Pavlovian Society 2021

Sept 30 - Oct 2
Ann Arbor, MI

Program

Thursday, September 30th - Huron Room, Graduate Hotel, Ann Arbor

- 6pm – 10pm: Opening Reception - Snacks and Cash Bar

Friday, October 1st – 4th Floor Rackham Building, University of Michigan

- 7:30: Breakfast, Rackham 4th floor - Assembly Room and East/West conference rooms
  *** Move to the Rackham Amphitheater ***
- 8:20 am: Welcome and Opening Remarks by Natalie Tronson
- 8:30 am: Tracey Shors, "Ruminating on Memory" (virtual)
- 9:10 am: "Now you CB, now you don't - endocannabinoid modulation of associative learning processes" – Symposium chaired by Rebecca Shansky and featuring:
  - Leah Mayo: "Endocannabinoid function as a target to promote fear extinction in humans" (virtual)
  - Kylie Huckleberry: "Endocannabinoid-mediated sex differences in context-evoked fear generalization" (virtual)
  - Samuel Bacharach: "The role of VTA cannabinoid receptor-1 in modulating dopamine and sign-tracking in rats"
- 10:10: Coffee Break
- 10:25 am: Kate Wassum, "Corticoamygdala contributions to reward learning and decision making"
- 11:00 am: "Threat avoidance: Novel models, new insights" – Symposium chaired by Anthony Burgos Robles and featuring:

  - Maria Diehl: "Acquisition and expression of platform-mediated active avoidance under social conditions in male and female rats"
  - Fabricio Do-Monte: "Neural correlates and determinants of approach-avoidance conflict in the prelimbic prefrontal cortex"
  - Anthony Burgos Robles: "Novel tasks to revisit prefrontal involvement in threat avoidance and safety learning"
  - Avishek Adhikari: "Coordination of escape and spatial navigation circuits orchestrates versatile flight from threats"

- 12:20 pm: Lunch (on your own) / Executive Committee Meeting (West Conference Room)

- 1:50 pm: "Naturalistic learning behavior" – Symposium chaired by Andrew Fink and Carl Schoonover and featuring:
  - Ahmed El Hady: "Learning to forage in uncertain environments"
  - Marissa Applegate: "Flexible use of memory by food-caching birds"
  - Matthew Rosenberg/Tony Zhang: "Rapid learning, sudden insight, efficient exploration, and resistance to perturbation in mice navigating complex environments"
  - Athena Akrami: "From high-throughput semi-automated training to naturalistic study of working memory." (virtual)

- 3:10 pm: Jeansok Kim,"Fear conditioning in natural settings?"

- 3:30 pm: Coffee Break

- 3:45 pm: Discussion Panel: How to Move Forward after COVID featuring Tracey Shors, Michael Drew, Shelly Flagel, and Amy Arguello (hybrid)

- 4:40 to 4:45 pm: Move to Breakout Groups - Rackham East & West Conference rooms; Assembly hall;

- 4:45 pm: Breakout Discussion Groups (with Snacks)
  - Table 1 - Complex roles of dopamine in learning. Hosts: Nicole Ferrara & Gunes Kutlu (hybrid)
  - Table 2 - What's up with fear conditioning? Hosts: Justin Moscarello, Moriel Zelikowsky, Stephen Maren (hybrid)
  - "Table" 3 - Models of post-traumatic stress disorder. Host: Dayan Knox (Virtual only)
  - Table 4 - Science careers outside academia. Hosts: Katie Leaderbrand, Jennifer Perusini (hybrid)

  - Table 5 - Sex as a biological variable / Sex differences in learning. Hosts: Abha Rajbhandari, Rebecca Shansky (hybrid)
  - Table 6 - Are spikes good for anything aside from calcium entry? Host: Kamran Diba

- 5:30 pm: Poster Session, Rackham Assembly Hall (with Snacks)
- 7:00 pm: Dinner (on your own)

Saturday, October 2nd - 4th Floor Rackham Building, University of Michigan

- 7:30 am: Breakfast, Rackham 4th floor - Assembly Room and East/West conference rooms

  *** Move to the Rackham Amphitheater ***
- 8:25 am: Welcome and Opening Remarks by Natalie Tronson
- 8:30 am: Gavan McNally, "Punishment" (virtual)
- 9:05 am: "Exploring the richness of associatively-activated event representations through mediated learning"
- Symposium chaired by Alex Johnson and featuring:
  - Alex Johnson: "Optogenetic stimulation of ventral tegmental area dopamine cells augments representation-mediated devaluation"
  - Giovanni Marsicano: "Cannabinoid CB1 receptors control incidental learning" (virtual)
- 9:45 am: Marieke Gilmartin, "Prelimbic encoding of threat-related stimuli across the estrous cycle"
- 10:05 am: Coffee Break
- 10:20 am: "The increasingly diverse role of midbrain dopamine neurons in reinforcement learning" – Symposium chair by Benjamin Seitz and featuring:
  - Benjamin Seitz: "Prediction(less) Errors: Probing the role of midbrain dopamine in backward conditioning"
  - Aaron Blaisdell: "The role of dopamine and higher order conditioning in rational inference"
  - Ronald Keiflin: "From prediction to action: Dissociable roles of ventral tegmental and substantia nigra dopamine neurons in reward learning"
  - Anna-Lena Schlenner: "Midbrain dopamine neurons represent outcome omission as an event of opposite valence" (virtual)

  - Rachel Lee: "Explaining dopaminergic response heterogeneity as a reflection of cortical state representation" (virtual)

- 11:55 am: Women in Learning Luncheon (at Pizza House) Registration required.
- 1:45 pm: "The predictive and causal relationship between cognitive endophenotypes and substance use"
- Symposium chaired by Caitlin Orsini and featuring:
  - Hongjoo Lee: "Role of conditioned orienting and endogenous hormones in female drug-preference" (virtual)
  - Donita Robinson: "Adolescent ethanol exposure reduces flexibility in animal models of behavioral choice" (virtual)
  - Donna Calu: "Probing SUD vulnerability: Amygdala contributions to sign- and goal-tracking flexibility differences"
  - Caitlin Orsini: "Chronic cocaine causes age-dependent increases in risk taking in rats of both sexes"
- 2:55 pm: Coffee Break
- 3:15 pm: Presidential Symposium featuring:
  - Kent Berridge: "Value Beyond Prediction: When motivational value decouples from predicted outcome value."
  - Hilary Marusek: "Childhood trauma exposure, endocannabinoid signaling and fear extinction recall in children and adolescents"
  - Jonathan Morrow: "Individual differences in human Pavlovian conditioned approach behaviors: sign-tracking and goal-tracking"
- 4:30 pm: Past President Lecture by Catharine Rankin, "Questioning the dual process theory" (virtual)
- 5:15 pm: Virtual Poster Session, GatherTown (With Snacks available at Rackham)

7:00 pm: Break - Return to Graduate Hotel

- 7:30 pm: Banquet - Terrace Ballroom, The Graduate Hotel. Featuring a discussion panel "Pavlov on the Conditional Reflex: Papers, 1903-1936" with Olga T. Yokoyama (Translator) and Daniel P. Todes (Historian), facilitator and speaker Michael Fanselow
- Awards Ceremony & Closing

ABSTRACTS

Abstracts are grouped into three categories: Symposia & Invited Speakers, In-Person Posters (p. 22), and Virtual Posters (p. 52). Within each category, abstracts are arranged alphabetically by first author.

Symposia & Invited Speakers

Applegate MC, Aronov D - *Flexible use of memory by food-caching birds* - Columbia University - A hallmark of human episodic memory is "flexibility" – the ability of a single memory to influence different behaviors in pursuit of different goals. It is unknown to what extent memory in other animals is similarly flexible. To address this question, we studied spatial behaviors of a specialist food-caching bird, the black-capped chickadee. These birds naturally pursue different goals at different moments in time as they explore an environment, cache food, and later retrieve caches. We designed a behavioral setup to engage these behaviors and track them automatically. We also used probabilistic modeling to disentangle the contributions of memory-guided and non-mnemonic strategies to the behavioral choices made by chickadees in this setup. We find that memories of the contents of individual cache sites are used by chickadees in a context-dependent manner. During caching, chickadees avoid sites that already contain food, resulting in an even distribution of caches throughout the environment. During retrieval, they instead efficiently navigate to such occupied sites to obtain food. Therefore, a single memory can be used by a chickadee to achieve at least two unrelated behavioral goals. These results demonstrate memory flexibility in an animal in a tractable spatial paradigm. - NSF GRFP (MCA), NIH T32 EY013933 (MCA), NIH Director's New Innovator Award: DP2-AG071918, New York Stem Cell Foundation – Robertson Neuroscience Investigator Award, and the Beckman Young Investigator Award

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Bacharach SZ, Stapf CA, Cheer JF, Calu DJ - *The role of VTA cannabinoid receptor-1 in modulating dopamine and sign-tracking in rats* - University of Maryland, School of Medicine - Sign-tracking rats show enhanced cue sensitivity prior to drug experience that predicts greater discrete cue-driven drug seeking compared to goal-tracking rats. Cue-evoked dopamine (DA) in the nucleus accumbens (NAc) is a neurobiological signature of sign-, but not goal-tracking. Here, we examine an important, yet understudied regulator of the dopamine system; endocannabinoids, which bind the cannabinoid receptor-1 (CB1). We use pharmacological, optogenetic, and fiber photometry approaches to test the hypothesis that ventral tegmental area (VTA) CB1 receptor signaling regulates NAc DA levels to control sign-tracking. We trained rats in a Pavlovian lever autoshaping task to determine their sign- or goal-tracking groups before testing either the effect of VTA CB1 inhibition or the effect of NAc dopamine terminal inhibition on sign-tracking behavior. We first show that intra-VTA injections of rimonabant, a CB1 receptor inverse agonist, during Pavlovian lever autoshaping reduces sign-tracking. Specifically, rimonabant decreases behavior towards the

lever and increases behavior towards the foodcup in sign-tracking rats. Second, we use transgenic TH::Cre rats expressing light-activated inhibitory halorhodopsin in the VTA to demonstrate that dopaminergic projections from VTA to NAc contribute to lever, but not foodcup directed behaviors. Optogenetic inhibition of dopaminergic terminals in the NAc specifically decreased lever-directed behaviors of sign-tracking rats. Lastly, to understand if CB1 inhibition decreases sign-tracking through a dopaminergic mechanism, we use fiber photometry in combination with the dopamine sensor, GRABDA to test the effects of intra-VTA rimonabant on NAc DA dynamics during sign-tracking behaviors. Surprisingly, we find that rimonabant-induced decreases in sign-tracking are not related to cue-evoked NAc dopamine activity. Through these experiments, we aim to elucidate novel molecular and circuit-based mechanisms that contribute to addiction susceptibility in sign-tracking rats. -

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Blaisdell AP - *The role of dopamine and higher-order conditioning in rational inference* - UCLA - Many types of cognition are thought of as the product of rational processes, often contrasted with behavior governed by bottom-up associative processes. Examples include cognitive maps and causal inference. Instead of viewing rational and associative processes as antagonistic, they can be conceived as mutually supportive. I discuss research from three domains: spatial cognition, timing, and causal reasoning, and show how associative processes largely supported by dopaminergic activity can build the representational architecture upon which rational processes of inference and insight can emerge. This synergistic, synthetic framework where rational processes build on associative ones should displace the antagonistic framework that dominates certain sectors of psychology. Further, I discuss how this framework shifts our understanding of dopamine away from simple reinforcement learning and towards supporting more complex, rational cognition. -

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Burgos-Robles A - *Novel tasks to revisit prefrontal involvement in threat avoidance and safety learning.* - University of Texas at San Antonio - Growing evidence implicates the medial prefrontal cortex (mPFC) in both threat avoidance and safety learning. However, the role for distinct mPFC divisions – infralimbic (IL) and prelimbic (PL) – still remain a matter of debate. We revisit the role of these brain regions using novel behavioral paradigms in mice. The first paradigm is based on a modification of the platform-mediated avoidance task in which animals need to step on a platform to avoid tone-signaled shocks (Diehl, Bravo-Rivera, Quirk, 2019). While previous versions of this task included appetitive components (e.g., lever-pressing for food), the new version excludes them, thus eliminating possible confounds from motivational conflict. The new task also forces active avoidance responses and eliminates the possibility of passive avoidance strategies by introducing a motorized platform that only becomes available when animals need it to avoid shocks. The second paradigm implements thermal threats and is based on spatial learning strategies. For this, animals need to distinguish zones within an acrylic apparatus that have either a significantly colder and unpleasant temperature (0°C, deemed as "threat zones") or a significantly warmer but

pleasant temperature (30°C, deemed as "safety zones"). Interestingly, using optogenetic-mediated inhibition approaches, these novel tasks have produced consistent results for the IL and PL divisions of mPFC. In short, while IL activity is necessary for threat avoidance and safety learning, PL activity is not required for these learning processes during normal conditions. However, PL manipulations are capable of reversing deficits produced by prolonged exposure to psychological stressors such as social isolation. Together with previous findings, our results are consistent with the notion of dissociable roles for the IL and PL divisions of mPFC for the flexible adaptation of a wide range of threat-related behaviors. -

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Busquets-Garcia A, Ferreira G, Marsicano G - *Cannabinoid CB1 receptors control incidental learning* - Inserm - NeuroCentre Magendie - The ability to form associations between different stimuli in the environment to guide adaptive behavior is a central element of learning processes, from perceptual learning in humans to Pavlovian conditioning in animals. Like so, classical conditioning paradigms that test direct associations between low salience sensory stimuli and high salience motivational reinforcers are extremely informative. However, a large part of everyday learning cannot be solely explained by direct conditioning mechanisms — this includes to a great extent associations between individual sensory stimuli, carrying low or null immediate motivational value. This type of associative learning is often described as incidental learning and can be captured in animal models through sensory preconditioning procedures. Recent data from our labs provide evidence for the role of cannabinoid receptors in such higher-order learning tasks. This evidence favors a number of contemporary hypotheses concerning the participation of the endocannabinoid system in psychosis and psychotic experiences and provides a conceptual framework for understanding how the use of cannabinoid drugs can lead to altered perceptive states. -

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Calu, DJ, Kochli DE, Bacharach, SZ, Keefer SE - *Probing SUD vulnerability: Amygdala contributions to sign- and goal-tracking flexibility differences* - University of Maryland School of Medicine - While not all individuals who try recreational drugs develop Substance Use Disorder (SUD), those that do are vulnerable to specific triggers that drive drug seeking even in the face of negative consequences. Preclinical evidence in rats suggests that sign- and goal-tracking individual differences predict differences in drug relapse vulnerability to discrete and contextual cues. These unique relapse vulnerabilities persist despite negative consequences of drug seeking actions. We focus on behavioral flexibility differences of sign- and goal-tracking rats, which are evident both before and after drug experience. We have established that discrete cue-triggered relapse vulnerable sign-tracking rats are less flexible than goal-tracking rats even before drug experience. While extended Pavlovian training promotes sign-trackers' ability to use state-dependent information to appropriately guide responding to cues, sign-trackers' persistent flexibility deficits relate to their inability to use cues to infer current outcome value based on prior experience. We've recently found basolateral amygdala (BLA) communication with the nucleus accumbens core (NAcC) prevents

flexible behavior in sign-tracking rats. When we use chemogenetics to inhibit BLA-NAcC communication, sign-tracking rats show greater flexibility after outcome devaluation. The same manipulation has the opposite effect in goal-tracking rats that rely on BLA-NAcC communication to optimally express their flexible behavioral phenotype. We had originally hypothesized the flexibility of goal-trackers would be mediated by amygdala-cortical projections, and while we find a necessary role for find BLA-insular cortex (IC) communication in the expression of sign- and goal-tracking behaviors, this pathway does not support the behavioral flexibility of goal-tracking rats. In contrast, an ongoing study suggests chemogenetic inactivation of BLA-IC also promotes flexibility in sign-tracking rats, similar to manipulations that disrupt amygdala-striatal communication. Together these results inform our understanding of the brain circuits driving sign- and goal-tracking differences before drug experience, which may aid in circuit investigation of sign- and goal-trackers' distinct relapse vulnerabilities observed after drug experience. -

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Diehl MM - *Acquisition and expression of platform-mediated active avoidance under social conditions in male and female rats.* - Kansas State University - Active avoidance of danger is essential for survival. Active avoidance has been studied in male rats using the platform-mediated avoidance (PMA) task, in which a tone-signaled footshock can be avoided by stepping onto a safe platform (Bravo-Rivera et al., 2014). To determine how social cues may impact avoidance, we modified the PMA task to study avoidance under social conditions, in which male and female Naïve Learner rats undergo PMA conditioning in the presence of a same-sex partner separated by a perforated plexiglass barrier. To determine whether previous experience of the social partner affected acquisition and expression of avoidance in Naïve Learners, we varied the partner's previous experience (Avoidance-Experienced or Avoidance-Naïve). Following 10 days of social partner PMA conditioning, Naïve Learners underwent a PMA session alone on Day 11 to determine whether avoidance responses would differ in the absence of their partner. Since PMA has only been studied in males, we were first interested in whether there were any sex differences in PMA acquisition under solo conditions. We found that females (n=27) spent more time avoiding compared to males (n=29), whereas males showed higher levels of freezing during early conditioning sessions. This suggests that females display more active responses whereas males display more passive responses to danger, agreeing with fear conditioning studies examining sex differences (Gruene, et al., 2015). When comparing acquisition during solo vs. social conditions, rats under social conditions (n=20) showed lower freezing levels during early conditioning sessions compared to rats under solo conditions, suggesting that partners have a social buffering effect. On Day 11, females with an Avoidance partner (n=4) spent more time avoiding compared to females with a Naïve partner (n=6), whereas males with a Naïve partner (n=6) spent more time freezing compared to males with an Avoidance partner (n=4). These data suggest that females use social cues from their Avoidance partner that promote avoidance, whereas males use social cues from their Naïve partner that increase fear responses, when later confronted with danger in isolated contexts. Future studies aim to examine the neural correlates of these behavioral differences to understand how social interactions influence avoidance. - University

of Kansas K-INBRE Developmental Research Grant (P20 GM103418) and Kansas State University CNAP (P20GM113109)

El Hady, A - *Learning to forage in uncertain environments* - Princeton University - Foraging is a ubiquitous behavior performed by all animals as search for food is crucial for survival. When the animal is foraging throughout its environment searching for resources, it is employing a variety of cognitive computations from decision making to planning to learning in addition to adjusting its bodily dynamics. Foraging as a behavior allows studying cognitive dynamics in a natural context and opens up the opportunity for evolutionary comparison across species. In this talk, I will provide a conceptual framework for an integrative understanding of patch foraging focusing on recently developed mechanistic theoretical models, that delineate the potential decision strategies an animal might employ to decide when and how to leave a patch of food across environments with different statistics. Moreover, I will delve into learning mechanisms underlying patch foraging in environments with different statistics. I will discuss how these models can be extended to the social foraging realm. I will also contextualize the theoretical models in relation to field data from a variety of species and their potential to design large scale naturalistic experiments in traditional laboratory animal models. -

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Fernandez-Leon, J.A; Engelke, D.S; Aquino-Miranda, G.; Goodson, A.; Do-Monte, F.H. - *Neural correlates and determinants of approach-avoidance conflict in the prelimbic prefrontal cortex* - The University of Texas Health Science Center at Houston - The recollection of environmental cues associated with threat or reward allows animals to select the most appropriate behavioral responses. Neurons in the prelimbic cortex (PL) respond to both threat- and reward-associated cues. However, it remains unknown whether PL regulates threat-avoidance vs. reward-approaching responses when an animals' decision depends on previously associated memories. Using a conflict model in which rats retrieve memories of shock- and food-paired cues, we observed two distinct phenotypes during conflict: i) rats that continued to press a lever for food (Pressers); and ii) rats that exhibited a complete suppression in food seeking (Non-Pressers). Single-unit recordings revealed that increased risk-taking behavior in Pressers is associated with persistent food-cue responses in PL, and reduced spontaneous activity in PL glutamatergic (PL-GLUT) neurons during conflict. Activating PL-GLUT neurons in Pressers attenuated food-seeking responses in a neutral context, whereas inhibiting PL-GLUT neurons in Non-Pressers reduced defensive responses and increased food approaching during conflict. Our results establish a causal role for PL-GLUT neurons in mediating individual variability in memory-based risky decision making by regulating threat-avoidance vs. reward-approach behaviors. - This work was supported by NIH grants R00-MH105549 and R01-MH120136, a Brain & Behavior Research Foundation grant (NARSAD Young Investigator), and a Rising STARs Award from UT System to F.H.D-M.

