish the conditioned fear response. The purpose of the present study was to minimize the return of persistent fear using the retrieval/extinction paradigm in conjunction with a neurometabolic enhancer, USP methylene blue (MB), shown previously to be effective in enhancing memory consolidation, including enhancement of fear extinction memory consolidation. MB serves as an auto-oxidizing compound that at low doses (0.5-4 mg/kg) improves brain mitochondrial respiration. Using a 2x2 factorial design, we predicted that both presentation of a retrieval CS (vs. no retrieval CS) before extinction and administration of MB (vs. saline) post-extinction would enhance the retention of the extinguished conditioned response. Accordingly, we expected that the combination of the retrieval CS and the post-extinction MB administration would result in the highest overall suppression of the conditioned response. Our results suggest that when subjects were re-introduced to the US after extinction and were subsequently tested for reinstatement of the fear response, MB treatment inhibited the return of conditioned fear responses in subjects that did not receive the retrieval CS, whereas the saline controls showed a significant return of fear in the no retrieval condition. It appears that all subjects in the retrieval condition reach floor levels of the fear response after reinstatement, regardless of MB treatment. Therefore, MB had beneficial effects facilitating extinction for treatment groups displaying less effective extinction.

(53) An unexpected role of adult hippocampal neurogenesis in trace fear conditioning • Drew, M.R. (University of Texas at Austin), Seo, D.O. (University of Texas at Austin), Carillo, M.A. (Columbia University), Thakkar, K. (University of Texas at Austin) • Engaging in a trace conditioning task rescues

newborn hippocampal neurons from death (e.g., Gould et al., 1999; Shors, 2004), but are these newborn neurons necessary for trace conditioning? Several studies suggest that arresting adult neurogenesis impairs trace conditioning, but these studies used methods that arrest cell proliferation throughout the body, not just neurogenesis in the hippocampus. We revisited the role of adult neurogenesis in trace conditioning using methods that may provide a more specific arrest of adult hippocampal neurogenesis. One method is targeted, low-dose x-irradiation. The second is a novel transgenic mouse, the DCX-TK mouse, in which an inducible suicide gene is expressed specifically in newborn neurons and their lineage-restricted progenitors. Irradiated and sham-irradiated mice were conditioned 6–8 weeks following irradiation using delay or trace fear conditioning procedures. Irradiated and control mice displayed similar levels of tone fear in both delay and trace procedures. However, an unexpected difference in context-elicited fear emerged. Control but not irradiated mice displayed less context fear after trace conditioning than after delay conditioning. Moreover, in the trace procedures, irradiated mice exhibited significantly more context fear than controls. To confirm this unusual phenotype, DCX-TK and WT mice were trace conditioned 3 weeks after the inducible arrest of neurogenesis. Similar to irradiated mice, DCX-TK mice displayed normal fear of the trace CS but increased context fear compared to WT controls. The results suggest that adult neurogenesis is not absolutely necessary for trace conditioning. However, the arrest of neurogenesis may change the neural and psychological mechanisms through which trace and context memories are acquired or expressed.

(54) Involvement of noradrenergic transmission in the immediate extinction deficit in rats • Seemann, J.R., Fitzgerald, P.J., Maren, S. (Texas A&M University) • Individuals with post-traumatic stress disorder (PTSD) show deficits in their ability to “extinguish” traumatic fear memories. In rats, this form of learning can be modeled with extinction procedures in which an innocuous cue that has been paired with an aversive footshock is presented alone many times. Previous studies in our laboratory have shown that recent fear is particularly resistant to extinction—an immediate extinction deficit that has parallels with the extinction impairments in PTSD. Work by Arnsten and colleagues suggests that the neurotransmitter norepinephrine (NE) is pathologically elevated in response to psychological stress. Here we examined whether variable agonism or antagonism of α and β adrenoceptors might mitigate the immediate extinction deficit. Immediately after conditioning one group of rats (Immediate) received a systemic injection of the alpha 1 noradrenergic receptor antagonist, prazosin (Exp. 1); cirazoline, an α -1 agonist and α -2 antagonist (Exp. 2); propranolol, a β -1 and 2 antagonist (Exp. 3); or vehicle. Thirty minutes later the immediate groups underwent extinction. Additional groups (Delay) received drug or vehicle prior to an extinction session 24 hours after conditioning. All groups were tested for extinction retention 24 hours later. Prazosin elevated freezing during the extinction session, particularly in the “immediate” rats, but did not strongly influence retention. Cirazoline significantly impaired within session extinction in the delay animals. Propranolol impaired the retention of delay extinction, while enhancing the retention of immediate extinction. In a subsequent experiment (Exp. 4), propranolol’s effect on immediate extinction was related to interference with consolidation of the original fear memory. In summary, contrary to our expectations, NE antagonism of alpha adrenoceptors acutely augmented fear and did not mitigate the immediate extinction deficit, while blockade of beta adrenoceptors appeared to do the opposite, though these effects may be due to impairment in the consolidation of the original fear memory.

(55) Conditioned orienting behavior predicts attenuation of conditioned responses after satiation • Olshavsky, M.E., Monfils, M-H., Lee, H.J. (The University of Texas at Austin) • When a neutral conditioned stimulus (CS) is paired with an unconditioned stimulus (US), animals often acquire CS-directed responses, for example, approaching/orienting to a light predictive of food (Brown and Jenkins, 1968; Holland, 1977). Under certain conditions, only a subset of animals acquires CS-directed behaviors (aka sign-tracking) in addition to, or at the cost of, developing US-directed behaviors (aka goal-tracking) that ultimately lead to the obtainment of a rewarding US. CS-directed behaviors likely reflect enhanced attentional, emotional, and/or motivational processing of the cue (Holland, 1977; Robbins and Everitt, 1996; Cardinal et al., 2002) and represent how the cues themselves can acquire incentive value (Robinson and Berridge, 2001). Thus, CS-information processing is likely to be different among animals displaying enhanced CS-directed behaviors, as our earlier studies suggest (Olshavsky et al., 2012 a&b, 2011). In the current study, we examined whether reducing US value might have greater influence on CS-information processing among the rats that acquired

CS-directed behaviors. In our study, when a light was repeatedly paired with food, some rats acquired robust CS-directed orienting response (“Orienters”) while others did not (“Nonorienters”). However, both groups displayed comparable US-directed food cup behavior. After conditioning, the rats were allowed to be satiated with the US. Then, they were presented with the CS again. Only the satiated Orienters showed significantly reduced conditioned responses, both orienting and food cup approach, compared to the Orienters that were not satiated. Thus, reducing US value significantly lowered both CS- and US-directed responses among Orienters only.

(56) Touch screen operant testing implicates adult hippocampal neurogenesis in reversal learning but not spatial discrimination learning • Swan, A.A., Darr, A., Turns, C., Raad, M., Shue, F., Patel, Y., Mallick, S., Patel, Y., Karnkowska, B., Nguyen, M., & Drew, M.D. (University of Texas at Austin) • The dentate gyrus is implicated in pattern separation, which is the separation of overlapping patterns of neural activation into orthogonal representations. This coding is believed to enable behavioral discrimination among similar stimuli, a process we term “pattern discrimination.” To investigate the role of DG in pattern discrimination, we used touch-screen equipped operant chambers in which a pair of square-shaped stimuli was presented at either a large or small spatial separation on the screen. Mice were reinforced to nose-poke the initial correct location (left or right) until a behavioral criterion was met, and then a reversal occurred. A previous study found that neurogenesis-arrested animals exhibited deficits in this task at the small, but not large, spatial separation. However, it was unclear whether the deficit reflected an impairment in the ability to discriminate spatial locations or an impairment in reversal learning. To investigate the role of adult neurogenesis in these distinct processes, we assessed pattern discrimination in transgenic mice in which adult neurogenesis was inducibly arrested. Relative to controls, mice without ongoing neurogenesis had deficits at both the large and small spatial separations in the touch screen task. This effect was present 5 weeks but not 1 week after the arrest of neurogenesis, suggesting that the deficit depends on the loss of adult-born neurons at least 5 weeks old. Moreover, the deficit was more pronounced after a reversal than prior to one. The results suggest that adult neurogenesis enables cognitive flexibility rather than pattern discrimination/separation per se. Furthermore, this work confirms that touch-screen operant testing is a very flexible and sensitive method for assessing cognitive ability in mice.