Fink AJP*, Axel R, Schoonover CE* - *Latent learning of odor sequences in primary olfactory cortex* - Columbia University - Classical results in experimental psychology (Lashley, Blodgett/Tolman, Sokolov, Brogden, Groves/Thompson, Berlyne) have documented that animals continuously learn complex features in their sensory environment. This faculty does not depend on external reinforcement to drive learning, only the existence of structure in the world. How does an organism learn the world's statistics when the vast majority of the time it is neither rewarded nor punished for doing so? We have developed a behavioral paradigm for the head-fixed mouse that permits observation of exploration and latent learning simultaneous with longitudinal recording of populations of single units. Mice presented a sequence of two neutral odorants A-B initially investigate the stimuli and then cease responding following repeated experience over three days. Recordings in primary olfactory (piriform) cortex show that over this same time period the magnitude of the population response to the A-B sequence decreases markedly, with responses during the B epoch nearly abolished. However, presentation of B alone elicits strong activity; and if B is omitted from the A-B sequence, the piriform responds vigorously to its absence, producing a response that matches the expected timing of the omitted stimulus even though there is no odorant molecule in the nares. Taken together, these concordant behavioral and electrophysiological results show that response magnitude in both behavior and in piriform activity scales inversely with the degree to which an event is predictable. This suggests that activity in piriform does not strictly reflect physical stimuli, but rather a model of the statistics of the olfactory environment, formed over the course of latent learning. Familiar stimuli that contain little new information are filtered out, whereas the content of unanticipated events is transmitted to regions downstream. -

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Fry, B.R., Russell, N., Pence, N.T., Johnson, A.W. - *Optogenetic stimulation of ventral tegmental area dopamine cells augments representation mediated devaluation* - Department of Psychology and Neuroscience Program, Michigan State University - We examined representation mediated devaluation, in which a reward-paired conditioned stimulus (CS) mediates a devaluation to an unconditioned stimulus (US), following pairing with the illness-inducing agent LiCl. On successfully demonstrating the transient early-evaluative nature of this phenomenon in C57 mice, we used optogenetics to examine whether activating ventral tegmental area (VTA) dopamine cells would promote mediated devaluation. Tyrosine hydroxylase-Cre mice were injected with Cre-dependent ChR2 or eYFP control in VTA, followed by Pavlovian training in which mice received 16 pairings with an auditory CS and sucrose solution US. During the aversion stage, the CS was presented alone and combined with contemporaneous delivery of 473 nm blue light directed to the VTA. Immediately upon completion, half the mice were injected with 0.6M LiCl. The next day, all mice were tested for consumption of the sucrose solution US. Those that previously received CS-LiCl pairings displayed a reduction in the palatability of the sucrose solution as indicated by reduced cluster size. Prior activation of VTA dopamine cells during CS alone presentations further

enhanced mediated devaluation, in a D2R-dependent manner. These effects were specific to mediated devaluation, as other measures including CS and context evoked conditioned approach behaviors were unaffected by prior LiCl administration and/or VTA dopamine cell activation. These results suggest dopamine cells may encode detailed facets of reinforcement beyond that typically recognized with reward prediction error, and suggest mediated devaluation can be used to study conditioned hallucinations in animal models. - DK111475

Gilmartin MR - *Prelimbic encoding of threat-related stimuli across the estrous cycle.* - Marquette University - The prelimbic cortex is necessary for the association of a neutral conditional stimulus (CS) and an aversive footshock unconditional stimulus (UCS) that are separated in time, as in trace fear conditioning. We have previously shown that a subset of PL cells shows sustained firing in response to the CS and that optogenetic silencing of prefrontal activity during the trace interval between the cue and shock prevents learning (Gilmartin & McEchron, 2005; Gilmartin et al., 2013). Recently, we have uncovered sex and sex hormone differences in the prefrontal cortical contribution to trace conditioning (Kirry et al., 2018; 2019). In one study, the estrous cycle gated the memory-impairing effects of a muscarinic antagonist in prelimbic cortex (Kirry et al., 2019), which suggested that circulating ovarian hormones may modulate prefrontal encoding during aversive learning. We thus assessed neuronal encoding of trace conditioning in females trained in either proestrus (high circulating ovarian hormones) or metestrus (low circulating ovarian hormones). We found that compared with metestrus, proestrus-trained rats showed enhanced population encoding of the CS and trace interval and greater firing in response to the shock UCS. Strikingly, putative inhibitory interneurons were responsive to the shock-UCS only in proestrus and only if the UCS was preceded by a CS. Given that proestrus is associated with reduced fear expression at test, these patterns suggest that sex hormonal state at the time of training influences the recruitment of local inhibitory circuits to shape prelimbic encoding and its influence on downstream targets to modulate the strength of learned fear. - This study was supported by Whitehall Foundation Research Grant 2014-08-67 (MRG), National Science Foundation IOS:1558121 (MRG), National Institute of Mental HealthR15MH118601 (MRG) and the Charles E. Kubly Mental Health Research Center (MRG).

Huckleberry KA 1, Mejdell M 1, Calitri R 1, Winter A 1, Colom-Lapetina J 1, Abettan J 1, Shepard N 1, Singh A 1, Morena M 2, Nastase AS 2, Hill MN 2, Shansky RM 1 - *Endocannabinoid-mediated sex differences in context-evoked fear generalization* - 1 Behavioral Neuroscience, Northeastern University, Boston, MA, USA; 2 Hotchkiss Brain Institute, Calgary, Alberta, Canada - As many as 10.1% of the US adult population experiences symptoms of post-traumatic stress disorder (PTSD) at some point within their lifetime. There is a marked gender disparity in PTSD diagnoses, with women comprising at least two-thirds of PTSD patient populations. Women's increased susceptibility to PTSD could be due to sex differences in how contextual information regulates the expression of

fear. The hippocampus processes contextual information and provides valence to contexts via the ventral hippocampus's (vHPC) projections to the basolateral amygdala (BLA), an area required for fear conditioning (FC). One novel and under-explored mechanism for regulating activity within this circuit could be via local endocannabinoid (eCB) signaling. FC selectively increases hippocampal anandamide (AEA) in females but not in males. However, AEA can act as a ligand for either the CB1 or TRPV1 receptors. Systemic administration of the CB1 receptor antagonist AM251 prior to FC results in increased context fear generalization in females but not in males. This effect is reversed by treatment with the TRPV1 antagonist Capsazepine. We hypothesize that the combination of FC-induced AEA release and CB1R blockade results in excess TRPV1 binding in females, thereby compromising context encoding. To further explore this possibility, we want to explore potential sex differences in presynaptic CB1R density. We injected retrograde viruses into either the BLA or vHPC in order to label neurons projecting to these regions. Using immunohistochemistry, we will then quantify the density of CB1 receptors on these presynaptic neurons. We are also probing potential postsynaptic sex differences in TRPV1 expression. Because painful stimuli (such as a footshock) can produce rapid insertion of TRPV1 receptors into the cellular membrane, we use western blots to compare TRPV1 levels in the membrane vs cytoplasm of the dorsal hippocampus, ventral hippocampus, and amygdala following FC, CS-only exposure, or directly from the home cage in order to quantify TRPV1 membrane insertion in females versus males. We found no significant differences between any of the groups. These data suggest that our observed sex differences are not due to sexual dimorphisms in FC-induced TRPV1 membrane trafficking and instead could be attributable to potential sexual dimorphisms in CB1 receptor expression. -

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Keiflin R - *From prediction to action: Dissociable roles of Ventral Tegmental and Substantia Nigra Dopamine Neurons in reward learning* - UC Santa Barbara - Dopamine neurons in the ventral tegmental area (VTA) and the substantia nigra (SNc) encode and broadcast reward prediction errors—a fundamental parameter in associative learning. These neurons also display a high degree of anatomical and functional organization. Specifically, VTA dopamine neurons participate in Pavlovian reward prediction and incentive motivation while SNc dopamine neurons contribute to movement invigoration. One striking exception to this regional specialization is the seemingly uniform role of VTA and SNc dopamine neurons in simple instrumental self-stimulation preparations. Indeed, several studies reported that the phasic optogenetic stimulation of dopamine neurons constitutes a potent reinforcer of instrumental action, regardless of the location of the stimulation (VTA or SNc). We hypothesized that this functional similarity in self-stimulation was only apparent and that VTA and SNc dopamine neurons do in fact make distinct contribution to instrumental reinforcement. To test this hypothesis, we trained rats to perform an instrumental action for the optical stimulation of either VTA or SNc dopamine neurons; we then conducted a series of behavioral manipulations to probe the nature of the associative processes engaged in the self-stimulation behavior. We observed that the stimulation of these two neural populations engages largely dissociable learning processes. Specifically, the cues paired with VTA dopamine stimulation

became imbued with incentive salience which facilitated spatially and temporally organized sequences of reward-seeking behaviors. In contrast, the activation of SNc dopamine neurons promoted the repetition of only the most proximal movement that immediately preceded the stimulation; the resulting self-stimulation behavior was highly sensitive to disruptions and lacked traditional features of motivated behavior. Overall, these results are largely consistent with the actor-critic architectures for reinforcement learning. - NIH DA035943; UCSB Academic Senate Grant

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Lee HJ & Hilz EN - *Role of conditioned orienting and endogenous hormones in female drug-preference* - University of Texas at Austin - Cue-directed behavior is considered to reflect enhanced attention or motivation towards environmental cues predictive of a rewarding outcome. High cue-directed behavior is associated with factors such as impulsivity and heightened dopamine function that may put one at a greater risk for developing substance abuse disorder; however, much knowledge in this area is based on studies using primarily male subjects. We have begun to examine cue-directed behavior as a phenotype predictive of amphetamine preference in female rats, and expanded this to consider the influence of endogenous ovarian hormones associated with the rat estrous cycle. Our lab uses conditioned orienting as form of cue-directed behavior and consistently observes individual differences wherein a subset of rats exhibit greater orienting behavior towards a light cue preceding delivery of a food pellet. We recently showed that the orienting phenotype was associated with greater amphetamine-conditioned place preference (CPP) and resistance to extinction of that preference. In a follow up study, we examined the role of ovarian hormonal states on amphetamine CPP. By tracking the estrous cycle of the female rats, we conditioned and tested for amphetamine preference either during a high hormonal state (i.e., proestrus) or low hormonal states (i.e., metestrus/diestrus). Rats conditioned and tested in proestrus showed greater amphetamine CPP and emitted more ultrasonic vocalizations in response to amphetamine. Furthermore, we observed greater FOS expression in dopamine cells of the substantia nigra among rats with the orienting phenotype and rats in the proestrus stage of the estrous cycle; interaction of behavioral phenotype and hormonal state produced the greatest FOS expression. We also considered the potential influence of hormonal contraceptives (HCs, commonly used among women); a subset of intact female rats were implanted with the HC levonorgestrel and we observed their behavioral and neural results to be similar to the rats tested in low-endogenous hormonal states. Together, our results show that cue-directed behavior (as measured by conditioned orienting) in females might predict vulnerability to substance abuse and that endogenous hormonal levels may play a key modulatory role. -

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Marusak HA,Peters, C, Iadiapolo, A, Rabinak, CA - *Childhood trauma exposure, endocannabinoid signaling, and fear extinction recall in children and adolescents* - Wayne State University - Fear-based disorders, including anxiety and posttraumatic stress disorder (PTSD), frequently begin during childhood and adolescence, which highlights the need to

identify underlying neurodevelopmental mechanisms. Research in adults indicates that fear-based disorders are characterized by deficits in fear extinction and dysfunction in underlying frontolimbic circuitry. Here, we examine the impact of childhood trauma exposure and the endocannabinoid (eCB) system on fear extinction and frontolimbic circuitry in youth. We focus on childhood trauma exposure and the eCB system because trauma is a leading risk factor for fear-based disorders, and given recent studies demonstrate the key role of the eCB system in modulating fear extinction in adults and animal models. Forty-eight youth (23 female, ages 6-17 years) completed a novel two-day virtual reality fear extinction experiment. On day one, participants underwent fear conditioning and extinction. Twenty-four hours later, participants completed a test of extinction recall during fMRI. Conditioned fear was measured throughout the experiment using skin conductance responses (SCRs) and fear-related behavior, and activation in fear-related brain regions was estimated during recall. Genetic variation in the eCB system was estimated from saliva for the FAAH C385A polymorphism (rs324420), which is associated with elevated eCB signaling. Trauma exposure was measured using the UCLA PTSD RI screener for DSM-5. Almost half of youth (45%) reported prior trauma exposure and almost half (47.5%) carried the FAAH A allele, associated with higher eCB signaling. There were no effects of trauma exposure or FAAH on conditioned fear during fear conditioning or extinction learning. During extinction recall, however, trauma-exposed youth kept more distance from the previously extinguished and the safety cue, suggesting poor differentiation between threat and safety cues. Trauma-exposed youth also failed to approach the previously extinguished cue over the course of extinction recall. The effects on fear-related behavior during extinction recall were accompanied by higher activation to the previously extinguished cue in fear-relevant brain regions, including the dorsal anterior cingulate cortex and anterior insula, in trauma-exposed relative to control youth. There were also effects of FAAH during extinction recall, such that A-allele carriers demonstrated lower neural response in the dorsomedial prefrontal cortex, a region implicated in conditioned fear responding, relative to CC youth. Relative to the CC group, A-allele carriers also demonstrated lower SCRs during extinction recall, but this effect did not reach significance. There was no interaction between FAAH and trauma exposure on conditioned fear or neural response. These data suggest that trauma exposure and the eCB system modulate fear extinction and frontolimbic circuitry during development, and may contribute to susceptibility to fear-based disorders. - K01MH119241

Mayo LM, Heilig M - *Endocannabinoid function as a target to promote fear extinction in humans* - Linköping University - The endocannabinoid (eCB) system is a neuromodulatory system involved in stress and threat responding and has recently gained interest as a potential therapeutic target for disorders characterized by dysregulation of these processes, such as post-traumatic stress disorder (PTSD). In particular, preclinical evidence suggests that the eCB ligand anandamide may play a critical role in the extinction of conditioned fear responses. Here, we use an experimental approach to determine how variation in eCB levels, conferred either through genetic variation (N = 75) or pharmacological intervention

(N = 45), impact conditioned fear learning in healthy humans using a fear-potentiated startle paradigm. We find that elevated peripheral levels of the eCB ligand anandamide are associated with enhanced extinction of conditioned fear and greater recall of fear extinction when tested 24hrs later. We have subsequently initiated an ongoing clinical to assess the efficacy of potentiating anandamide levels together with prolonged exposure therapy, a common PTSD therapy based on the premise of extinction learning, in PTSD patients (current N = 31; target N = 90). We pharmacologically enhance anandamide levels via inhibition of its main degradative enzyme, fatty acid amide hydrolase (FAAH), over 12 weeks. In addition, all patients receive an 8-week prolonged exposure treatment, with the goal of enhancing the effects of prolonged exposure via facilitated extinction learning and enhanced consolidation as a consequence of elevated anandamide. If successful, this would provide a novel treatment approach for PTSD patients. -

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McNally GP - *Punishment* - UNSW - Punishment is learning about the adverse consequences of our actions. Although once a prominent topic in the study of learning, interest in punishment waned. Meanwhile, the use of old (fines, sanctions, boycotts, social exclusion, incarceration) and new (dislikes, unfollowing) punishers to shape human behavior continues unabated. I will describe experiments directed toward answering three questions about punishment: Under what conditions is punishment learned? What is learned during punishment? And how does punishment guide behavior? The answers to these questions help distinguish punishment from other forms of aversive learning, speak to understanding compulsive behaviours, and provide insights into the nature of decision processes in aversive learning. -

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Morrow JD - *Individual differences in human Pavlovian conditioned approach behaviors: sign- and goal-tracking* - University of Michigan - The process by which reward cues become attractive and motivating is central to the development of addiction. Individual differences in Pavlovian conditioned approach behaviors known as sign- and goal-tracking are known to predict addictive-like behavior in animals but have not been well-characterized in humans. In rats, sign-trackers primarily engage in cue-triggered approach toward reward-associated cues, indicating that for these individuals the cues themselves have acquired incentive value. In contrast goal-trackers direct their cue-triggered approach toward the location of reward delivery, and for these individuals the cues have acquired predictive value but not incentive value. Finally, some rats are intermediate responders who engage in both sign- and goal-tracking roughly equally, perhaps indicating engagement of two types of learning processes simultaneously. In this study, we directly translated a rat Pavlovian conditioned approach task for use in human subjects. We used a retractable lever as a conditioned stimulus that predicts reward delivery into a different physical location (reward magazine). Physical contacts as well as eye-gaze directed toward the lever or magazine were recorded as outcome measures. Subjects also completed questionnaire-based measurements of trait impulsivity.