(57) Running wheel escape learning in the earthworm and possible time-of-day effect • Wilson, W. J., Paxton, H. R., Baguzis, M. J. (Albion College) • Yerkes (1912) suggested that an earthworm could learn a T-maze, but Rosenkoetter & Boyce (1975) suggested that pheromonal signals, not learning, were responsible for the maze behavior. Few have studied instrumental learning in the earthworm since that discovery. We assessed escape learning in *Lumbricus terrestris* by allowing worms to turn off an aversive white light by crawling in a “running” wheel. Sessions lasted 11.67 hrs: 40-min acclimation then 11 hrs during which the light would turn on and remain on until the Escape worm turned the wheel approximately 3.5 cm. This response turned off the light for a period of 60 sec; any subsequent response occurring before the minute had elapsed would reset the 60-sec timer. Yoked control worms received the same light as the Escape worms regardless of their responses. Responses of both worms were recorded. Escape worms, in which light offset was contingent on responding, crawled more than Yoked worms. The effect appears greatest in worms whose sessions began around 0830, and was less pronounced in the pairs of worms whose sessions began at around 2030, despite the fact that worms were housed and run in a basement room with few obvious zeitgebers. We continue to examine the potential time-of-day effect, and will report our complete results at the meeting. When offset of an aversive light is contingent on the earthworm's crawling response, that response increases. This represents clear evidence of instrumental learning in the earthworm. Support from Albion College Foundation for Undergraduate Research, Scholarship, and Creative Activity

(58) Intra-basolateral amygdala infusions of the GAP Junction blocker carbenoxolone impairs auditory and contextual fear conditioning • Nocera, N.A. (UCLA), Bissiere, S. (Monash University), Fanselow, M.S. (UCLA) • Parvalbumin containing interneurons are electrically coupled by connexin36 gap junctions resulting in coordinated activity of these inhibitory neurons. Hippocampal gap junctions play a critical role in the network activity necessary for learning. For example, blocking hippocampal gap junctions

causes a reduction in theta activity and contextual fear conditioning. The amygdala is also rich in these gap junctions but to date a functional role has not been established for these electrical synapses in learning and memory. Therefore we infused the gap junction blocker carbenoxolone into the basolateral amygdala of rats either prior to, or immediately after, auditory fear conditioning and then tested both contextual fear and tone fear. Consistent with previously reported findings in the hippocampus administration at either time point abolished contextual fear conditioning. However, unlike hippocampal application, pretraining or pretesting infusions into the BLA abolished both acquisition and expression of tone fear. Post-training infusions also attenuated later expression of tone fear. These findings implicate gap junctions in the acquisition and consolidation of fear memory.

(59) Neural correlates of appetitive Pavlovian conditioning • Jorge Avila, Veronica Sebastian, Peter Serrano (Hunter College) & Andrew Delamater (Brooklyn College) • Through Pavlovian conditioning, a cue can come to access representations of a biologically significant event. The formation of such an association depends on the way the stimuli are delivered in relation to one another. In this experiment, rats received deliveries of a tone and sucrose. For half of the rats the delivery of the sucrose was contingent upon the presentation of the tone. The rest received random presentations of the tone and sucrose, such that the delivery of sucrose was equally likely during each second of the session. Responding to the tone was then assessed in the absence of sucrose, at which point rats were sacrificed to measure expression of the immediate early gene c-FOS. Results showed that activity in the substantia nigra and nucleus accumbens differed according to type of training, within tyrosine hydroxylase positive neurons. Thus the striato-nigral pathway may reflect differences in the

(60) Acquisition of action tendencies in complex learning situations • Kryptos, A.-M., Eftting, M., Kindt, M. (University of Amsterdam) & Beckers, T. (University of Amsterdam, KU Leuven) • According to bio-informational theory, a behavioral response is a basic component of complex emotional responding. Previous research has shown that an avoidance tendency towards a predictor of danger can be acquired following traditional differential fear conditioning. A series of studies have examined the subtlety of these acquired action tendencies following a complex learning paradigm, where both elemental and compound stimuli have been conditioned. Participants received forward blocking (A+, AB+) and protection-from-overshadowing (C-, CD+) training. Responding to the secondary cues (B and D) was of primary interest. We expected that participants would be slower in avoiding a redundant predictor of danger (the blocked B) than a superior predictor of danger (the protected-from-overshadowing D) in a symbolic approach-avoidance task, while faster in approaching B than D. The data suggests that differential action tendencies may be conditioned even to stimuli, whose threat value can only be inferred through their associations with other previously encountered stimuli. The paradigm might offer a more ecologically valid analogue procedure for the acquisition of phobic fear and opens new avenues for further research into fear responding.

(61) Treatment with 5HT and NMDA induces stepping in spinally transected adult rats while maintaining spinal cord plasticity • Strain, M.S., Lee, K.H., Niemerski, A.L., Huang Y.J. & Grau, J.W. (Texas A&M University) • Previous research suggests that drug treatments (e.g., 5HT) that exert a protective effect on spinal plasticity can generate locomotor behavior. Some of these drugs (e.g., 5HT) have been used in combination with locomotor training to help facilitate recovery. A common drug cocktail used to induce locomotion involves a combination of 5HT and NMDA. However, few tests of the effect of this drug cocktail on an in vivo rat model have been tested. Further, work has shown that acute treatment with NMDA can inhibit adaptive plasticity, and implicated the NMDA receptor in the regulation of nociceptive behavior. Here we sought evidence that a 5HT/NMDA cocktail can induce locomotor behavior in adult Sprague-Dawley rats without inducing behavioral signs of nociceptive sensitization impairing spinal plasticity. After an intrathecal (i.t.) injection of a drug cocktail consisting of 5HT (0, 25, 100 and 400 nmol) and/or NMDA (0, 0.5, 2, and 8 nmol), the Hindlimb activity of fully transected rats was recorded for a 30-min and scored via DVD playback. Mechanical reactivity was examined using von Frey microfilaments at time 0 and 2 hours following injection. The next day, spinal plasticity was assessed using an instrumental learning paradigm (Grau et al., 1998, *Behav Neurosci*, 112, 1366), which measured learning as an increase in flexion duration reducing net shock exposure. Results showed that serotonin induced a dose-dependent increase in activity. Stepping behavior was only seen

at the highest combined dose of 5HT and NMDA. Importantly, no combination affected mechanical reactivity, or had a long-term effect on adaptive spinal plasticity. This work supported by HD058412 to J. Grau.

(62) Interaction between pre- and post-shock context exposure in single-trial contextual fear conditioning • Bernier, B., Lacagnina, A., and Drew, M.R. (University of Texas at Austin) • Contextual fear conditioning (CFC) is a hippocampus-dependent form of Pavlovian conditioning in which an animal learns to associate a specific context with an aversive stimulus such as a footshock. CFC requires experience with the context preceding shock presentation; a shock delivered immediately after placing the animal within a novel context often produces little conditioning. This phenomenon, known as the immediate shock deficit (ISD), has been thought to result from the animal's inability to form a mental representation of the context in the limited time prior to shock delivery. Experiments from our lab suggest another mechanism that may contribute to the ISD: rapid extinction of context memory. We found that immediate shock presentation can produce significant fear conditioning when the post-shock interval is short (30 sec). However, when the post-shock interval was extended to 3.5 minutes mice exhibited little contextual fear when tested 24 hours later. When mice were retrained following 5 sessions of extinction, mice that were initially conditioned with 30 sec or 3.5 min post-shock intervals exhibited equivalent savings, suggesting that post-shock context exposure causes extinction, not erasure of context fear memory. Interestingly, pre-exposure to the context on the day before conditioning, but not on the same day, prevented immediate extinction, preserving fear conditioning in animals experiencing the 3.5 min post-shock exposure. Together, these findings suggest that consolidation-dependent memory processes associated with context pre-exposure can inoculate against immediate extinction. CFC appears to involve both learning and immediate extinction processes; the relative dominance of these processes may depend on the the timing and amount of context exposure before and after the US.