We found significant inter-individual variation in the extent to which subjects interacted with the "sign" (lever) or the "goal" (magazine) during lever presentation. Latent profile analysis revealed three groups, partially predicted by impulsivity scores and analogous to the three categories used to describe rats tested on a similar task. These results demonstrate that sign- and goal-tracking behavior can be measured in humans, and variation in these behaviors appears to correlate with a known risk factor for addiction. This approach may be used in future studies to translate valuable insights gained from animal studies into new clinical treatment strategies. - NIDA P50 DA037844; Frances and Kenneth Eisenberg Emerging Scholar Award from the A. Alfred Taubman Medical Research Institute; Spiegel Family Fund

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Orsini, C. A., Blaes, S.L., Shimp, K.G., Betzhold, S.M., Setlow, B. - emphChronic cocaine causes age-dependent increases in risk taking in rats of both sexes - The University of Texas at Austin, University of Florida - Chronic cocaine users frequently exhibit maladaptive decision making, overweighting the rewards and underweighting the potential adverse consequences of their choices. We have previously shown that chronic cocaine self-administration in young male rats causes an increase in risk taking that persists well into abstinence, suggesting that cocaine itself may cause lasting alterations in risk-based decision making. This talk will present data from studies that were designed to extend these previous findings by examining whether biological sex and route of administration influence the effects of cocaine on risk taking. In Experiments 1 and 2, rats were trained in a risky decision-making task in which rats made discrete choices between two levers, one that delivered a small, "safe" food reward and another that delivered a large, "risky" reward that was accompanied by mild footshock, the probability of which increased from 0% to 100% during the course of each test session. Rats then underwent two weeks of passive cocaine injections or long-access cocaine self-administration, followed by three weeks of abstinence and re-testing in the decision-making task. In Experiments 3 and 4, rats immediately underwent cocaine exposure, via passive injections or self-administration, followed by three weeks of abstinence before being trained in the decision-making task. In Experiments 1 and 2, neither passive cocaine nor cocaine self-administration affected rats' risk-taking behavior. These negative results contrasted sharply with previous findings; cocaine administration in the current studies, however, began at a considerably older age (15-25 weeks) than in prior experiments that employed a similar experimental design but began cocaine self-administration at an earlier age (11 weeks). Experiments 3 and 4 were conducted to address the possibility that rats are more sensitive to the effects of cocaine at earlier ages, by starting cocaine administration at 9-11 weeks. Under these conditions, risk taking was increased by both passive cocaine injections and cocaine self-administration relative to control conditions, and this effect was evident in both female and male rats. The results replicate previous work showing that chronic cocaine self-administration can cause a long-lasting increase in risk-taking behavior, and extend the prior results to both sexes and to passively-administered cocaine. Moreover, the results suggest there is a limited window of young adult development during which cocaine can affect risky decision making. Specifically, initiation of cocaine exposure at 11

weeks of age or earlier causes lasting increases in risk taking, whereas initiation of cocaine exposure at 15 weeks of age or later has little or no effect. Future studies will examine the neurobiological mechanisms underlying these developmentally dissociable effects, the understanding of which will reveal potential therapeutic targets to attenuate the effects of cocaine on risk taking in both sexes. -

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Rankin, CH, Giles AC, McDIarmid, TA, Ardiel, EL, Yu, AJ - *Questioning the Dual Process Theory* - University of British Columbia - A little over 50 years ago Groves and Thompson (1970) published the Dual Process Theory of non-associative learning in which they postulated that "two hypothetical processes, one decremental (habituation) and one incremental (sensitization) are assumed to develop independently in the nervous system, and interact to yield final behavioral outcome." The behavioral data collected up to that time all supported this hypothesis. The Dual Process Theory was revisited in 2009 (Rankin et al) and experts who studied habituation in C. elegans, Drosophila, Aplysia, rodents and humans all agreed that the basic principles of the Dual Process Theory still fit the behavioral and physiological data collected between 1970 and 2009. In my lab we began studying habituation in C. elegans in 1990 and tested the behavioral predictions of the Dual Process Theory and they were supported by our data. In 2011 we published the development of the Multi Worm Tracker that allowed us to track up to one hundred worms at a time and to parse the data into many components ((ie probability, duration, latency and speed of response) rather than simple yes/no probability measures or a single measure of response magnitude. This allowed us to study hundreds of strains of worms with mutations in identified nervous system genes for many components of behavior including response probability, response duration, response latency and response speed simultaneously. What we found surprised us- different components of the behavior do not always show the same patterns of plasticity. For example, mutations in one gene might eliminate habituation of response probability, but leave habituation of response duration intact. In a study of 524 strains of worms with mutations in nervous system genes we found many more genes affected habituation of response probability than affected response duration. These data suggested there was more than one mechanism underlying habituation. Recently we have applied the same sort of analysis to response sensitization with similar results. In the Dual Process Theory dishabituation was considered a special case of sensitization- we have found genes that play a role in sensitization and not dishabituation, and vice versa. In the Dual Process theory sensitization was considered to be an organism wide arousal process affecting all behaviors- our data suggests this is not the case, but that some components of a behavior can sensitize while others do not. What does this data mean for the Dual Process Theory? Does the genetic data mean that we must rethink how we view non-associative learning? Are these observations relevant for investigating other types of behavior in learning studies? - Natural Sience and Engineering Research Council of Canada

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Rankin CH, Gies, AC, Yu, A, McDiarmiD, T - *Questioning the Dual Process Theory* - University of British Columbia - A little over 50 years ago Groves and Thompson (1970) published the Dual Process Theory of non-associative learning in which they postulated that "two hypothetical processes, one decremental (habituation) and one incremental (sensitization) are assumed to develop independently in the nervous system, and interact to yield final behavioral outcome." The behavioral data collected up to that time all supported this hypothesis. The Dual Process Theory was revisited in 2009 (Rankin et al) and experts who studied habituation in C. elegans, Drosophila, Aplysia, rodents and humans all agreed that the basic principles of the Dual Process Theory still fit the behavioral and physiological data collected between 1970 and 2009. In my lab we began studying habituation in C. elegans in 1990 and tested the behavioral predictions of the Dual Process Theory and they were supported by our data. In 2011 we published the development of the Multi Worm Tracker that allowed us to track up to one hundred worms at a time and to parse the data into many components ((ie probability, duration, latency and speed of response) rather than simple yes/no probability measures or a single measure of response magnitude. This allowed us to study hundreds of strains of worms with mutations in identified nervous system genes for many components of behavior including response probability, response duration, response latency and response speed simultaneously. What we found surprised us- different components of the behavior do not always show the same patterns of plasticity. For example, mutations in one gene might eliminate habituation of response probability, but leave habituation of response duration intact. In a study of 524 strains of worms with mutations in nervous system genes we found many more genes affected habituation of response probability than affected response duration. These data suggested there was more than one mechanism underlying habituation. Recently we have applied the same sort of analysis to response sensitization with similar results. In the Dual Process Theory dishabituation was considered a special case of sensitization- we have found genes that play a role in sensitization and not dishabituation. In the Dual Process theory sensitization was considered to be an organism wide arousal process affecting all behaviors. Our data suggests this is not the case, but that some components of a behavior can sensitize while others do not. What does this data mean for the Dual Process Theory? Does the genetic data mean that we must rethink how we view non-associative learning? Are these observations relevant for investigating other types of behavior in learning studies? -

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Robinson, DL - *Adolescent ethanol exposure reduces flexibility in animal models of behavioral choice* - Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill - Binge alcohol exposure during adolescence can persistently disrupt performance in several models of decision-making and approach. Our working hypothesis is that binge alcohol alters adolescent development such that top-down control over decision making is impaired and cognitive flexibility is reduced. For example, adult rats with a history of adolescent alcohol exposure are likely to exhibit more sign-tracking behavior and less goal-tracking behavior than control-exposed rats. Moreover, adolescent alcohol-exposed rats also exhibit deficits in reversing an attentional set than do their control-exposed siblings. Both of these phenotypes

involve cue-evoked behavior, suggesting that adolescent binge alcohol may impact the ability of reward-associated cues to guide behavioral choice even into adulthood. Specifically, reward-predictive cues drive approach even after they no longer predict reward availability. Furthermore, our data suggest that changes in resting-state functional connectivity MRI among prefrontal and subcortical regions contribute to the behavioral phenotypes. These findings have implications on substance use disorder, including the persistence of drug seeking triggered by drug-associated cues and contexts. -

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Rosenberg M, Zhang T, Perona P, Meister M - *Rapid learning, sudden insight, efficient exploration, and resistance to perturbation in mice navigating complex environments.* - Division of Biology and Biological Engineering, California Institute of Technology, United States; Division of Engineering and Applied Science, California Institute of Technology, United States - Animals are capable of rapidly learning complex tasks. Unconstrained behaviors of mice exploring a complex labyrinth can evoke 2000 navigation decisions per hour, in which the animal discovers the location of a reward and performs 10-bit decisions after only 10 reward experiences. Mice often display discontinuous improvement in navigation to the reward, suggesting moments of sudden insight about the arrangement of the labyrinth. Furthermore, animals retain these abilities after a number of perturbations: whisker removal, olfactory neuron ablation, cortical absence, and maze modification. These observations raise new questions about the algorithms for rapid learning and their neural implementation in the brain. - Markus Meister: Simons Foundation (543015); Pietro Perona: , Simons Foundation (543025), National Science Foundation (1564330), Google

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Schlenner AB, Gardner MP, Iordanova MD - *Midbrain dopamine neurons represent outcome omission as an event of opposite valence* - Concordia University Montreal - A key role ascribed to dopamine (DA) rests with learning about rewarding events. It remains unclear, however, how DA regulates learning about aversive events. Using behavioural electrophysiology we recorded from DA neurons in the ventral tegmental area (VTA) during a Pavlovian task in which auditory cues were trained as predictors of either an appetitive sucrose reward or aversive footshock. Our analyses confirmed a role for VTA DA neurons in tracking reward prediction error (RPE), that is, elevation in firing rate (FR) to the reward predictor and depression in FR at time of reward omission in a correlated fashion. Further, our goal was to determine whether DA firing would accurately represent stimulus type, as well as the type of outcome that was omitted. We found that reward and aversion predicting stimuli were indeed perfectly predicted by the DA population. The omitted outcome on the other hand was classified as an event of opposite valence. -

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Seitz, B.M., Hoang, I.B., Blaisdell., A.P., & Sharpe, M.J. - *Prediction(less) Errors: Probing the role of midbrain dopamine in backward conditioning* - UCLA - Midbrain dopamine has become almost synonymous with the concept of prediction error in

temporal-difference reinforcement learning. Central to this proposal is the notion that reward-predictive stimuli elicit expectations about the value of predicted rewards, and those expectations are compared to the actual reward received. Prediction errors occur when there is a mismatch between the predicted and actual reward. The dopamine prediction error is thought to reflect this computation, facilitating the backpropagation of value from the predicted reward to the reward-predictive stimulus, thus reducing future prediction errors. One assumption of this proposal is that the dopamine signal can only be involved in anticipatory learning, when the initially neutral cue is followed by the rewarding outcome. We asked whether dopamine neuronal activity would also be necessary for learning that a neutral cue reliably follows a rewarding outcome. Using a backward conditioning procedure, we show rats are capable of learning outcome-specific associations between rewards and backward cues, and that dopamine neuronal activity in the ventral tegmental area (VTA) is necessary for this learning to occur. We discuss how these findings inform current debates over the role of dopamine in learning and suggest midbrain dopamine neurons are capable of supporting the acquisition of associations between contiguously occurring events, regardless of whether those events contain just cues, just outcomes, or cues that precede outcomes. -

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Shors, TJ - *Ruminating on Memory* - Rutgers University - In this lecture, I will focus on rumination, which describes thought processes that are repetitive and autobiographical in nature. Ruminative thoughts are generally negative and tend to be more prevalent in women than in men, especially when reflecting on the past (Shors et al., 2017). And within individuals, these thoughts are oftentimes accompanied by trauma-related cognitions, as well as symptoms of anxiety and depression (Shors et al., 2017; Millon, Chang, Shors, 2018). As such, rumination may serve as a proxy for stress-related mental health, as was recently suggested through factor analyses (Millon and Shors, 2021). If these kinds of thoughts are so bad, can we learn to generate fewer of them in our everyday life? To some extent, yes. Brain training can help reduce their prevalence, along with some of the accompanying symptoms of stress, anxiety and depression. In particular, I will discuss a brain fitness program that combines mental and physical (MAP) training. This program, referred to as MAP Train My Brain combines focused-attention meditation and aerobic exercise. Weekly engagement over 6-8 weeks lessened rumination in a number of populations, according to self-report (Alderman et al., 2016; Shors et al., 2018; Lavadera et al., 2020; Millon et al., 2021). This training program also reduced the prevalence of stress-related symptoms that had yet to arise, as reported by K-12 teachers who were preparing to go back into the classroom during the coronavirus pandemic (Demmin, Silverstein, Shors, 2021). Because ruminative thoughts often revisit stressful or traumatic memories, their presence may interact with updating and editing processes in the brain to create yet more memories. Thus, a lifestyle that incorporates mental and physical exercise may help reduce the tendency to ruminate, and thereby lessen the impact of stressful and/or traumatic memories on our everyday life. -

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Wang W, Schuette PJ, Nagai J, Tobias BC, Cuccovia V Reis FM, Ji S, de Lima MAX, La-Vu MQ, Maesta-Pereira S, Chakerian M, Leonard SJ, Lin L, Severino AL, Cahill CM, Canteras NS, Khakh BS, Kao JC, Adhikari A - *Coordination of escape and spatial navigation circuits orchestrates versatile flight from threats* - Dept. of Psychology, UCLA - Naturalistic escape requires versatile context-specific flight with rapid evaluation of local geometry to identify and use efficient escape routes. It is unknown how spatial navigation and escape circuits are recruited to produce context-specific flight. Using mice, we show that activity in cholecystokinin-expressing hypothalamic dorsal premammillary nucleus (PMd-cck) cells is sufficient and necessary for context-specific escape that adapts to each environment's layout. In contrast, numerous other nuclei implicated in flight only induced stereotyped panic-related escape. We reasoned the dorsal premammillary nucleus (PMd) can induce context-specific escape because it projects to escape and spatial navigation nuclei. Indeed, activity in PMd-cck projections to thalamic spatial navigation circuits is necessary for context-specific escape induced by moderate threats but not panic-related stereotyped escape caused by perceived asphyxiation. Conversely, the PMd projection to the escape-inducing dorsal periaqueductal gray projection is necessary for all tested escapes. Thus, PMd-cck cells control versatile flight, engaging spatial navigation and escape circuits. -

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Wassum KM, Sias AC, Lichtenberg NT, Malvaez M, Morse AK, Sepe-Forrest L, Greenfield VY, Wang S, Goodpaster CM, Pennington ZT, Murphy MD, Wikenheiser AM, Holley SM, Cepeda C, Levine MS - *Corticoamygdala contributions to reward learning and decision making* - UCLA - To make adaptive decisions we must cast ourselves into the future and consider the outcomes of our potential choices. This prospective consideration is informed by our memories. I will discuss our lab's recent work investigating the neural circuits responsible for encoding and retrieving reward memories for use in the considerations underlying decision making. We have taken a multifaceted approach to these investigations, combining neural recording and interference methods with behavioral tools rooted in the rich traditions of learning theory. Our results are generally indicating that the bidirectional orbitofrontal cortex - basolateral amygdala circuit regulates the encoding and retrieval of sensory-specific, state-dependent reward memories. The cognitive symptoms underlying many psychiatric disorders result from a failure to appropriately learn about and/or anticipate potential future events, making these basic science data relevant to the understanding and potential treatment of mental illness. - NIDA, NIMH

Zambetti PR, Schuessler BP, Lecamp BE, Kim EJ & Kim JJ - *Pavlovian fear conditioning does not readily occur in rats in naturalistic environments* - University of Washington - Classical or Pavlovian fear conditioning, which offers the obvious advantage of simplicity in both the control of conditioned stimulus (CS) and unconditioned stimulus (US) presentation and the analysis of specific conditioned response (CR) and unconditioned response

(UR) behavior in a controlled laboratory setting, has been the standard behavioral model in basic and translational fear research. Yet despite 100 years of experiments, the utility of fear conditioning has not been trans-situationally validated in real-life contexts. Our laboratory has recently investigated whether auditory fear conditioning readily produces associative fear memory that guides future behavior in animals performing goal-oriented behavior in an ecologically-relevant environment. To do so, female and male Long-Evans rats trained to leave the safety of a nest to search for food in an open arena were presented with a delayed pairing of tone CS and electric shock US to their dorsal neck/body region that instinctively elicited escape UR. On subsequent test days, the tone-shock paired animals exhibited neither freezing nor flight fear CR to the CS and successfully procured food in the arena. In contrast, animals that previously encountered a looming artificial owl paired with a shock, simulating a realistic predatory strike, instantly averted foraging and fled to the nest when presented with a tone for the first time. These results illustrate the survival function and precedence of a nonassociative, sensitization-like process, rather than associative fear conditioning, in life-threatening situations that animals are likely to encounter in nature. -

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In-Person Posters

Ordered alphabetically here. Number indicates the location where poster should be mounted - typically follows alphabetical order except for posters that were requested to be grouped together.

1. **Andrus M, Kim E, Lattal M** - *Optogenetic stimulation of D1-cells in the intercalated cell mass of the amygdala promotes appetitive responding* - OHSU - The main intercalated cell (ITC) mass is a cluster of GABAergic neurons situated between the basolateral and central nuclei of the amygdala. To date, these ITCs have been primarily characterized in their role in regulating fear-associated learning. Very few, if any, investigations have assessed the contributions of ITCs to appetitive learning. This is somewhat surprising, given that the ITCs are a site of D1-dopamine receptor expression, which indicates a potential role in appetitive learning. We used an optogenetic approach in male and female D1-Cre transgenic Long Evans rats to evaluate the role of D1-receptor expressing ITC cells in appetitive learning. We transfected a Cre-dependent channelrhodopsin into the ITCs bilaterally (ML: ±4.6, DV: -8.3, AP: -1.88), and fiber optic cannulas were implanted. Surgeries were done with the same virus in both Cre+ and Cre- animals. Animals were trained to perform a Seek-Take chain lever-pressing task for food reward. This task allows for the establishment of a high level of stable lever pressing across sessions, with presses on a Seek lever (variable interval 10 s schedule) leading to the insertion of a Take lever. A press on the Take lever will cause a food pellet to be delivered to the subject as the Take lever retracts the Seek lever is again presented. To assess the contribution of D1-ITC cells to reward seeking, we programmed laser stimulation to occur as a consequence of every Seek press during cued 5-minute windows, alternating with 5-minute cue-off windows with no stimulation within 30-minute sessions. Cre+, but not Cre-, subjects increased their Seek-lever responding during cue periods. This effect was seen

both when food reward continued to be delivered, and in reward extinction, when the Take lever yielded no food pellet delivery. In a second experiment, we again observed increased appetitive responding during laser stimulation periods when there was a 30% probability that Seek responding would lead to a mild footshock, demonstrating that the optogenetic stimulation could overcome the suppressive effects of punishment. There are several possible mechanisms that could explain these findings (e.g., cue-reward associations, an unconditioned rewarding property of D1-ITCs stimulation, disinhibiting an inhibitory circuit on a consummatory modal action pattern in the central amygdala) that can be partly clarified by deeper investigation into microcircuit dynamics. - T32: DA007262 R01: DA047981

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3. **Biddle M, Knox D, Collins B, Mohammadmirzaei M** - *The role of estrogen receptor manipulation on persistent fear memory induced by traumatic stress exposure* - University of Delaware - Post-traumatic stress disorder (PTSD) is a psychiatric disorder with higher incidence rates in women than men, though research into the underlying mechanisms behind this sex difference are lacking. A possible explanation may be the effects of fluctuating estrogen hormones which coincide with the menstrual cycle in women. While there are risks associated with studying this in humans, by utilizing a rodent model we are able to provide insight into the role estrogen receptors may play in mediating the effects of traumatic stress. The Single Prolonged Stress (SPS) model of PTSD is a validated animal model of traumatic stress and consistently impairs the ability to regulate/inhibit fear memory (i.e. persistent fear memory), when used with male rats. This effect is not consistently observed in female rats, possibly due to similar hormonal fluctuations coinciding with the estrous cycle of female rats. Using the SPS model, we examined the effects of three different estrogen-based pharmacological manipulations on female rats, measuring two individual defensive behaviors (freezing and darting). In Experiment 1, we found that SPS induced persistent fear memory (measured using freezing), and this effect was not affected by antagonism of nuclear estrogen receptors. In Experiment 2, we found no SPS effects on persistent fear memory, nor any combined effect as a result of activation of G-protein coupled estrogen receptors (GPERs) and SPS on persistent fear memory. In Experiment 3, we found that SPS induced persistent fear memory (measured using darting), and that this effect was attenuated through administration of 17β-estradiol prior to SPS. Since the integration of female rats into SPS-based PTSD research, there have been sex-based discrepancies in the literature regarding behavioral outcomes. Our results suggest that SPS effects on persistent fear memory are attainable in female rats, but may present themselves as a shift in behavior (freezing vs. darting). Furthermore, increasing estrogen levels prior to SPS may attenuate persistent fear memory induced by SPS. -