(63) Timing in the absence of supraspinal input: Integration of fixed spaced stimulation • Lee, K.H., Strain, M.M., Huang, Y.J., Grau, J.W. (Texas A&M University) • A basic property of neural systems involves the abstraction and storage of information regarding the temporal distribution of events over time, though how the nervous system is able to abstract and store that information is not always clear. Prior work has shown that spinal neurons can discriminate fixed (FT; ISI: 2 s) versus variable (VT; ISI: 0.2-3.8 s) shock and these two forms of stimulation have divergent effects; variable stimulation inhibits learning about response-outcome (instrumental) relationships whereas fixed stimulation fosters instrumental learning and both prevents and reverses the adverse consequences of variable stimulation. Interestingly, the beneficial effect of FT stimulation is only observed after extended training (>540 shocks). Additionally, fixed spaced shocks need not occur as a single block of continuous shock to produce beneficial effects, two blocks of 360 FT shocks separated by 24 hrs also produce the FT effect. Further, when two blocks of 360 FT shocks are administered across two dermatomes (tail and leg), the FT effect still occurs, implicating that the FT shocks are being integrated. Additionally, subjects given randomly alternating stimulation were able to acquire the instrumental learning response. These results imply that spinal mechanisms are not only able to produce savings across time; it can also "infer" missing stimuli from a fixed spaced schedule of stimulation. Future studies aim to determine what spinal mechanisms underlie these effects.

(64) A Bayesian context fear learning algorithm/automaton • Krasne, F.B (UCLA) • A computational neural model composed of hippocampus, cortex, and amygdala has been constructed that emulates many aspects of context fear conditioning, as well as predicting unknown features. It is assumed that the attributes of a context are observed serially in random order and are generally incomplete. The model utilizes commonly discussed ideas about hippocampus and associated circuitry. However, it adds conjectured circuitry that controls aspects of the functioning of the hippocampus, cortex, and amygdala. This circuitry computes the Bayesian Weight of Evidence that the automaton is or is not in a familiar place based on comparisons between observed attributes of the context it is currently in and attributes associated with representations of places it has formerly been. The outcome of these computations control the functioning of the hippocampus and related circuitry to determine whether a new hippocampal representation of the current context will be created, whether an existing representation will be updated with information about newly observed contextual attributes, whether new conditioning will be allowed, and whether previously learned fear will be expressed.

(65) Aging reduces basal neuronal activation as measured by immediate early gene expression within medial prefrontal cortex and hippocampus • Sehgal, M., Bula, T. S., Detert J. A. & Moyer Jr., J. R. • We recently demonstrated that extinction of trace fear conditioning is impaired during normal aging and that these deficits are accompanied by altered intrinsic excitability of mPFC neurons (Kaczorowski et al., 2012). Since aging-related changes in the expression of memory permissive genes (e.g. immediate early genes or IEGs) may be useful markers altered basal neuronal activity and early cognitive decline, we studied Zif-268 and Arc expression within distinct subregions of mPFC (IL and PL) and hippocampus (dorsal and ventral) during normal aging. Beginning in middle age, both Western blots and immunohistochemistry revealed a significant decrease in Zif-268 expression in IL and PL. Within hippocampus, Zif-268-ir neurons were significantly decreased in both CA1 and DG of dorsal hippocampus and DG of ventral hippocampus during aging. Western blots indicated a trend toward an aging-related decrease in Zif-268 expression within ventral but not dorsal hippocampus. Aging-related changes in Arc expression were observed within mPFC, but only in aged IL. Earlier changes were observed in dorsal hippocampus, with Arc expression being significantly reduced in both middle-aged and aged rats. These studies illustrate differential and region-specific changes in the expression of Zif-268 and Arc that emerge during normal aging. Given the role of IEG expression in neuronal plasticity, the early emergence altered expression of IEGs may serve as markers for early onset of cognitive decline. Finally, these data necessitate an in-depth analysis of how changes within mPFC and hippocampus interact and contribute to various aging-related impairments such as those observed in trace fear conditioning and extinction.

(66) Neural firing of the amygdala central nucleus during lower than expected rewards • Iordanova, M.D. (University of Maryland), Deroche, M.L.D (Johns Hopkins University), Esber, G.R., Sadacca, B., Schoenbaum, G. (National Institute of Drug Abuse) • The firing pattern of neurons in the central nucleus of the amygdala (CeA) was examined during learning two conditions, extinction and overexpectation, which lead to a loss of conditioned responding to a previously reinforced cue. Rats were trained to expect a reward following the presentation of each of three auditory cues and one visual cue. Subsequently, one of the auditory cues was paired with the visual cue to generate the expectation of double reward, yet only a single reward was delivered, yielding the overexpectation condition. In addition, the other two cues were presented in compound with a second visual cue, which was never reinforced. Each one of those compounds resulted in the delivery of either a single or no reward, yielding the control and extinction conditions. Compared to the control compound, neural firing in the CeA during the overexpectation and extinction compounds was higher and lower, respectively. Interestingly, the difference in neural firing seen to the overexpectation and control compounds was reversed at time of reward and immediately post reward, showing greater firing to a seemingly identical outcome in the control condition compared to the overexpectation condition. Neural firing at time of reward omission in the extinction condition did not differ from baseline. These results suggest a role for the CeA in regulating learning on the basis of expected rewards and provide evidence that the loss of responding to predictors of reward seen in extinction and overexpectation is not processed identically at the neural level.

(67) The contribution of adult-generated granule cells in generalization of fear to novel auditory stimuli • Barrera, V. R., (UCLA), Yee, H. (UCLA), Hodul, M. S. (UCLA), Canelos, V. (UCLA), Iwamoto, K. (UCLA), Nakashiba, T. (MIT), Tonegawa, S. (MIT), Fanselow, M. S. (UCLA) • The dentate gyrus contains mature and immature populations of granule cells that have been functionally implicated in tasks that require the ability to form distinct memories for similar experiences by generating different memory engrams. Fear conditioned animals often show robust generalization to novel auditory cues presented at test and this effect can be degraded with post-training lesions of the dorsal hippocampus, or enhanced in conditional knockout mice that lack all post-natal neurogenesis. C57/BL6 mice were fear conditioned with one auditory stimulus (2800Hz, or 1000Hz, or white noise) and then tested for generalization of fear to the trained or one of the untrained stimuli. Next, we used DG-TeTx triple transgenic mice in which the output of mature GCs can be selectively silenced by the conditional expression of tetanus toxin (TeTx), while leaving immature GCs intact in combination with focal x-irradiation to eradicate immature GCs. The pattern of generalization observed did not conform to a linear domain such as frequency but may be more abstract in nature, where mice learn a rule that

tones signal aversive outcomes and then apply this rule to novel stimuli. These suggest an interplay between mature and immature GCs in the dentate is crucial for the generalization of fear to novel stimuli.