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5. **Blair RS, Nagaya N** - *3alpha-androstanediol, an androgenic neurosteroid, does not confer state dependence to contextual fear in male rats* - Psychological and Brain Sci., Inst. For Neurosci., Texas A&M Univ. - Sex steroids and their neuroactive metabolites can modulate

emotional behavior in both humans and rodents. We have previously shown that allopregnanolone (ALLO), a progesterone metabolite, can confer state dependence to contextual fear when infused into the bed nucleus of the stria terminalis (BNST) of adult male rats. The testosterone metabolite, 3alpha-androstanediol (3alpha-diol), is similar to ALLO such that it produces anxiolysis and acts as a positive allosteric modulator of the GABAA receptor. In the present study, we hypothesized that 3alpha-diol, like ALLO, may confer state dependence to contextual fear via modulation of GABAergic tone. To this end, we bilaterally infused 3alpha-diol (7.3 μg/μL) or vehicle (VEH; 30% 2-hydroxypropyl-beta-cyclodextrin) into the BNST of adult male rats (n = 31) 10 min prior to Pavlovian fear conditioning with 5 tone (CS; 2 kHz, 10 s, 80 dB)-footshock (2 s, 1 mA) pairings. On subsequent days, fear retention was tested by separate exposures to context (10 min) and cue (4 CS-alone trials in a novel context) following infusion of either the same or different drug (3alpha-diol or VEH). Acquisition of conditioned fear was facilitated by pre-training infusions. However, expression of contextual and cued fear was similar for all animals, regardless of drug treatment. Further, no significant interaction was found between drug infused on conditioning and testing days, suggesting that 3alpha-diol does not confer state dependence to either contextual or cued fear. Additionally, animals were subjected to extinction training and testing sessions consisting of 27 CS-only presentations in a novel context. After infusion of either 3alpha-diol or VEH, rats received extinction training followed by an extinction retention test 24 h later. Acquisition and retention of extinction did not differ between drug treatment groups. Overall, these findings suggest that, unlike the neurosteroid ALLO, 3alpha-diol does not confer state dependence to contextual fear via the BNST but may modulate acquisition of fear learning. - College of Liberal Arts, Texas A&M University

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6. **Brockway ET, Simon S, Drew MR** - *Divergent activity among hippocampal projections to IL and BLA during recall of context fear and extinction* - UT Austin; Center for Learning and Memory - Fear acquisition and extinction have long been considered separate learning processes. Indeed, fear and extinction memory ensembles are distinct in the hippocampal dentate gyrus (DG). Our lab has recently shown that extinction suppresses activation of a fear memory representation in DG and establishes a distinct ensemble representation of the extinction memory. Whereas artificial stimulation of the fear memory ensemble increases fear, stimulation of the extinction ensemble reduces fear. However, how fear and extinction memory representations in the hippocampus differentially influence fear recall and behavior is not well understood. Here we investigated whether recall of context fear and extinction memories activate different populations of ventral hippocampal projection neurons. Cholera-toxin subunit B (CTb) was used to retrogradely label ventral hippocampal neurons projecting to the infralimbic cortex (IL) and basolateral amygdala (BLA) in each mouse. Mice were context fear conditioned, and then split into three groups that received either (group 1) 10 days of extinction, (2) no extinction and a fear retrieval test on the 10th day, or (3) no extinction and were taken from their home cage on the 10th day as a non-exposure control. Brains were collected after the last behavioral session and labeled for c-Fos (IHC) to assess activity among the IL- and BLA-projecting ventral hippocampal neurons. Our results show

that ventral hippocampal c-Fos activation by area is similar between fear and extinction recall groups, but that c-Fos activity among specific projection neurons differs between fear and extinction recall. The fear extinction group shows more activity among projections to IL than BLA (calculated as % CTb neurons labeled with c-Fos), and the fear retrieval group shows the opposite with higher BLA- than IL-projection activity. Further experiments aim to assess the role of context exposure in this paradigm by including a group of mice that receives 10 days of preexposure to the context before fear conditioning. Overall, these results demonstrate that context fear and extinction retrieval activate different proportions of ventral hippocampal projections, and potentially provides a mechanism for hippocampal memory representations to signal the valence of contextual memory to other fear-related regions. -

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7. **Brunswick CA, Bodinayake KK, Wright DS, Stuart EM, Lo CF, Heimann AC, Lakhia SS, Kwapis JL** - *Circadian gene Per1 may modulate memory formation within the retrosplenial cortex* - Penn State University - The link between the circadian system and memory performance is well-characterized and widely accepted. Circadian rhythms are endogenous cycles governed by transcriptional patterns of circadian genes within the suprachiasmatic nucleus (SCN). Little research has investigated the roles of these genes beyond the SCN. One such circadian gene—Period1 (Per1)—has recently been implicated in gating memory formation within the hippocampus. Here, we investigated the link between Per1 expression and memory performance in another memory-relevant structure, the retrosplenial cortex (RSC). First, we sought to uncover how Per1 expression within the RSC is affected by memory formation. Male C57BL/6J mice were trained in contextual fear conditioning and were then sacrificed at various timepoints following training. Per1 mRNA expression in the RSC was increased by learning and peaked one hour following training, consistent with a potential memory-relevant role for Per1 within the RSC. To determine whether retrosplenial Per1 exerts circadian control over memory formation, we next examined diurnal oscillations in both learning-induced Per1 within the RSC and RSC-dependent memory performance. Two cohorts of male C57BL/6J mice were trained in object location memory (OLM) at six timepoints spanning the circadian cycle (Zeitgeber Times: ZT1, ZT5, ZT9, ZT13, ZT17, ZT21). One cohort of animals was used to investigate how performance in OLM varies throughout the circadian day, while the other was sacrificed an hour post-training to examine how learning modulates Per1 expression. We found a significant effect of circadian timepoint on memory performance, with better memory observed during the day than at night. Learning-induced Per1 expression within the RSC also varied with timepoint and peaked during the day. Notably, learning had no effect on the circadian cycling of Per1 in the SCN. Overall, these results suggest that Per1 in the RSC may exert circadian control over memory performance independent of its role in the SCN. -

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9. **Chu A, Michel CB, Gordon NT, DuBois AM, Williams DC, McDannald MA** - *Towards a comprehensive temporal ethogram of rat behavior during Pavlovian fear discrim-*

ination. - Boston College - In Pavlovian fear conditioning, a neutral cue is paired with an aversive stimulus, such as foot shock delivery. One result of this predictive relationship is that cue presentation will elicit a suite of defensive behaviors. The most commonly studied defensive behavior is freezing, a 'passive' defensive behavior defined by the absence of movement. Recent studies have revealed that shock-associated cues can also elicit 'active' defensive behaviors, such as darting, characterized by increased movement. Thorough ethograms of rodent defensive behavior have been confined to contextual fear conditioning procedures and lack temporal specificity. The goal of the current study is to construct a complete ethogram of rat behavior across Pavlovian fear discrimination. To do this, we tested 24 rats (12 females and 12 males) in a conditioned suppression procedure. Briefly, rats were trained to nose poke for food rewards. Rats then received 16 fear discrimination sessions in which three auditory cues predicted unique foot shock probabilities: danger ($p = 1.00$), uncertainty ($p = 0.25$), and safety ($p = 0.00$). Poke-reward and cue-shock contingencies were independent. During each trial, we captured individual images at a rate of 5 frames/s before and during cue presentation (2 s pre-cue, 10 s during cue, 12 s total). Initial observations show that rats exhibit a mixture of freezing and active behaviors, such as locomotion, during danger and uncertainty cue presentation. We will present our most up to date findings for behaviors such as freezing, locomotion, grooming, nose poking, and eating across cue presentation and across the 16 fear discrimination sessions. Our hand-scoring efforts will reveal fine organization of behavioral responding and form the basis of a data set to train neural networks for behavior classification. Successful application of neural networks to behavior classification will permit highly-detailed, large-scale analysis of behavior to more completely reveal neural circuits supporting diverse threat behavior. -

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2. **Derman, R.C., Bindas, S., Wood, M.A., Lattal, K,M** - *Contribution of basolateral amygdalar histone deacetylase-3 to stress-induced changes in instrumental motivation.* - Oregon Health and Sciences University - Behavior is regulated by interplay between aversive and appetitive learning and motivational processes. Under some circumstances one process can come to persistently dominate and influence the other. In humans, highly stressful events can lead to post-traumatic stress disorder (PTSD) which carries with it long-lasting comorbid aberrations in aversive and appetitive motivational processes. Treatment of PTSD and its accompanying comorbidities has been challenging, though a combination of behavioral and pharmaceutical approaches show promise. Behaviorally, re-exposure to the cues associated with trauma can weaken fearful reactions through extinction. Pharmacologically, epigenetic mechanisms, such as histone acetylation, can persistently alter fearful memories. Here, we adapted the Stress-Enhanced Fear Learning (SEFL) approach to study (1) effects of 'trauma' on appetitive learning and motivation, and (2) the role of basolateral amygdalar (BLA) histone deacetylase 3 (HDAC3) in fear extinction and its impact on SEFL and appetitive learning and motivation. Our lab has previously found that HDAC3 inhibition promotes histone acetylation and promotes extinction and many studies have established the BLA as a key nucleus in appetitive and aversive learning. In two experiments, rats were put through a highly stressful contextual fear conditioning session in which 15, 1mA foot shocks

were delivered within a 90-min session. In Experiment 1, rats then underwent appetitive Pavlovian conditioning, extinction and renewal testing, followed by instrumental training and Progressive Ratio (PR) testing to evaluate motivation for natural reward. Shocked vs control rats did not differ notably during Pavlovian conditioning, extinction, or renewal testing, however we found that shocked rats showed markedly lower responding in PR testing, notably this testing was conducted 45 days after the initial treatment. A 90-min extinction session of the original fear context modestly though reliably enhanced responding in PR testing. In Experiment 2, rats underwent surgery to deliver either a null virus, a virus that expresses a point mutant (PM) of HDAC3 that interferes with its function, or a virus that expresses wild type (WT) HDAC3 into the BLA. Following surgery and a 3-week viral incubation period, rats underwent shock, fear extinction, SEFL treatment and testing, and finally instrumental training and PR testing. Manipulation of HDAC3 lowered fear responding (freezing) during both extinction and SEFL testing. Consistent with these data, HDAC3 manipulation also enhanced responding during PR testing for motivation for natural reward. Collectively these data demonstrate that highly stressful events have long-lasting impacts on motivation for natural reward, that extinction of the original stressor can alleviate this effect, and that BLA HDAC3 contributes to this extinction process. - T32 DA007262; R01 DA047981

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10. **DiFazio, LE; Greer, Z; Sharpe, MJ** - *The temporal dynamics of encoding fear memories in the BLA, and how they change following experience with rewards.* - UCLA - Decades of research has shown that the basolateral amygdala (BLA) supports the acquisition and storage of fear memories. Indeed, lesions or pharmacological inactivation of BLA during either encoding or retrieval of a fear memory reduces associated fearful behavior (Cousens & Otto, 1998, Behavioral Neuroscience; Maren, Aharonov & Fanselow, 1996, Behavioral Neuroscience; Phillips & LeDoux, 1992, Behavioral Neuroscience). As a result, current models of fear learning generally argue that information about environmental stimuli (e.g. context, odor) and the fearful event (e.g. pain) arrive in the BLA and become associated so that we form a fear memory (Maren & Quirk, 2004, Nature Reviews Neuroscience; Pitkänen, Savander & LeDoux, 1997, Trends in Neurosciences). The development of optogenetics has given us the opportunity to investigate the temporal dynamics of BLA's role in fear encoding. Specifically, we took advantage of the temporal specificity of optogenetics to investigate the effect of inhibiting glutamatergic neurons in the BLA during either a stimulus (i.e., tone) preceding an aversive event or during the aversive event (i.e., shock) itself, during Pavlovian fear conditioning. Contrary to a framework that argues information about the stimulus and aversive outcome arrive in the BLA and become associated to form a new fear memory, we found that only inhibition of BLA neurons during the shock-predictive cue and not the shock itself disrupted learning. This demonstrates that the BLA is necessary for learning about the fear-predictive cue, but processing of the aversive event itself remains intact when BLA activity is inhibited. Additionally, most rodent research on fear learning is conducted on rats with no substantial learning experiences outside of their home cage. Given the massive impact of prior stressful or traumatic events on future fear learning, we chose to investigate how prior experiences with reward learning might alter the role of the

BLA in learning about the shock-predictive cue. This is of particular interest following recent evidence that the lateral hypothalamus becomes necessary for fear learning following experience with reward learning (Sharpe et al., 2017, Nature Neuroscience). To test this idea, half our rats learned to associate stimuli and rewards. Then, in a different context, the rats learned that a novel stimulus predicted arrival of shock and BLA principal neurons were inhibited during the stimulus (and not shock). Here, we found that BLA inhibition no longer impacted formation of the new fear memory in rats who experienced reward learning, while they remained important for fear memory formation in the control group without reward experience. Together, these results show that glutamatergic neurons in the BLA are necessary for learning about the shock-predictive cues and that reward learning can change the role of BLA in learning about fear-predictive cues. -

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13. **Donovan EN, Avila C, Parikh VV, Fenollar-Ferrer MC, Blakely RD, Sarter MF** - *Disrupted choline clearance and sustained acetylcholine release in vivo by a common choline transporter coding variant associated with poor attentional control in humans* - Department of Psychology, University of Michigan, Ann Arbor, MI, USA; Department of Psychology & Neuroscience Program, Temple University, Philadelphia, PA, USA; Section of Human Genetics, National Institute of Deafness and Other Communication Disorders, Bethesda, MD, USA; Stiles-Nicholson Brain Institute and Department of Biomedical Science, Charles E. Schmidt College of Medicine, Florida Atlantic University, Jupiter, FL, USA; Department of Psychology, Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI, USA - Transport of choline via the neuronal high-affinity choline transporter (CHT; SLC5A7) is essential for cholinergic terminals to synthesize and release acetylcholine (ACh). In humans, we previously demonstrated an association between a common CHT coding substitution (rs1013940; Ile89Val) and reduced attentional capacity as well as attenuated frontal cortex activation. Moreover, studies by our collaborators have demonstrated over-representation of this variant in ADHD families as well as an association with depression. Here, we used a CRISPR/Cas9 approach to generate mice expressing the I89V substitution and assessed, using in vivo cortical choline biosensing, CHT-mediated choline transport, and ACh release. CHT-mediated clearance of choline in mice expressing one or two Val89 alleles was reduced by over 7-fold relative to wild type (WT) mice, suggesting dominant-negative effects. Choline clearance in CHT Val89 mice was further reduced by neuronal inactivation. Deficits in ACh release, 5 and 10 min after repeated depolarization at a low, behaviorally relevant frequency, support an attenuated reloading capacity of cholinergic neurons in mutant mice. The density of CHTs in total synaptosomal lysates and neuronal plasma-membrane-enriched fractions was not impacted by the Val89 variant, indicating a selective impact on CHT function. Consistent with this hypothesis, structural modeling revealed that Val89 may attenuate choline transport by changing the ability of choline to induce conformational changes of CHT that support normal transport rates. Our findings suggest that diminished, sustained cholinergic signaling capacity in the frontal cortex underlies perturbed attentional performance in individuals expressing CHT Val89. Our

work supports the utility of the CHT Val89 mouse model to generate behavioral and neuroscientific insights into the risk and manifestation of numerous psychiatric and neurological disorders in which cholinergic dysfunction plays a contributing role. -

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14. **Edwards CM, Dolezel T, Rinaman, L** - *Ghrelin signaling plays a role in fasting-induced suppression of passive avoidance behavior and A2 noradrenergic neuron activation in the nucleus of the solitary tract (NTS) in male rats* - Florida State University - Competing motivational drives coordinate behaviors essential for survival. Previous work from our lab has demonstrated that negative energy balance suppresses innate anxiety-like behaviors. Recently, we extended these findings to show that conditioned passive avoidance behavior is reduced in male (but not female) rats following an overnight fast. Levels of ghrelin, the "hunger hormone," are elevated during negative energy balance, and some evidence suggests that ghrelin signaling plays a role in learning and memory. To explore whether ghrelin signaling is involved in the ability of food deprivation to suppress passive avoidance behavior, male rats were trained in a task in which they learn to avoid a context paired with a single mild footshock. The fasting-induced increase in ghrelin signaling was then pharmacologically antagonized with a ghrelin receptor antagonist (GRA) prior to the retention test. Fasted rats treated with GRA displayed more passive avoidance behavior compared to fasted, saline-injected controls. The prolactin-releasing peptide (PrRP)+ subpopulation of hindbrain A2 noradrenergic neurons integrates signals from the body, especially from the gut, and projects to a variety of forebrain regions considered to modulate motivated behavior. We recently demonstrated that PrRP neurons are activated in rats after re-exposure to the shock-paired context, and that this activation is "silenced" by overnight fasting. In the present study, we demonstrated that GRA pretreatment partially restores the ability of the shock-paired context to activate PrRP neurons in fasted rats. Our results support the view that ghrelin signaling participates in the ability of negative energy balance to reduce expression of passive avoidance behavior and to activate PrRP hindbrain neurons in male rats. -

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15. **Felix-Ortiz AC, Garza MC, Hajali DT, Arriaga DT, Lopez SM, Burgos-Robles A** - *Contribution of medial prefrontal cortical areas and ventral hippocampal inputs to threat and safety learning using naturalistic thermal stimuli.* - University of Texas at San Antonio - Growing evidence implicates the infralimbic (IL) and prelimbic (PL) subregions of the medial prefrontal cortex (mPFC) in threat and safety learning. However, these roles have been mostly evaluated using paradigms involving electric shocks and discrete cues predicting the presence or absence of shocks. Thus, the contribution of IL and PL during more naturalistic paradigms remain unclear. For this study, we developed a novel paradigm in which mice learn to discriminate distinct spatial locations within an acrylic apparatus that simulate either threat zones (floor at -5°C) or safety zones (floor at 30°C). We also implement a prolonged social isolation stress paradigm to investigate learning deficits in this novel paradigm during disease-like states. Using optogenetic-mediated inhibition approaches, our findings

so far indicate that IL and PL play crucial roles for the learning of safety zones. Furthermore, we observed robust deficits in safety learning after social isolation stress. Interestingly, while IL inhibition during safety training mimics stress-related deficits, PL inhibition during safety training prevents stress-related deficits. Finally, our experiments suggest involvement of ventral hippocampal (vHPC) inputs to mPFC during this task and during stress-related deficits. Collectively, these findings provide substantial evidence suggesting crucial participation of the IL and PL divisions of mPFC, as well as vHPC inputs for threat and safety learning when experiencing naturalistic thermal stimuli. In addition, these findings reveal important roles of these brain regions for the modulation of learning deficits associated to prolonged psychological stress. -

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16. **Ferrara NC, McMichael W, Padival M, & Rosenkranz JA** - *Dopamine 1 receptor activity modulates social behavior and cortical influence on amygdala activity in an age-dependent manner.* - 1Cellular and Molecular Pharmacology/RFUMS, 2The College of Wooster - Adolescence is characterized by transitions in social engagement and ongoing cortical maturation, which likely contribute to the age-dependent changes in the regulation of emotions. Engagement in social behavior and brain maturation influence each other and are critical for appropriate social responding later in life. The basolateral amygdala (BLA) is critical for social behavior and receives extensive inputs from the prefrontal cortex (PFC). During adolescence, PFC inputs to BLA increase in strength and more readily activate intra-BLA inhibitory circuits. Understanding the way in which PFC-BLA circuits are regulated to alter social behavior, particularly in an immature circuit, is essential for a clear understanding of neuropsychiatric disorders characterized by abnormal social behavior. One way in which PFC-BLA circuits are regulated is through dopamine 1 receptors (D1R), but much less is known about D1R regulation of social behavior and what this does in the still maturing PFC-BLA circuit. Here, we investigated the relationship between D1R modulation of PFC driven changes in BLA activity and social behavior in adults and adolescents. We found that D1R activation resulted in elevated zif268 expression, used as a proxy for cellular activity, as well as increased glutamate sensitivity in the BLA. D1R agonism facilitated PFC-driven inhibition in adolescents but not in adults, suggesting that while D1R agonism can increase BLA neuronal activity, it has a distinct impact on the maturing PFC-BLA circuit. Further, we found that D1R agonism had opposing effects on social interaction, where age-specific social behaviors were increased in adolescents but were modestly reduced in adults, but D1R agonism uniformly increased BLA activity in both adults and adolescents during social engagement. These results begin to provide insight to the changes in D1R modulation of cortico-amygdala circuitry from adolescence to adulthood that contribute to the refinement of social behaviors. - NIMH R01 MH118237 (JAR), F32MH122092 (NCF)

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17. **Fry BR, Fex V, Sotomayorreinat FC, Sawa A, Niwa M, Johnson AW** - *Circuit-specific inhibition of dopaminergic signaling associated with phantom gustatory sensations in*

Disrupted-in-Schizophrenia-1 mice - Department of Psychology, Neuroscience Program, College of Natural Science, Michigan State University; Departments of Psychiatry and Behavioral Sciences, and Neuroscience, The Johns Hopkins University; Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham School of Medicine - Disrupted-in-Schizophrenia-1 (DISC-1) is a gene which encodes a protein involved in a number of intracellular processes, particularly those critical for early neural development. In mice, perturbations of the DISC-1 genetic locus lead to a syndrome which mimics many of the core endophenotypes associated with schizophrenia. These mice have been shown to display impaired reality testing as revealed by heightened perceptual processing of an absent sucrose solution in the presence of an auditory conditioned stimulus (CS). These impairments were dopamine-dependent and associated with elevated insular cortex (IC) activity. In the current study, we examined whether these effects required activity of ventral tegmental area (VTA) dopamine cells projecting to the IC. To achieve this, we bred DISC-1 mice to mice selectively expressing Cre-recombinase within cells expressing the dopamine transporter (DAT-Cre). These DISC-1 x DAT-Cre mice received bilateral infusions of a retrograde Cre-dependent Flp-O recombinase virus in IC, followed by bilateral VTA injections of a Flp-dependent hM4Di inhibitory DREADD virus. Through this intersectional approach, intraperitoneal (IP) injections of clozapine-N-oxide (CNO) allows for circuit-specific inhibition of dopamine cells projecting to IC. Following surgery, mice underwent Pavlovian conditioning in order to learn an association between a white-noise CS and delivery of sucrose solution. Subsequently, mice received IP injections of vehicle or CNO on separate test days, at which point the capacity of the CS to transfer sensory responses typically evoked by the sucrose was examined by presenting the CS alone with water. Our findings will offer insight into the genetic and neurobiological mechanisms underlying dopamine-mediated reality testing. - NIH Grant: R01DK111475 awarded to Alexander W. Johnson

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36. **Goldberg LR, Sebastian A, Albert T, Gould TJ** - *C57BL/6 subtrain differences in conditioned fear and hippocampal transcriptome.* - Penn State University - Accumulating evidence supports significant phenotypic and genomic differences between the closely related C57BL/6 (B6) substrains, C57BL/6J (B6J) and C57BL/6N (B6N). Inconsistent differences in conditioned fear between B6J and B6N have been reported. These inconsistences may be due to B6N substrain selection (B6NJ vs B6NCrl) or fear conditioning paradigm characteristics. Here, we aimed to determine if B6J and B6NJ substrains differed in contextual fear conditioning and learning-induced hippocampal transcriptome. Male B6J and B6NJ (PND60, n=8 per group) were fear conditioned with 1 pairing, 2 pairings, or 4 pairings of footshock and auditory cue, and then were tested for hippocampus-dependent contextual fear learning or hippocampus-independent cued fear learning. A 2-way ANOVA of Strain and number of Pairings for contextual fear learning revealed a significant effect of Strain ($F_{1,41}=48.83$, p¡0.001), a significant effect of Pairings ($F_{2,41}=4.99$, p¡0.05), but no interaction of Strain and Pairings. For all number of Pairings, B6NJ showed enhanced contextual freezing compared to B6J. A 2-way ANOVA of Strain and number of Pairings for cued fear learning revealed a significant effect of Strain ($F_{1,41}=5.46$, p¡0.05), but no significant effect of

Pairings or interaction of Strain and Pairings. For all number of Pairings, B6NJ showed enhanced cued freezing compared to B6J. To investigate baseline and learning-induced changes in hippocampal transcriptome that may underlie the observed behavioral differences between B6J and B6NJ, dorsal (DH) and ventral hippocampi (VH) were dissected for RNAsequencing. Samples were collected from B6J and B6NJ male mice from three groups (n=6 per group): direct from home cage, 30 minutes after 2-pairings of shock and cue, 30 minutes after 2 exposures to cue alone. Preliminary analyses of B6J and B6NJ home cage transcript level expression identified 336 DH and 153 VH differentially expressed transcripts. Enrichment analyses using Enrichr identified 7 enriched GO Biological Processes (adjusted p-value ¡0.05), including regulation of synapse maturation, glutamatergic synaptic transmission, and regulation of nervous system development. Continued analyses include learning-induced differential transcriptome expression between B6J and B6NJ in both hippocampal regions. Together, these behavioral and transcriptomic findings confirm differences in conditioned fear between the commonly used B6 substrains, B6J and B6NJ, and begin to uncover the underlying hippocampal transcriptome differences. - Funding: U01DA04163205

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18. **Grunfeld I, Denholtz E, Nahmoud I, Aubry A, Datta S, Chattarji S, Burghardt N, Likhtik E** - *Chronic Social Defeat Stress Leads to Over-Generalized Fear: a Role for Impaired Safety Learning and Disrupted Communication Between the Amygdala and Prefrontal Cortex* - The Grad. Ctr., CUNY, Hunter College, Biol. Dept., Hunter College, Psychology Dept., Mt. Sinai, Icahn School of Medicine, Tata Institute of Fundamental Research, National Centre for Biological Science - Chronic stress leads to generalization of fear to non-threatening cues, a key symptom in numerous psychiatric disorders. We previously showed that in the absence of stress, successful discrimination of non-threatening from threatening cues is associated with suppression of the basolateral amygdala (BLA) by the medial prefrontal cortex (mPFC). In contrast, BLA activity is not under mPFC control during fear generalization. Here, we used the chronic social defeat stress (CSDS) model to investigate the contribution of the BLA and mPFC to stress-induced fear generalization in 129 Sv/Ev mice. We found that 10 days of CSDS decreased dendritic spines in the prelimbic (n=3 mice) and infralimbic (n=4 mice) subregions of the mPFC relative to non-stressed controls (n=3-4, p¡0.0001). In contrast, CSDS enhanced consolidation of auditory fear conditioning (n=18-19, p¡0.0001), and increased expression of the immediate-early gene Arc in the BLA during fear memory retrieval (n=5, p¡0.05), supporting differential effects of stress on these brain regions. We then tested the effects of CSDS on discrimination learning using an auditory differential fear conditioning task, in which one tone was paired with a shock (CS+) and a second tone was unpaired (CS-, 2kHz, and 8kHz, counterbalanced). The majority of non-stressed animals successfully discriminated between the tones (n=14/19, 73.69%), freezing at least 10% more to the CS+ than the CS-. However, the majority of stressed mice generalized fear to the CS- (n=11/17, 64.7%), freezing similarly to the two tones. To investigate whether this reflects an effect of stress on safety learning, mice were trained on a salient safety learning task, where the presence of a 1-sec light and a 30-s tone signaled

the explicit absence of shock. Whereas non-stressed controls reliably decreased freezing during the safe tone (n=10, p¡0.05 compared to pre-tone), the CSDS group did not (n=11, p¿0.05). Analyses of local field potential recordings in the mPFC and BLA reveal that in non-stressed mice, the safety CS evoked an increase in mPFC theta power from pre-tone (p¡.0001), whereas stressed mice didn't show this increase (p¿0.05). In contrast, the safety CS evoked an increase in BLA theta-power in stressed mice (p¡0.0001), whereas no such increase was seen in the BLA of non-stressed mice (p¿0.05). We conclude that chronic stress differentially impacts the mPFC and BLA and alters theta-range communication in this circuit during periods of explicit safety, potentially diminishing the ability to discriminate between threatening and non-threatening cues. - NIH R21MH114182, NIH R01MH118441, PSC-CUNY

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19. **Gupta, TA & Sanabria, F** - *Investigating the role of the dorsal hippocampus in an opportunity-cost model of interval timing* - Arizona State University - Cognitive processes such as decision-making and reward-seeking are facilitated by the capacity of interval timing, or timing intervals in the seconds-to-minutes range. Interpretation of interval timing data generated from animal models is complicated by ostensible motivational effects which arise from the delay-to-reward imposed by interval timing tasks, as well as overlap between timed and non-timed responses. When to-be-timed intervals are long, requiring response-initiation often results in schedule strain, which can increasingly confound timing data with motivation. Further, evidence from lesion studies suggests that memory capacity required for timing these longer intervals require a functional hippocampus (Gupta et al., 2019; Jacobs et al., 2013). Using intracranial infusions of GABA agonists in the dorsal hippocampus during testing in the timing-with-opportunity-cost task (Sanabria, Thrailkill, & Killeen, 2009), a current study investigated the specific role of the hippocampus in temporal entrainment of motivated behavior to long intervals. -

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11. **Hoang, I.B., Munier, J.J., Greer, Z., Millard, S.J., Wassum, K.M., Izquierdo, A., Sharpe, M.J.** - *The role of the hypothalamic-midbrain circuit in reward learning and how this changes with drug exposure.* - UCLA - Every day, we are exposed to cues that we have learned predict important events. These cues drive adaptive behavior, so that we approach rewards and avoid unpleasant or painful experiences. However, reward-predictive cues gain excessive control over an individual's actions in cases of drug addiction, which can perpetuate drug use and induce relapse. Previously, we have shown that the hypothalamic-midbrain circuit regulates learning about reward-paired cues (Sharpe et al., 2017, Current Biology; Sharpe et al, 2021, Nature Neuroscience). Here, we examined the specific role of the hypothalamic-midbrain circuit in reward learning and investigated how drug exposure might influence this function. We first trained rats to self-administer methamphetamine and then tested their behavior during specific Pavlovian to Instrumental Transfer (sPIT). We found that rats with a history of methamphetamine self-administration showed an enhancement

in sPIT relative to drug-naïve rats, which was correlated with their drug intake. Further, this enhancement in sPIT was accompanied by sensitization of the hypothalamic-midbrain circuit, revealed by increases in the willingness of drug-exposed rats to work for optogenetic stimulation of this pathway. To probe the general function of the hypothalamic-midbrain circuit in sPIT, we disconnected the hypothalamic-midbrain pathway as rats were learning the specific cue-reward contingencies during sPIT. Here, we found evidence that dopamine neurons in the midbrain send information to the lateral portion of the hypothalamus to reinforce specific cue-reward associations that drive the sPIT effect. Further, GABAergic neurons in the lateral hypothalamus then relay an expectation for the sensory-specific reward back to the midbrain to regulate ongoing learning. Together, these data reveal that the hypothalamic-midbrain circuit accumulates specific reward memories and that this function is enhanced following drug experience, increasing the power of reward-predictive cues over decision making. -

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33. **Hoffman AN, Makridis AS, Trott JM, & Fanselow MS.** - *Anxiety, fear, panic: A novel approach to assessing defense across the predatory imminence continuum* - UCLA Psychology; Staglin Center for Brain and Behavioral Research - The defensive behavior system is organized as a sequence of innately programmed behaviors with the goal of thwarting predation and increasing survival. These modes of defense spatially and temporally map the interaction with the predator or threat, known as the predatory imminence continuum (PIC). Ranging from low threat to predator contact, the PIC categorizes defense modes as pre-encounter, post-encounter, and circa-strike. These modes correspond to states of anxiety, fear, and panic, respectively. Previous research has shown that a prior stress event can lead to over expressed and sensitized fear such as in the model of Stress Enhanced Fear Learning (SEFL). The present experiment sought to examine if overexpression of all defensive responses along the PIC within subject, including anxiety-like behavior, freezing, and panic-like responses, may also result following a significant stressor. Female and male mice were exposed to an acute traumatic stress event that consisted of a series of 10 pseudorandomly presented unsignaled 1.0mA/1sec foot shocks (or no shocks). Mice were then subsequently tested on a battery of tasks to assess stress effects on pre-encounter (anxiety-like), post-encounter (fear), and circa-strike (panic-like) behaviors, in ascending order along the PIC. In a modified open field with a light gradient, we analyzed anxiety-like behavior post trauma as assessed by average velocity and zone preference behavior. Mice were then fear conditioned to a single mild footshock (1.0mA/2 sec) and tested for contextual fear the following day. Lastly, panic-like behavior was examined through mild auditory startle exposing subjects to 16 trials of 10sec/75dB white noise in the initial trauma context. Results revealed that following stress, mice exhibited increased anxiety-like behavior shown through reduced average velocity within the modified open field. Furthermore, stressed mice showed increased fear following the single mild footshock, consistent with our SEFL model. Interestingly, there was an increase in reactivity to the white noise during panic testing trials with stressed mice exhibiting a more robust panic-like response than controls. Therefore, traumatic stress exposure has been shown to influence the defensive states of anxiety, fear,

and panic across the predatory imminence continuum. This research could therefore work to reveal how such responses become maladaptive in human clinical populations following traumatic stress, potentially leading to a leftward shift in the predatory imminence continuum. - Supported by R01MH062122 (MSF); Staglin Center for Brain and Behavioral Health (MSF); BBRF Young Investigator Grant (ANH)

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20. **Keefer SE, Kochli DE, Gyawali U, Calu DJ** - *Role of basolateral amygdala to insular cortex and to nucleus accumbens projections in mediating individual differences in flexibility in sign- and goal-tracking rats* - Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA - Goal-tracking (GT) rats are motivated by the value of a reward and are sensitive to devaluation of that reward. However, sign-tracking (ST) rats are more motivated by cues associated with rewards and are less sensitive to changes in reward value. GT rats remain devaluation sensitive independent of the amount of training, while ST become sensitive to devaluation only after extended training, suggesting distinct, potentially shifting neural mechanisms that decipher reward value. Our lab demonstrated disconnection of the basolateral amygdala (BLA) and anterior insular cortex (IC) decreases goal-tracking behaviors, and previous research demonstrated disconnection of the BLA and nucleus accumbens (NAc) decreases sign-tracking behaviors. We predicted 1) inactivating BLA-IC would abolish devaluation sensitivity in GT rats and 2) inactivating BLA-NAc would unmask devaluation sensitivity in ST rats. Additionally, after extended training, we predicted inactivating the BLA-IC would block devaluation sensitivity in both GT and ST rats. First, for BLA-IC inactivation, we injected inhibitory DREADDs (hM4D-mcherry) or control (mCherry) virus bilaterally into the BLA and implanted bilateral cannulae into the IC for clozapine-N-oxide (CNO; 1mM, 0.25 μ l/side) infusions to temporarily inactivate BLA terminals during devaluation tests. After limited training (sessions 5/6) and extended training (after sessions 18/19), we used a within subject satiety-induced outcome devaluation procedure and sated rats on training pellets (devalued condition) or homecage chow (valued condition; counterbalanced). All rats received bilateral CNO infusions into the IC prior to brief non-reinforced test sessions. We replicated previous results and show control GT rats are sensitive to outcome devaluation, while control ST rats are not. Contrary to our hypothesis, BLA-IC inactivation did not interfere with devaluation sensitivity in GT behaviors, but did render ST behaviors sensitive to devaluation after limited training. Interestingly, devaluation sensitivity in ST behaviors from BLA-IC inactivation may be more prominent in male rats than female rats after extended training, and further analyses are ongoing. For BLA-NAc inactivation, we injected contralateral BLA and NAc with either inhibitory DREADDs (hM4D-mcherry) or control (mCherry) virus. We used similar devaluation testing as before, except rats received systemic clozapine injections (0.1 mg/kg) to disrupt communication between the BLA and NAc prior to brief non-reinforced test sessions. As predicted, BLA-NAc inactivation promoted devaluation sensitivity in ST rats, and, surprisingly, disrupted devaluation sensitivity in GT rats. Together, these results demonstrate BLA-IC and BLA-NAc mediate behavioral flexibility and furthers our understanding of individual differences by recognizing behavioral and neural differences that relate

to addiction vulnerability. - NIDA R01DA043533 to DJC, NIDA F32DA053772-01 to SEK, McKnight Memory and Cognitive Disorders Award to DJC (McKnight Foundation), Brain and Behavior Research Foundation NARSAD Young Investigator Grant # 24950 to DJC