(68) Characterization of hippocampal neural activity using catFISH during acquisition and extinction of conditioned taste aversion • Sánchez-Carrasco, L., (Universidad Nacional Autónoma de México), , Lozano-Flores, C., (UNAM Juriquilla), Salazar, J., Martínez, D., Valadez, A., Enríquez, K. (UNAM), Ramírez-Amaya, V. (UNAM Campus Juriquilla) • To achieve extinction, a new association that interferes with the previous one is build (Rescorla, 2001). The post-acquisition effect (León, Callejas-Aguilera, Rosas, 2012) as well as renewal (Bouton, 1994) in conditioned taste aversion (CTA) suggests that new associations and extinction integrate context information. To evaluate this hypothesis, we trained rats in CTA learning by pairing sucrose with lithium chloride (LiCl). One group was sacrificed immediately after the acquisition trial (Acq-Fwd), another, the Acq-2 group was sacrificed after two conditioning trials. Groups Ext-1 and Ext-5 received one day of recovery and then were expose to sucrose for either 1 or 5 consecutive days, and then sacrifice after sucrose and LiCl exposure, which occurred the next day after the last extinction trial. Animals with water restriction (WD-Ctrl) and animals sacrificed from their home cages were used as controls. The brains were processed for in situ hybridization for Arc mRNA to perform “catFISH” image analysis. The behavioral results indicated progressive extinction of the taste aversion association throughout the five trials. The “catFISH” results revealed activation of CA1 hippocampal neurons from Ext-5 animals, but particularly clear were the CA1 neurons from those trained in CTA for two trials (Acq-2). These cells were predominantly double activated cells, suggesting that after the second experience with the flavor, associative neural activity occurred in the CA1 hippocampal network. This suggests that during sustained acquisition and extinction the hippocampal network process associative information, which may explain how context information is incorporated into the taste-visceral association.

(69) Context pre-exposure, inter-trial intervals and contextual conditioning in appetitive Pavlovian conditioning • Carranza-Jasso, R. (UNAM), Urcelay, G. (Cambridge Univ), Nieto, J. (UNAM) & Sánchez-Carrasco, L. (UNAM) • During the past decades, a most relevant research topic in the associative learning theory has been the analysis of the factors that propitiate learning about the context where the experimental task takes place. Research in this matter has shown that a central factor related to the learning between events and between context and such events is the inter-trial interval (ITI) length. Particularly, ABA renewal allows exploring the effect of contextual conditioning in the subsequent extinction and recovery of the trained response therein. In order to determine the effect of using different ITI lengths (50s vs. 1440s) over the Context-US association’s strength during the acquisition phase and the effect of this strength in the subsequent response, four experiments were designed. First we explored if the different ITIs revealed differential contextual conditioning (Exp1). We also sought to determine whether imposing a context-pre-exposure phase previous to the acquisition phase (Exp. 2) as well as if extinguishing the acquisition context (Exp. 3) or manipulating the number of context-food trials in the pre-exposure phase (Exp. 4) had any effect on contextual conditioning. We randomly assigned 12 Wistar strain rats per group (6 males and 6 females). The results show that high levels of contextual conditioning can be found if short ITI lengths are used during training and that such conditioning doesn’t interfere with the CS response level. The context pre-exposure was shown to be an important variable to observe this contextual conditioning and such context conditioning was found to be very susceptible to be extinguished. Experiments funded by CONACYT Graduate Scholarship 249617 and PAPIIT projects IN304411, IN307113 and IN307413. We would like to thank Alexis Martínez-Ramírez, Alma Delia Pérez-López & Arlette Carrillo-Sulub for helping with the conduction of experimental sessions.

(70) The use of noise in the establishment of escape and avoidance nose poke responding in mice • Lewon, M., Hogue, S., Porter, M., Van Dam, S., Stites, M., Senko, A., & Parrott Hayes, L. J. • Previous research has indicated that noise can be used as an aversive stimulus to establish escape responding in mice and rats. Most of these studies, however, have required subjects to move to a particular area within the experimental space (e.g. a platform) to terminate the noise. The present study investigated whether or not escape and/or avoidance responding on a nose poke apparatus could be established and maintained with mice via a signaled avoidance procedure in which loud noise served as the putative aversive stimulus. The rates of

escape and avoidance responding were examined across three different conditions in which the type, intensity, and method of noise presentation were varied. Our results indicated that escape nose poke responding could be established, though the rates of escape responding decreased both within and across training sessions. Escape responding in subsequent sessions temporarily recovered when the type of noise or method of noise presentation was varied. Consistent avoidance responding was not observed. These results support the existing literature indicating that noise is a relatively mild aversive stimulus and suggest that both intra- and inter-session habituation appears to attenuate the extent to which the termination of noise functions as a reinforcer.