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38. **Kutlu MG, Zachry JE, Melugin P, Isiktas A, Joffe ME, Grueter B, Conn PJ, Siciliano CA, Calipari ES** - *D1 and D2 medium spiny neurons in the nucleus accumbens core signal valence-independent associative learning parameters* - Vanderbilt University - Value-based decision-making is at the core of nearly all motivated behaviors and requires the ability to associate outcomes with specific actions and make adaptive decisions about future behavior. At the core of value-based decision-making and reinforcement is the nucleus accumbens (NAc) which is integrally involved in learning, selecting, and executing goal-oriented behaviors. The NAc is a heterogeneous population primarily composed of D1 and D2 medium spiny projection (MSN) neurons that are thought to have opposed roles in behavior, with D1 MSNs promoting reward and D2 MSNs promoting aversion. However, this framework is largely based on ex vivo recordings showing cell-type specific plasticity after reward/drug exposure. Here we focused on defining the temporal dynamics of D1 and D2 MSNs in response to a variety of stimuli across contexts to define how information is processed in these populations. We tested the role of D1 and D2 MSNs in behavioral paradigms that require processing of stimulus valence, salience, prediction, and timing using optogenetics, patch-clamp electrophysiology, fiber photometry, and cellular resolution calcium imaging. First, we tested whether activation of D1 and D2 MSNs is reinforcing using an optogenetic intra-cranial self-stimulation task. Then, we recorded cellular activity at the population and single neuron level during operant and Pavlovian learning tasks with rewarding and aversive outcomes. Additionally, we examined how inhibition of these two populations at temporally specific time points (cue versus outcome presentation) affects learning. Finally, we tested if aversive learning induces plasticity on both D1 and D2 MSNs using patch-clamp electrophysiology. First, we found that optical stimulation of both D1 and D2 MSN populations supported intracranial self-stimulation. Next, using patch-clamp electrophysiology, we discovered that both D1 and D2 MSNs underwent plasticity following aversive learning demonstrating that plasticity within these populations was not determined by the valence of the experience. To define the information that is encoded within these populations, we recorded their in vivo activity during reinforcement schedules and Pavlovian learning paradigms that dissociate stimulus value, outcome, cue learning, and action from one another. We demonstrated that D1 MSNs responded to the presence and intensity of unconditioned stimuli – regardless of valence. Conversely, D2 MSNs responded to the presentation of predictive cues independent of whether these cues signaled positive or negative outcomes. We also found that learning was disrupted when D1 MSNs were inhibited at the time of the outcome, and D2 MSNs were inhibited during cue presentation – showing a causal role of these signals in learning. Overall, these results provide foundational evidence for the discrete information encoded within D1 and D2 MSN populations in the NAc. The information encoded within these populations goes beyond simply valence encoding and shows that these populations do not have opposing actions. These results will significantly enhance

our understanding of the involvement of the MSN sub-populations within the NAc in both basic learning and memory as well as how these neurons contribute to the development and maintenance of substance use disorders. -

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21. **Magalhães GE, Garza MC, Felix-Ortiz AC, Diehl MM, Burgos-Robles A** - *Active avoidance learning during a dynamic platform avoidance task requires processing in the medial prefrontal cortex and ventral hippocampal inputs.* - University of Texas at San Antonio & Kansas State University - Active avoidance is a complex behavior learned by animals to evade harm associated with imminent threat. Although previous studies have implicated the medial prefrontal cortex (mPFC) in active avoidance, the functional role of the infralimbic (IL) and prelimbic (PL) subregions remains unclear due to contradicting results. We revisit this question using a modified version of the platform-mediated avoidance task in which animals learn to avoid tone-signaled shocks by stepping onto a non-electrified platform (Diehl, Bravo-Rivera, and Quirk, 2019). In contrast to the established task, our new version isolates active avoidance responses while eliminating appetitive components and motivational conflict. Furthermore, the new version incorporates a motorized platform that is inserted into the apparatus just before the beginning of each tone-shock trial, while retracted shortly after the termination of trials. Thus, the platform is only available when animals need it for active avoidance responses. This also eliminates the possibility of passive avoidance confounds (e.g., animals staying on the platform during the entire task including the inter-trial intervals). Using this task in combination with optogenetic-mediated inhibition strategies, our findings indicate that animals undergoing IL inhibition exhibit significant impairment in active avoidance learning. Despite recent findings on PL established using the older version of the task (Diehl et al., 2018), our new findings suggest no significant alterations in active avoidance learning with PL inhibition. In addition, while examining the contribution of specific inputs, we observed significantly slower rates of active avoidance learning during inhibition of ventral hippocampal inputs to mPFC. Collectively, these findings align with the idea of significant contributions by IL but not PL for active avoidance learning. In addition, these findings suggest that the ventral hippocampus may be gating contextual information to the mPFC during avoidance learning. -

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39. **Meyer HC, Gerhard DM** - *Spacing extinction trials across days impacts fear regulation in adult, but not adolescent, male mice* - Weill Cornell Medicine - Fear regulation changes as a function of age and adolescence is a key developmental period for the continued maturation of fear neural circuitry. A consistent finding in the literature is diminished extinction retention in adolescents. However, these studies often directly compare adolescents to adults using a single protocol and therefore provide little insight into learning parameters that improve adolescent fear regulation. Studies in adults highlight the benefits of spaced learning over massed learning. These findings have been extended to fear regulation, with adult rodents exhibiting improved extinction learning and retention when cues are distributed over days versus a single session. However, similar studies have not been performed

in adolescents. Here, we systematically examine the impact of trial spacing across days on fear regulation. Adolescent or adult male mice were exposed to one of three extinction paradigms that presented the same number of trials but differed in the temporal distribution of trials across days (one day, two days, or four days). We found that introducing consolidation events into the protocol improves adult extinction learning and short-term extinction retention but these effects disappear after two weeks. For adolescents, all three protocols were equally effective in reducing freezing across extinction training and improved retention at both short-term and long-term fear recall time points relative to extinction-naive mice. These findings suggest that extinction protocols that incorporate consolidation events are optimal for adults but additional booster training may be required for enduring efficacy. In contrast, protocols incorporating either massed or spaced presentations show immediate and enduring benefits for adolescents. - National Institutes of Mental Health (NIMH) Pathway to Independence Award (K99MH119320)

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12. **Millard, SJ, Greer, Z, Hoang, I, Wassum, K and Sharpe, MJ** - *The cognitive basis of intracranial self-stimulation of midbrain dopamine neurons* - Department of Psychology, University of California, Los Angeles - The finding that dopamine neurons support intra-cranial self-stimulation (ICSS) has been taken as evidence that phasic firing of these neurons is reinforcing. However, these studies typically stimulate dopamine neurons at frequencies (upwards of 50Hz) well outside of those seen in physiological settings. For example, phasic dopamine exhibited during learning as a prediction error is seen at much lower frequencies (12-20Hz). In addition, there are almost no studies investigating the cognitive basis of ICSS. That is, we do not know why dopamine neurons will support ICSS at any frequency. To address this, we used the specific Pavlovian-to-Instrumental transfer (PIT) procedure to investigate if dopamine stimulation supports ICSS because it acts as an internal representation of a goal that subjects are motivated to work for, or whether it simply reinforces the ICSS response (as would be predicted by the value hypothesis of dopamine). On this backdrop, we made comparisons in the frequency at which dopamine was stimulated (20hz or 50hz), to reveal any differences in how these distinct frequencies may support ICSS. We found that a physiologically-relevant frequency of dopamine stimulation (20hz) did not support ICSS or the PIT effect. However, at the supra-physiological frequency (i.e. 50Hz), dopamine stimulation supported robust ICSS and subsequently, specific PIT. These results reveal that physiological frequencies (20Hz) do not function as a reward, insofar that they will not support ICSS or PIT. However, dopamine stimulation at a supra-physiological frequency support robust ICSS because it functions as a specific goal, which is beyond the realm of our physiological everyday experience. -

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40. **Moaddab M, Ray MH, McDannald MA** - *Responding to predicted and surprising foot shock outcome in ventral pallidum and nucleus accumbens core* - Boston College - The ventral striatopallidal system, a basal ganglia network, includes the ventral pallidum

(VP) and the nucleus accumbens. The involvement of different nodes within the ventral striatopallidal system in mediating reward has been well-studied, yet little is known about the contribution of such circuitry to threat processing. Recently, we reported the VP and nucleus accumbens core (NAcc) as neural sources of threat signals. We did this using a discrimination procedure consisting of cues predicting unique foot shock probabilities: danger (p = 1.00), uncertainty (p = 0.25), and safety (p = 0.00). We showed dynamic relative threat signaling during cue presentation within the VP, and specific threat signaling within the NAcc. Here, our aim was to examine firing patterns following the delivery and omission of foot shock outcomes in each region. We observed diverse neural signals for aversive outcome in each region. Further, we found the VP and NAcc to be composed of very similar functional neuron types. Many VP and NAcc neurons are shock responsive, showing equivalent responding following danger and uncertainty shock. However, many other VP and NAcc show differential firing to surprising and predicted foot shock on uncertainty shock and danger trials. This firing pattern is indicative of positive prediction error – a signal to strengthen cue-shock associations. Additionally, both the VP and NAcc contain neurons showing differential firing to surprising and predicted foot shock omission on uncertainty omission and safety trials. This firing pattern is indicative of negative prediction error – signal to weaken cue-shock associations. Neither regions contain a substantial number of neurons showing a fully signed prediction error. Our observation of similar aversive outcome responding raises the possibility that both the VP and NAcc receive inputs from the same areas involved in foot shock-related processing. A complete analysis of VP and NAcc outcome firing will be presented. -

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22. **Mohamed F, Patel S, Ogale N, Santos L, Duesman S, Rajbhandari P, Rajbhandari AK** - *Sex differences in brain and body interactions in stress-associated energy metabolism* - Farzanna Mohamed- University of Michigan. Ohters-Icahn School of Medicine at Mount Sinaithers - While stress related conditions like post-traumatic stress disorder (PTSD) involve an inability to regulate fear responses, increasing observations indicate that such disorders may in fact be brain and body disorder. Homeostatic dysregulation of sympathetic functions in the brain and body axis can lead to enhanced fear, dysregulated autonomic functions and systemic metabolic dysfunctions. Using the stress enhanced fear learning (SEFL) as an assay of dysregulated fear, we have investigated the role of sympathetic neuromodulators, such as the pituitary adenylate cyclase activating peptide (PACAP) and its receptor PAC1 in regulation of fear and energy metabolism and associated sex differences. Human and animal studies have linked PACAP/PAC1 to PTSD diagnosis and symptom severity and regulation of energy metabolism. We have shown that PACAP innervates the brown adipose tissue (BAT), a metabolically active type of fat tissue that converts food substrates into heat energy. PAC1 is abundant in BAT. These two findings suggest a role for PACAP/PAC1 in BAT thermogenic regulation, a key aspect of energy metabolism required for any behavior. We hypothesized that traumatic stress enhances metabolism on the short term for high energy demand. On the long run, it can lead to decreased metabolism, thereby inducing metabolic syndromes. Using mice (males and females) with floxed PAC1

receptors and employing a range of techniques, including viral-mediated PAC1 knockdown, SEFL, metabolic physiology, immunohistochemistry, in situ hybridization, and qRT-PCR, we show PAC1 in the locus coeruleus (LC) regulates SEFL and energy metabolism in a sexually dimorphic manner. LC is a major source of sympathetic pathway to the forebrain, with enriched PAC1 expression. We show that PAC1 receptor deletion in the LC leads to sustained SEFL, enhanced energy expenditure and oxygen consumption in females. In males, we find either the opposite effect or no changes in these measures. Taken together, our results indicate that PACAP/PAC1 is an important neuropeptidergic system in the brain sympathetic node that links the brain and the body to regulate traumatic stress and associated metabolic changes. - Brain and Behavior Foundation, WhiteHall Foundation, Akira Arimura Foundation

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4. **Mohammadmirzaei N., Biddle M., Boyapati S., Hekmatyar K., Cai X., Kulkarni P., Knox D.** - *Sex differences in the effects of traumatic stress on the Mu-opioid receptors, brain volume and connectivity within reward circuits.* - University of Delaware - Post-traumatic stress disorder (PTSD) and opioid use disorder (OUD) are two disorders that frequently co-occur. Women are more likely than men to develop PTSD after trauma exposure and OUD after PTSD. OUD is characterized by changes in the mu-opioid receptor (MOR), volume of key nodes within the reward circuit, and connectivity among nodes within reward circuits. As a result of traumatic stress exposure, male and female brains may undergo different changes in MORs within reward circuits, volume of neural substrates within reward circuits, and functional connectivity within the reward circuit. These could be mechanisms by which traumatic stress leads to higher OUD susceptibility in females. The single prolonged stress (SPS) model was used to examine the effect of traumatic stress on MOR function, brain volume and functional connectivity in reward circuits. We defined the reward circuit as comprising of the medial prefrontal cortex (mPFC), amygdala (Amy), dorsal and ventral hippocampus (DH and VH), nucleus accumbens (NAc), and ventral tegmental area (VTA). Western blot (WB) and immunohistochemistry (IHC) were used to assay MOR function. For volumetric and connectivity analyses, 3D T2-weighted magnetic resonance imaging (MRI) and resting-state functional connectivity imaging (R-fMRI), using a 9.4T Brucker magnet, were performed. Our preliminary molecular data showed sex differences in the MOR expression in the mPFC, NAc, Amy and DH following SPS exposure. In male rats, SPS exposure decreased MOR expression in the mPFC, NAc, Amy, and DH, but increased MOR expression in these brain regions in females. Our preliminary fMRI data also revealed sex differences in functional connectivity within reward circuit nodes following SPS exposure. While SPS exposure increased the functional connectivity between the central Amy, CA1 of VH, and dorsal dentate gyrus (dDG) in female rats, it decreased the functional connectivity between subregions of mPFC including anterior cingulate cortex (ACC), infralimbic cortex (IL), and prelimbic cortex (PL) in males. Furthermore, we discovered an increase in the volume of VTA after SPS exposure in both male and female rats. Male rats showed increased volume in the CA2 and ACC in addition to the increased VTA volume after SPS exposure, whereas female rats showed increased volume in the NAc shell, central and basal Amy, CA1

of DH, and CA3 of VH. The study is ongoing, but sex differences in MOR function, volume, and functional connectivity within nodes of the reward circuit may be possible explanations for sex differences in the prevalence of OUD within PTSD. -

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23. **Morales I, Berridge KC** - *Identification of Novel Hedonic Hotspots in Orbitofrontal Cortex, Insula, Ventral Pallidum, and Anterior Cingulate that mediate 'liking' and 'wanting' for sweet reward.* - University of Michigan - Brain reward systems for 'liking' and 'wanting' may become dysregulated during eating disorders and addiction. Hedonic function or 'liking' is amplified by brain hedonic hotspots, or subregions of rat nucleus accumbens, ventral palladium, orbitofrontal cortex (OFC), insula during the taste reactivity test. Beyond previously identified neurochemical triggers, neuronal activation patterns and the larger circuitry that control hedonic function is not well understood. Here we present novel evidence that optogenetic stimulation of hedonic hotspots in rostromedial OFC, posterior VP GABAergic neurons, and caudal insult doubles 'liking' reactions to sweet sucrose, and suppresses the aversive impact of quinine that is normally 'disgusting'. Photostimulation of the hedonic hotspots also amplifies 'wanting', and generates laser self-stimulation. Importantly however, 'wanting', which can be generated outside of the hotspots, 'liking' effects are anatomically restricted. The same optogenetic manipulations in rostral VP, caudal OFC¡ and anterior IC fail to amplify 'liking', and even oppositely suppress hedonic reactions, despite still generating robust 'wanting' at some sites. Finally, we also identify the existence of anole hedonic hotspot in caudal anterior cingulate cortex (ACC) that has never been previously described. Our results show that optogenetic stimulation of caudal ACC doubles hedonic 'liking' reactions in rats, and also generates 'wanting' and laser self-stimulation. Overall, our findings suggest the existence of a novel hedonic hotspot in brain corticolimbic sites that form a functional hedonic circuit for 'liking' control. - MH125613 to IM, DA015188 and MH063649 to KB

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24. **Nishimura KJ, Stallings A, Drew MR** - *Behavioral and neural mechanisms of stress-induced fear sensitization* - Center for Learning & Memory, Dept Neurosci, UT Austin - Stressful experiences can lead to long-lasting alterations in emotional fear responses. Excessive and disproportionate fear that extends beyond contexts or situations related to the traumatic event resemble symptoms of hyperarousal, commonly observed in individuals with post-traumatic stress disorder. Although the neural circuitry underlying Pavlovian conditioned fear to a discrete stimulus has been well studied, the circuits recruited by a single stressful event to ubiquitously sensitize fear remain poorly understood. In the current study, we characterized the behavioral and neural mechanisms of stress-induced fear sensitization using a novel mouse model. Mice were assigned to a "Stress" group that received four 1-mA foot shocks or a "No Stress" group that received equivalent context exposure. Following 24 hours, animals exposed to stress exhibit a persistent phenotype of fear sensitization characterized by (1) decreased exploration in the open field, (2) potentiated unconditioned fear of a

novel tone, and (3) stress-enhanced fear learning (SEFL) in a novel context. Stress-induced fear sensitization to unconditioned tone and SEFL are resistant to extinction training in the stress context, indicating that non-associative learning facilitates this process. Furthermore, stressed animals do not exhibit differences in reflexive pain thresholds, suggesting that fear sensitization is not mediated by changes in peripheral pain processing. Next, we aimed to identify potential brain regions that mediate fear sensitization. Whole brain c-fos analysis was performed on a separate cohort of animals that underwent the stress-induced fear sensitization procedure. We identified several brain regions that were hyperactive in stressed animals including the posterior paraventricular thalamus, dorsolateral periaqueductal gray, and the lateral parabrachial nucleus. Lastly, we identified a novel role of the posterior paraventricular thalamus in the induction of stress-induced fear sensitization. Overall, the data indicate that fear sensitization is not contingent on the initial associative fear memory and is likely acquired and maintained through dissociable neural mechanisms. - R01 MH117426

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25. **Posillico CK, and Tronson, NC** - *Sex-specific type I interferon modulation of learning and memory* - Dept. of Psychology, University of Michigan, Ann Arbor - The neuroimmune system is necessary to carry out normal neural and cognitive functions including learning and memory. There are known sex differences in the neuroimmune response to various pathogens, but whether biological sex plays a critical role in how neuroimmune activation affects learning and memory processes is unknown. Here, we investigated the interaction of neuroimmune activation and hippocampal-dependent memory mechanisms in both male and female C57Bl/6 mice using intracerebroventricular administration of a synthetic viral mimic, polyinosinic:polycytidylic acid (poly I:C), and context fear conditioning. First, poly I:C treatment induced significant cytokine responses in the hippocampus of both sexes. Males had a greater magnitude of response than females for IL-1alpha, IL-1beta, IL-6, IL-10, IFNalpha, TNFalpha, CCL2, and CXCL10. Additionally, while both males and females showed increased expression of type I interferon beta, only males showed increased type I interferon alpha. Next, we found that poly I:C treatment four hours prior to training in context fear conditioning resulted in significant deficits in freezing responses in both males and females when tested three days later. Pre-training poly I:C appeared to blunt training-induced cFos in the dorsal hippocampus of males, but it had no effect on cFos levels in females. Thus, the same memory deficit in both males and females may be due to different and sex-specific mechanisms in the hippocampus. Next, we tested whether the sex difference in the type I interferon responses to poly I:C contributed to the sex difference in hippocampal mechanisms underlying memory deficits. We blocked type I interferon receptors (IFNAR) two hours prior to poly I:C treatment, followed by context fear conditioning four hours post-poly I:C. We found that IFNAR inhibition significantly rescued the poly I:C-induced deficit in males, but not females. This suggests that type I interferons play a more important role in modulating learning in males compared with females, and type I interferon signaling is a potential target for understanding sex differences in biological mechanisms of memory impairment induced by neuroimmune activation. Given that there are sex differences in the prevalence of debilitating memory disorders such as Alzheimer's disease and Post-Traumatic Stress disorder that

present with neuroimmune dysfunction, it is important to identify sex-specific mechanisms of memory deficits that may lead to more effective treatments. - University of Michigan Office of Research Grant to NCT

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26. **Raycraft, L.M.1, Bunney, M. 1, Nalla, A. 1, Pataro, A. 1, Kashy, D.A. 1, Noble, E.E. 2, Kanoski, S.E. 3, Johnson, A.W. 1** - *Excitation of lateral hypothalamic melanin concentrating hormone neurons influences motivation and peak interval responding in a sex and estrous cycle-dependent manner* - (1) Department of Psychology, Neuroscience Program, Michigan State University, (2) University of Georgia, (3) University of Southern California, Los Angeles - Interval timing, which refers to the ability to perceive small intervals of time in the magnitude of seconds to minutes, provides temporal contiguity and enables the learning of predictive relationships. In the present study, we examined whether lateral hypothalamic area (LHA) cells known to play a role in learned feeding behaviors could also influence interval timing. If so, the ability of these cells to alter temporal perception could provide a mechanism for their influence over feeding behaviors. Specifically, we examined whether chemogenetic excitation of LHA cells that produce the orexigenic neuropeptide Melanin Concentrating Hormone (MCH) would influence responding in a peak interval procedure. Male and female Sprague Dawley rats first learned to time a 20 s fixed interval (FI) criterion duration for sucrose reinforcement. Next, non-reinforced probe trials were intermixed, where the lever remained available for a period ¿60 s. During these trials, lever responding forms a slightly skewed normal distribution in which pressing peaks around the criterion duration (i.e., 20 s). Using a custom AAV packaged with excitatory DREADD hM3Dq expression under the control of the MCH gene promoter, we selectively excited MCH neurons in either the anterior or posterior region of the lateral hypothalamic area (LHA). Analysis of peak function and multilevel modelling revealed that MCH neuron excitation led to significant differences in peak interval responding during probe trials that depended on (1) the location of MCH neuron excitation within the LHA, (2) sex, and (3) estrous cycle stage. Altogether, these results indicate that subpopulations of MCH neurons in the LHA uniquely modulate interval timing and food-seeking depending on sex and estrous cycle stage. Moreover, these findings are the first to identify an influence of orexigenic neuropeptides on interval timing. - RO1DK111475

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27. **Reed S, Harlan EW, Campolattaro, MM, Lipatova, O** - *Stress influences on spontaneous recovery of a conditioned touchscreen response in male and female rats* - Christopher Newport University - The present study was designed to investigate how stress affects spontaneous recovery of a previously extinguished appetitively-conditioned touchscreen response in male and female rats. Forty adult Sprague Dawley rats were used in this experiment. Half of the rats were male and half were female. After pretraining, rats learned to perform a nose-poke response directed at a stimulus (e.g., a white square) that appeared on a touchscreen. Extinction training began on the session after each rat reached above an 80% correct

response criteria. Once a rat reached below a 23% response extinction criteria, they were placed on a three-week retention interval. After the retention interval, each rat was placed into the testing chamber for one session of stimulus-alone presentations in order to test for spontaneous recovery. A 30-min restraint stressor was administered to half of the male and half of the female rats immediately prior the spontaneous recovery test. There were no significant differences between male and female rats in acquisition or extinction of touchscreen responses. Interestingly, rats that were exposed to the stressor exhibited significantly greater spontaneous recovery compared to non-stressed rats early during the spontaneous recovery test trials. However, the stress-exposed rats also showed significantly lower responding during the latter blocks of response recovery than controls. These results suggest that elevation of the stress response system impairs the initial ability to inhibit a previously learned behavior, but potentially facilitates extinction learning in the longer term. We found high variability in conditioned responding in female rats that received stress exposure, and it is possible that stress-effects in female rats are dependent on the fluctuating levels of estrogen across the different phases of their natural estrous cycle. Future experiments are planned to address these questions. -

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28. **Schuh KM, Giffin KA, Choi HK, Tronson NC** - *COVID-like inflammation contributes to memory impairments and age-related cognitive decline* - University of Michigan - What is learned, and the strength of memory formation, are modulated by concurrent events including stress and illness. It is also clear that these events can lead to long-lasting changes in the brain that mediate persistent changes in memory formation. We have recently demonstrated that a mild, subchronic immune challenge leads to memory impairments months after the resolution of the immune challenge. These findings suggest that inflammatory signaling associated with SARS-COV-2 infection and COVID-19 illness, might trigger cognitive impairments and cognitive decline that is reported in patients of "Long COVID". In this project, we aim to determine whether inflammation triggered by single-stranded RNA viruses, like SARS-COV-2, cause memory impairments in young animals, similar to those observed after sepsis-like challenges. In addition, we examined whether either R848 or a sepsis-like immune challenge (lipopolysaccharide) increased risk for exaggerated cognitive decline in older animals. In young mice, we used our previously established subchronic systemic injection protocol (Tchessalova & Tronson, 2019, 2020) with a toll-like-receptor 7 agonist, R848, to mimic the specific inflammatory response to single-stranded RNA viruses. We observed that acutely, 400 ug/kg R848 caused a physiological sickness response, including fever, changes in locomotor activity, and weight loss in the 24 hours following injection in both males and females. We further examined learning and memory impairments in young and aged mice of both sexes, together with other Long-COVID-like symptoms including depression. In aged mice, we observed increased vulnerability to memory impairments after immune challenge, compared with young mice. Unlike young mice, in which memory impairments emerge after 8 weeks post-immune challenge, in aged animals, memory impairments were evident as soon as 2 weeks later. This work will be critical for understanding a lesser-studied inflammatory pathway in the brain, and its impact on memory; as well

as determining how COVID-19-like inflammation contributes to long-lasting changes in the brain and vulnerability to memory impairments and cognitive decline. - MDARC/Claude D. Pepper Pilot grant to NCT

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29. **Sheynin J, George SA, Aupperle RL, Smith R, Taylor S, Touthang J, Liberzon I, Abelson JL** - *Altered approach-avoidance processing in generalized anxiety disorder: Empirical and computational findings* - Department of Psychiatry and Behavioral Science, Texas A&M University Health Science Center, TX, USA (JS, IL); Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA (SAG); Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA (SAG, JA); Laureate Institute for Brain Research, Tulsa, OK, USA (RLA, RS, ST, JT); Oxley College of Health Sciences, The University of Tulsa, Tulsa, OK, USA (RLA). - Approach-avoidance conflict (AAC) is a state of opposing motivations, and an "imbalance" in approach-avoidance decision-making has been associated with psychopathologies. Here, we studied AAC resolution in patients diagnosed with generalized anxiety disorder (GAD; n=14), obsessive-compulsive disorder (n=10), social anxiety disorder (n=14), panic disorder (n=14), major depressive disorder (n=10) and healthy controls (n=20). Participants were given a validated computer-based AAC task, which creates decisional conflict between a negative affective stimulus with points attached and a positive affective stimulus with no points. In addition, we used active inference modeling approach to examine certainty or confidence in one's decisions (decision uncertainty) and sensitivity to negative outcomes versus rewards (emotional conflict) as two processes that might shape behavior on the task. Group differences were analyzed using independent samples t-tests. Relative to healthy controls, the GAD group showed lower approach behavior on both reward-only trials with only positive outcomes, and on conflict trials in which both positive and negative outcomes were presented. The computational model showed 73% accuracy in predicting behavior (chance level= 11%) and suggested that GAD patients had greater decision uncertainty and greater emotional conflict during task performance, compared to controls (all $p < .05$). None of the behavioral or computational variables differed for other patient groups. This study suggests that GAD is related to behavioral differences in response during approach-avoidance conflict, characterized by diminished overall approach behavior as well as increased decision uncertainty. These findings suggest that direct targeting of heightened sensitivity to negative outcomes (e.g., via exposure strategies), reduced valuation of rewards (e.g., via behavioral activation strategies), and decision-making functions (e.g., problem-solving strategies, exposure activities focused on making quick decisions) may be useful for enhancing treatment outcome for GAD. (JS and SAG contributed equally). - Support: Internal funds (Abelson); R01 MH123691 (Aupperle).

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8. **Smies CW, Bellfy L, Wright DS, Urban MW, Brunswick CA, Kwapis JL** - *The role of histone deacetylation in reconsolidation-based memory updating* - Penn State -

Memories are plastic records of experience that require maintenance in the form of updating to remain meaningful. Unlike memory formation, the molecular mechanisms of memory updating have yet to be fully elucidated. One key mechanism for memory formation is histone acetylation, the addition of acetyl groups to histone tails, which enables necessary gene expression. Blocking histone deacetylase 3 (HDAC3, an enzyme that reduces histone acetylation) during memory consolidation enhances gene expression and long-term memory formation in young and old mice (McQuown et al., 2011 & Kwapis et al., 2018). In addition, blocking histone acetylation disrupts both consolidation and reconsolidation of fear memories (Maddox et al., 2013). Here, we tested whether the HDAC3-specific inhibitor RGFP966 promotes reconsolidation-dependent memory updating in young and old male C57BL/6J mice using the novel Objects in Updated Locations (OUL) paradigm (Wright et al., 2020). In OUL, mice are trained on the locations of two identical objects. Next, this memory is updated by moving one object to a novel location. Memory is then indexed by the time spent investigating the three familiar locations in relation to a novel location to assess strength for the original and updated locations. We found that systemically inhibiting HDAC3 immediately following memory updating ameliorates age-induced deficits in memory updating. However, young mice that receive RGFP966 immediately following the update session show intact memory for the updated information but decreased memory for the initial training. Together, these data suggest that HDAC3 inhibition can enhance memory updating at the expense of the original memory. HDAC3 inhibition enhances the updated information without affecting the original memory in old animals that fail to update. In young animals demonstrating successful memory updating, RGFP966 strengthens the updated information, which then outcompetes the original memory. Thus, the updated and original memories are tightly linked and may compete for expression. -

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41. **Steinfeld MR & Bouton ME** - *An Examination of Responses Inhibition in Operant Conditioning* - University of Vermont - Evidence from extinction research on operant (instrumental) learning suggests that animals learn to inhibit a specific response in extinction, such that extinction of one response does not suppress other responses. Inhibition of operant behavior can be further studied using feature-negative (F-N) discrimination procedures. In such procedures, a response (R1) is reinforced during Cue A, but not when Cue A is compounded with Cue B (AR1+, ABR1-). This training allows B to inhibit R1. Previous research with this type of procedure suggests that cues trained to inhibit one response can also suppress other responses. However, there has been no comparison of the inhibitor's ability to inhibit the response it is trained with (same-response inhibition) versus the other response (cross-response inhibition). We set out to make such a comparison. In Experiment 1, rats learned two F-N discriminations in which different responses were occasioned and inhibited by unique cues (AR1+, ABR1-, CR2+, CDR2-). We tested each inhibitor with both its own response and the other response and found that cross-response inhibition was as strong as same-response inhibition. In Experiment 2, some rats performed two operants that were inhibited by differential inhibitors (AR1+, BR1-, CR2+, DR2-) while other rats received the F-N procedure. Consistent with a role for response-error correction, inhibition

was stronger after training with the F-N procedure. However, with the small amount of inhibition that was learned with the differential procedure, cross-response inhibition was again equal to same-response inhibition. Experiment 3 tested the idea that discrimination learning could result in the animal learning that a response is "inhibitable," making it susceptible to cross-response inhibition. Rats were given F-N training with R1, but did not receive inhibition training with R2 (AR1+, ABR1-, CR2+, -). While some cross-response inhibition still occurred (BR2 ¡ R2 alone), it was weaker than same-response inhibition, suggesting the previous inhibition learning with the target response allows it to be more easily inhibited. The results suggest that inhibitors do transfer across responses, but its strength depends on the animal having learned that the target response is itself "inhibitable." -

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42. **Strickland JA, McDannald MA** - *Neuropixels recordings reveal distributed signaling of threat probability and threat-elicited behaviour across rodent midbrain and pons* - Boston College - The generation of suitable threat signals in response to threat stimuli is generally considered to be the domain of the forebrain. These signals are then thought to be relayed to midbrain/pons regions to organize the behavioural aspects of fear and adaptive fear responding. Recording from the ventrolateral periaqueductal gray, a midbrain region commonly implicated in generating fear behavior, our lab has found signals for both threat probability and behavioural output. This suggests that threat probability signaling is not exclusive to forebrain regions. To more comprehensively map midbrain and pons threat signaling, we devised a system for multi-region, high-density recording using Neuropixels probes during a fear discrimination procedure in rats. The one-centimeter Neuropixels probe was implanted such that its path coursed through the superior colliculus, most periaqueductal gray subregions, all dorsal raphe subregions, median raphe and additional regions enroute. We recorded 1812 neurons across 21 regions from 9 rats. Analysis of firing during cue presentation showed that neurons could be clustered into discrete functional types, with each type distributed across at least several regions. Every functional cluster showed a unique temporal pattern of differential cue firing. From these clusters, a small functional network emerged: consisting of clusters showing short latency firing increases to danger, strong differential firing of danger and safety with firing patterns tightly correlated across clusters. Regression revealed these neurons to initially and rapidly signal threat probability, then giving way to signaling of threat-elicited behaviour. Additional analysis of the period following the outcome indicates these regions also play a role in threat outcome and prediction error signals, expanding even further the potential role of midbrain/pons networks in threat signaling and behaviour. These results suggest that networks of midbrain/pons regions not only generate threat-elicited behaviour in response to threat stimuli, but may also play a role in the generation of threat signals themselves. -

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30. **Sud N, Little B, Bhattacharya D, Escobar M** - *The Role of Prenatal ILK Activity on Spatial Memory* - Lake Erie College of Osteopathic Medicine, Oakland University - Using

a rodent model of fetal alcohol spectrum disorder (FASD), we previously reported that early exposure to moderate levels of alcohol leads to reduced synaptic plasticity and impairments in hippocampal-dependent spatial memory. These deficits were strongly associated to impaired function of Integrin Linked Kinase (ILK). ILK is a synaptic scaffolding protein that has important functions in learning, memory, and glutamatergic synapse development, and can be activated through stimulation of the Brain Derived Neurotrophic Factor (BDNF) receptor Tyrosine kinase B (TrkB). The present study aimed to determine whether the previously observed association between ILK and the memory deficits associated to FASD is indeed causal. Intraperitoneal injections of the ILK inhibitor, QLT0267, was administered to dams on alternate days through the gestational period. The offspring's spatial memory was assessed at approx. 1 month of age using a Y maze. QLT0267-exposed pups exhibited spatial memory deficits as compared to controls exposed to vehicle throughout gestational development. Although these spatial memory deficits were not associated to concurrent changes in hippocampal ILK or glutamatergic AMPA receptors, prenatal inhibition of ILK reduced expression of glutamatergic NMDA receptors. -

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31. **Totty M, Ramanathan K, Maren S** - *The nucleus reuniens of the thalamus is necessary for extinction retrieval and prefrontal-hippocampal theta synchrony* - Department of Psychological and Brain Sciences and Institute for Neuroscience, Texas A&M University, College Station, TX 77843-3474 - The nucleus reuniens of the thalamus (RE) is a small midline structure interconnecting the medial prefrontal cortex (mPFC) and hippocampus (HPC) that has been proposed to coordinate the precise retrieval of specific memories, such as fear extinction. One mechanism by which the RE may facilitate mPFC-HPC communication is by the coordination of theta oscillations. Previous work has revealed that pharmacological inactivation of the RE inhibits extinction recall, and RE inactivation impairs mPFC-HPC theta synchrony during work memory tasks. However, it is still unknown if the RE is critical for mPFC-HPC theta dynamics associated with extinction recall. Using auditory fear conditioning and extinction, we extend previous pharmacological findings by showing that optogenetic silencing of the RE using a red-shifted opsin (Jaws) impairs extinction retrieval 24 hours after extinction training. Importantly, we demonstrate that increased freezing due to RE inactivation is not non-specific as inhibiting the RE in animals that were never conditioned had no effect of freezing behavior. In an additional experiment, we show that RE inactivation also impairs mPFC-HPC theta synchrony during extinction recall. These data extend our current understanding of the role of the RE in the retrieval of fear extinction memories and coordinating mPFC-HPC theta synchrony. -

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32. **Trott JM, Hoffman AN, Zhuravka I, &. Fanselow MS** - *The associative nature of defensive behavior following serial conditioned stimulus fear conditioning* - UCLA - Fear conditioning is one of the most commonly used laboratory procedures to study learning and memory in addition to anxiety and fear-disorders. Fear conditioning results in the formation of an association between an initially neutral conditional stimulus (CS), such as a tone,

and an aversive unconditional stimulus (US), such as an electric footshock such that after conditioning the CS alone elicits a fear-induced conditional response (CR). For many years, freezing to the CS that predicts danger has been the standard objective measure of learning and fear. Freezing dominates as a defensive behavior in a large variety of species as many predators' visual systems use motion as a releasing stimulus for attack. Recently, a few studies have challenged this idea, suggesting that there can be other conditional behaviors elicited by fear-inducing stimuli, namely an increase in running, jumping, or darting. These results have been interpreted as a competition between "active" and "passive" defensive behavior. While this has clear technical implications, it also has theoretical implications. From a technical and methodological standpoint, only measuring freezing in fear conditioning studies in which alternative fear responses occur would result in missing instances of fear learning and suggests that prior research may need to be reexamined. Theoretically, understanding the antecedent causes of these differing defensive behaviors may help inform on the general transitions between emotional and behavioral states. We have replicated the results of these prior studies and showed that mice can indeed develop a darting response to a CS that predicts shock. However, we have also included important control conditions to assess the associative nature of such darting behavior which reveal that this running, jumping, and darting response is a result of non-associative processes and is often reduced by associative learning. Freezing behavior was the most reliable index of associative learning. We have further developed a rule which describes when and how animals may transition from freezing to darting. The rule is that, when afraid, animals will freeze until there is a sudden novel change in stimulation, then burst into vigorous flight attempts. This rule may also govern a transition between a fearful state, in which freezing dominates, to a panic-like state, in which darting behaviors appear. -

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37. **Vaaga CE, Raman IM** - *Activity and modulation of vlPAG neurons during innate freezing* - Northwestern University - Appropriate behavioral responses (i.e. freezing and flight) to threats within the environment are critical for survival. Such innate defensive behaviors require activation of the midbrain periaqueductal gray (PAG), however, the extent to which distinct sub-columns within the PAG are involved in conditioned vs. innate freezing remains controversial. Our recent work has demonstrated that putative freezing-related neurons (expressing the transcription factor Chx10) in the ventrolateral column of the PAG (vlPAG) are subject to modulation by local dopaminergic neurons, which receive input from cerebellar circuits. Whether these neurons in the vlPAG mediate innate freezing, however, is not known. Here we have begun to test the necessity and sufficiency of activity in vlPAG Chx10 neurons during innate fear responses evoked by visual looming stimuli. Under control conditions, both male and female mice responded to visual looming stimuli with prolonged freezing responses (males: 31.4 ± 4.0 seconds, females: 38.5 ± 3.6 seconds, unpaired t-test $p = 0.2$). In response to repeated stimuli, freezing responses showed robust habituation, but the pattern of habituation depended on the interval between successive stimuli. Habituation was slowed when stimuli were presented within the same behavioral session (inter-trial interval: $\sim$ 5 minutes), as compared to when stimuli were presented across behavioral sessions (inter-trial

interval: 24-48 hrs). To test necessity of vlPAG Chx10 neurons during innate freezing, we optogenetically inhibited these neurons during the presentation of a looming visual stimulus. Optogenetic inhibition significantly reduced the percent time spent freezing during the first 20 seconds post stimulus (WT: 87.2 ± 2.3%; Chx10::Arch: 41.3 ± 9.3%). These results suggest silencing Chx10 neurons occludes freezing responses, suggesting that vlPAG circuits are responsible for generating innate freezing behaviors. Freezing could also be elicited in head-fixed mice running on a treadmill, setting the stage for electrophysiological recordings of vlPAG neurons during behavior. -

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43. **Vasudevan K, Resser RL, Maren, S** - *Relapse of Extinguished Fear After Chemogenetic Reactivation of Hippocampal Fear Ensembles in Rats* - Texas A&M University Dept. of Psychological and Brain Sci. and Inst. for Neurosci. - Recent work in mice has suggested that distinct ensembles of dorsal hippocampal (DH) neurons represent memories of fear and safety after contextual fear conditioning and extinction, respectively (Lacagnina et al. 2019). It is unknown, however, whether similar representations are formed by auditory fear conditioning and whether these representations modulate responding to extinguished conditioned stimuli (CSs). Here, we used an activity-dependent Tet-OFF tagging system ("TetTag") to express an excitatory DREADD (AAV.cfos.tTA9.bGH, AAV.TRE.hM3Dq.mcherry.rBG) in DH neurons active during auditory fear conditioning in rats. Male and female Long-Evans rats were put on doxycycline (DOX) diet prior to infusion of the TetTag AAVs into the DH. Three weeks later, rats were removed from DOX, and the following day were placed in a novel context and presented with 5 tone-shock pairings (n=24) or 5 tone-only presentations (n=8) before being put back on DOX and returning to their homecage. Forty-eight hours later, all animals underwent fear extinction (45 tone-only trials or until fear response sufficiently diminished). Rats then underwent two counterbalanced extinction retrieval tests on subsequent days, in which either clozapine-N-oxide (CNO) or vehicle (VEH) was systemically administered. Freezing was used as an index of fear. During the retrieval tests, CNO administration increased conditioned freezing to the extinguished CS relative to VEH-treated rats or rats that received CNO but were not conditioned. Interestingly, CNO administration did not increase pre-CS freezing to the test context, suggesting that reactivating DH conditioning ensembles did not increase CS freezing by increasing fear to the test context. Rather, chemogenetic reactivation of DH ensembles tagged during auditory conditioning caused a relapse of extinguished fear to an auditory CS. These results imply that contextual representations formed in the DH during auditory fear conditioning can serve to modulate expression of conditioned fear to an extinguished CS. - NIH Grant R01MH065961

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34. **Wickens MM, Ferrario CR, Robinson TE** - *Increasing the intermittency of intermittent cocaine self-administration: development of a new model* - University of Michigan - When cocaine is self-administered in short periods interspersed by longer periods in which cocaine is not available ("intermittent access, IntA"), increased addiction-like behavior has

been reported by our lab and others compared to continuous access conditions (i.e., 1-6 hours). Addiction-like behavior occurs despite the total drug intake being much lower following IntA than continuous access, suggesting that increasing the degree of intermittency of cocaine self-administration may promote neuroadaptations that underlie addiction. To date, studies have used daily self-administration sessions for both continuous and IntA procedures. In our proposed model, we increased the intermittency of the IntA self-administration schedule by allowing rats to self-administer only twice per week. Thus, rats in this "intermittent squared" (IntA2) group underwent 10 days of intermittent cocaine self-administration distributed across 5 weeks, whereas rats in the IntA group were given 10 consecutive sessions. We then compared demand for cocaine between these groups. For analysis, we selected a subset of rats from each group that 1) acquired cocaine self-administration within 3 days, 2) completed all 10 sessions of intermittent self-administration, and 3) did not differ in total cocaine intake on day one of intermittent access. During intermittent self-administration, IntA rats maintained a steady number of infusions across 10 days and performed, on average, 1-2 active responses per minute during the drug available period. IntA2 rats showed a somewhat different pattern, with a steady increase in cumulative infusions and average responses throughout the first 4-5 sessions, followed by a steady decrease until day 10. On day 5 of intermittent self-administration, the IntA2 rats performed significantly more responses than the IntA rats, which is most clearly seen when comparing the average responses made during each minute of the drug available period. When cocaine demand was assessed, we found that although both groups prefer to administer the same amount of cocaine when price is low, the intermittent squared group showed a trend towards increased motivation to maintain their preferred level of cocaine intake. This may explain the early increase in responses during self-administration; although not significant, the intermittent squared group may capitalize on the infrequent availability of cocaine. Additionally, preliminary data suggests that the IntA2 may perform more responses during cue-induced reinstatement. Together, these results suggest that increasing the intermittency of the intermittent access procedure may enhance cocaine administration and select measures of motivation. Additional studies are in progress to replicate and extend these results. - T32DA07268

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35. **Wolcott N S, Redman W T, Sit K K, Goard M J** - *Chronic measurement of dendritic spine turnover in CA1 across the estrous cycle* - Department of Molecular, Cellular, and Developmental Biology, UC Santa Barbara, Santa Barbara, CA; Department of Dynamical Neuroscience, UC Santa Barbara, Santa Barbara, CA; Department of Psychological and Brain Sciences, UC Santa Barbara, Santa Barbara, CA; Neuroscience Research Institute, UC Santa Barbara, Santa Barbara, CA - Sex steroid hormones have long been thought to modulate structural and functional plasticity in the brain. Dendritic spines act as a primary driver of plasticity, yet technological constraints have limited chronic in vivo measurements of these dynamic structures. Previous studies suggest that dendritic spine turnover in the apical dendrites of subfield CA1 of the hippocampus is modulated by hormones such as estradiol and progesterone, which fluctuate over the course of the 4-5 day rodent estrous cycle. To address this, we tracked dendritic spine motility across multiple estrous cycles in

female mice. To reduce variability in estrous stage classification, we developed a novel unsupervised deep learning pipeline trained on a diverse set of 13,000 cytological images from mice and rats to classify estrous stage at expert levels. Apical dendritic spines in CA1 were recorded using a chronically implanted glass microperiscope that granted us optical access to the transverse plane of the hippocampus at submicron resolution. In naturally cycling mice, we observed a reliable 15.3% increase in apical dendritic spine density in late proestrous, followed by a 20.6% decrease in density in early estrous. In male mice, total turnover dynamics reflected 15.0% spine addition or subtraction across consecutive days, such that no net change in density was observed at 24hr increments. In our combined cohort we found only a mean 23.5% net loss in original spines after 10 days, indicating that majority of spine turnover takes place within an isolated population of transient spines. As a next step, we will use calcium imaging of hippocampal neurons while mice explore a floating environment to investigate how the estrous cycle modulates place field stability and remapping. Taken together, these findings inform our understanding of how structural and functional plasticity in hippocampal pyramidal cells are modulated by sex steroid hormones. - Many thanks to the Larry Hillblom Foundation, NSF NeuroNex, and NIH for supporting our work.

Virtual Posters

Anonymous - *Contextual cues influence the urgency of micturition: A subjective, introspective, and purely anecdotal analysis (n = 1)* - Institutional Affiliation Redacted - Contextual cues encountered in the presence of a full bladder were assessed in terms of their ability to exacerbate the urge to pee in a 60+-year-old man. The participant, experiencing the need to pee while his bladder was distended for prostate cancer radiation treatment, rated the strength of the urge to pee using a purely subjective introspective P-scale that offered five options: Meh, I Feel It, Please, Oo Oo Oo, and Outta My Way. While the urge to pee increased with time since ingestion of water, the urge was also increased or decreased by various contextual cues. The influence of these cues could be understood in terms of their past association with micturition or with its inhibition. - Supported in part by medical insurance provided by Medicare and Blue Cross Blue Shield.

Beale AD, Pribic MR, Rose JK - *Association of Competing Innate Responses* - Western Washington University - Recent studies examining association of opposing responses, contrasting emotional valences or counter motivational states have begun to elucidate how learning and memory processes can translate to clinical therapies for trauma or addiction. In the current study, association of opposing responses is tested in C. elegans. Due to its relatively simple and well-described nervous system, it was hypothesized that careful examination of post-conditioning responses would elucidate if oppositional associations result in a strengthening of one response over the other (alpha conditioning) or if a new response

emerges following pairing (beta conditioning). To test this, C. elegans were exposed to a mechanical vibration stimulus (to activate a locomotor reversal response) paired with a blue light (to activate a forward locomotor response). Post-conditioning responses to the vibration stimulus alone were then measured. Worms that received the stimulus pairing displayed a noticeable change in response to vibration at test in that they no longer performed a reversal response, but instead showed a significant increase in pause time (cessation of movement). This was similar to worms that had received vibration-alone during conditioning suggesting a habituation process. However, when other movement measures were examined, only the paired-conditioned worms showed significant decreases in these measures. Interestingly, this difference in response to vibration was more pronounced at 10-minutes post-conditioning. As well, paired-conditioned worms showed no changes in the omega-turn C. elegans avoidance response behavior. Understanding the dynamics of conditioned behavior resulting from pairing of oppositional responses could provide further insight into how learning processes may be applied. -

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Bowers RI - *Behaviour systems and plateaus of subjective time perception* - Bilkent University, Ankara - There is a temporal aspect to Timberlakean systems. The three-mode model of rat predatory behaviour posits that rat predation involves three qualitatively distinct motivational states. These three states are distinct not only in the form of behaviours produced, but occur in bouts with different time courses as well. One remaining question concerns whether these different time courses involve corresponding levels of subjective time perception. Initial results are described of an experiment using mid-session reversal, a technique for studying temporal sensitivity in animals, while biasing expression of specific motivational states. I discuss what the presence of motivational influences on subjective time perception means for an understanding of temporal cognition. -

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Cummings KA1,2, Bayshtok S1, Kenny PJ1, and Clem RL1 - *Ensemble encoding of conditioned fear by prefrontal somatostatin interneurons* - 1-Nash Family Department of Neuroscience and the Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY; 2-Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL - Memory has historically been thought to be encoded by sparse ensembles of excitatory projection neurons. Whether inhibitory interneurons are also recruited into these ensembles has remained largely unstudied. In recent unpublished work, we identified a prelimbic neural ensemble that is recruited following cued fear learning by using an activity-dependent neural tagging approach in wild-type male and female mice. We found that in addition to excitatory projection neurons, we also captured a sparse population of fear-activated somatostatin-expressing interneurons (SST-INs). To specifically capture this prefrontal SST-IN ensemble, we made novel use of an intersectional activity-dependent tagging strategy. Using this approach, we tagged a largely pure population of fear-activated SST-INs that: 1. exhibit unique circuit connectivity/plasticity properties as compared to

non-activated SST-INs; 2. are reactivated upon CS-evoked memory retrieval; and 3. are sufficient to drive fear expression upon optogenetic activation. We additionally observed that a positive experience, morphine administration, results in the recruitment of an ensemble of SST-INs that is both distinct from and functionally opposed to the fear-activated SST-IN ensemble. Overall, our data suggest that a potentially specialized ensemble of SST-INs may orchestrate fear memory encoding in the prelimbic cortex and reveal additional details about the nature of memory encoding by distinct interneuron populations. -

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Desrochers SS, Nautiyal KM - *Increased conditioned responding and taste reactivity is associated with impulsivity in mice lacking the serotonin 1B receptor* - Psychological and Brain Sciences, Dartmouth College - Impulsivity is a complex behavioral construct which remains poorly understood despite its prevalence in mental disorders such as ADHD, substance abuse, and conduct disorders. Breaking impulsivity down into its underlying components (e.g., impulsive action or inability to withhold responding, and impulsive choice or intolerance for delays) may lead to a better understanding of the behavioral and neural processes contributing to pathological cases. The present study uses a genetic model with mice lacking the serotonin 1B receptor (5-HT1BR), which has been implicated in impulsive behavior both in single-nucleotide polymorphism population studies in humans and in preclinical models. Our results show that mice lacking the 5-HT1BR had increased premature responding (a measure of impulsive action) in the 5-choice serial reaction time test (5CSRTT), both during training and under attentional constraints. In a lickometer test of hedonic responding, these mice also showed elevated licking for lower concentrations of a palatable rewards compared to controls. These results suggest that the impulsive action phenotype in the absence of the 5-HT1BR may be the result of increased taste reactivity and reward valuation. Therefore, we tested other behaviors which do not directly measure impulsivity and may be influenced by changes in reward processing. First, we examined Pavlovian Conditioned Inhibition and found intact inhibitory learning in mice lacking the 5-HT1BR (as assessed by the summation and retardation of acquisition tests), despite deficits in withholding responding during training. In a Pavlovian cue elicited responding experiment to test excitatory responding, mice lacking the 5-HT1BR responded normally to cues continuously paired with reward, but showed elevated responding when the cue and reward were on independent schedules. Then, in a conditioned reinforcement experiment, 5-HT1BR knockout mice had higher instrumental responding for a previously reward-paired cue, despite no differences in responding during the Pavlovian training. Together, these experiments highlight the importance of considering diverse behavioral tests in the study of impulsivity. - NIH R00 MH106731

Guerra DP, Moscarello JM - *The role of the BNST in the expression of signaled active avoidance in male rats* - Texas A&M University - Avoidance is a unifying behavior of many anxiety disorders. Here, we report a series of experiments using a signaled active avoidance (SAA) procedure to model avoidant behavior in rats. In SAA, the subject first acquires an

association between a tone (CS) and a shock (US) and then learns to perform an avoidance response during the CS in order to prevent US delivery. Over the course of SAA training, the US becomes less frequent and thus goes from being a certain/imminent threat to a possible/distal threat as the avoidance response is acquired. Though the bed nucleus of the stria terminalis (BNST) has been shown to mediate defensive responses to possible/distal threats, its role in SAA has yet to be examined. We used a chemogenetic approach to test the hypothesis that BNST is necessary for the expression of the avoidance response. Male rats received intra-BNST infusions of AAV containing the gene construct for either the inhibitory hM4Di DREADD or GFP. After recovery, animals received four days of SAA training in order to reach stable levels of avoidance. This was followed by two additional days of SAA training preceded by counterbalanced IP injections of either the DREADD ligand CNO or vehicle. We found that CNO decreased avoidance responses in hM4Di subjects but not in GFP controls, demonstrating that the BNST is necessary for the avoidance response. Because some subjects showed hM4Di expression in the medial septum (MS), we followed up with an anatomical control experiment specifically targeting this region. We found no evidence of a role for MS, indicating that the effects observed in our initial experiment were BNST-specific. We then compared chemogenetic activation (hM3Dq) and inhibition (hM4Di) of BNST in a test of avoidance under extinction conditions. Because stimuli were delivered in an invariant way that was not contingent upon the subjects' response, this design allowed us to test all groups under common conditions. After AAV infusions and recovery, subjects received six days of avoidance training. 24 hours later, all subjects were exposed to 10 unreinforced CSs preceded by either CNO or vehicle. We found that CNO decreased avoidance in hM4Di subjects and increased avoidance in hM3Dq subjects relative to GFP and vehicle controls, confirming that BNST is not only necessary for normal levels of avoidance but is also sufficient to potentiate the response. Our results suggest that the BNST is an essential structure in avoidant behavior. -

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Hafenbreidel M, Briggs SB, Arza M, Tiller A, Hall AB, Lin L, Khan S, Sillivan SE, Pandey S, Cameron MD, Rumbaugh G, Miller CA - *Corticotrophin Releasing Factor Receptor 2 in the basolateral amygdala enables Nonmuscle Myosin II-mediated disruption of methamphetamine-associated memory* - Scripps Research Institute - Substance use disorders (SUD) are perpetuated by associative memories that induce motivation to seek drug. We previously reported that inhibition of the actin motor ATPase nonmuscle myosin II (NMII) with the small molecule inhibitor blebbistatin (blebb), results in actin depolymerization and dendritic spine loss in the basolateral amygdala (BLA) and a retrieval-independent disruption of methamphetamine (METH)-associated memories and METH seeking. The effect is unique to BLA and specific to METH, as it does not interfere with other associative memories for foot shock, food reward or many other commonly abused drugs, including cocaine (COC). To investigate the source(s) of this selectivity, we used RNA-seq following conditioned place preference (CPP) conditioning to determine transcriptional differences between METH- and COC-associated memories. One gene selectively upregulated in METH-treated mice in BLA, but not hippocampus or nucleus accumbens, was crhr2, which encodes

Corticotrophin Releasing Factor receptor 2 (CRF2). Besides its role in the stress response, its ligand, CRF, is released in BLA following COC or METH administration, and CRF receptor 1, but not CRF2, is involved in COC-associated memory. Interestingly, CRF2 has been linked to NMII regulation outside the CNS, but its role and potential interaction with NMII in METH-associated memories was unknown. To test for this interaction, we first established the impact of CRF2 inhibition after METH CPP conditioning. Mice received intra-BLA infusions of vehicle or the CRF2 antagonist Astressin-2B (AS2B) after the final METH conditioning session. CRF2 inhibition was not sufficient to disrupt the memory, as both groups expressed a METH CPP when tested 48hr later. A second experiment established that, unlike AS2B, NMII inhibition with blebb (IP) shortly after the last METH conditioning session disrupted METH CPP expression. Taken together, these results enabled examination of the interaction between CRF2 and NMII. For this, mice received intra-BLA infusions of vehicle or AS2B after the last conditioning session, followed by blebb (IP) shortly after. METH CPP was disrupted in mice treated with vehicle and blebb, replicating our previous results. However, METH-associated memory was protected in mice that received AS2B and blebb. These results suggest that CRF2 is required in combination with NMII activity in BLA to render METH-associated memories and seeking-like behavior selectively vulnerable to NMII inhibition in the absence of retrieval. Indeed, CRF2 may represent a general target for destabilizing memory via (yet to be identified) downstream effects on NMII. - UH3 NS096833 (CAM), R01 DA049544 (CAM)

Jean-Richard-dit-Bressel P, Lee JC, Ma C, Liew SX, Bradfield LA, Killcross S, Weidemann G, Lovibond PF, McNally GP - *Punishment insensitivity in rodents and humans is driven by failed punishment association learning* - UNSW Sydney, Australia; University of Technology Sydney, Australia; Western Sydney University, Australia - Propensity to avoid detriment (i.e. sensitivity to punishment) differs markedly across individuals; a common assumption is that this is due to differences in motivation or behaviour control. We investigated potential causes of punishment insensitivity in rats and humans using a conditioned punishment paradigm that concurrently assesses punishment, reward-seeking, and Pavlovian fear. Rats (n = 48) were trained to press two continuously-presented levers for food (VI30s). In punishment phase, lever-pressing still yielded food, but pressing the "punished" lever yielded a stimulus co-terminating with footshock (VI60sec CS+), whereas the unpunished lever yielded an inconsequential stimulus (VI60sec CS-). We then developed a computer-based version of the task for humans. Participants (n = 135) could click on two continuously-presented planets for points (RR2), which in later blocks also yielded spaceships resulting in point loss (RR5 CS+) or no consequence (RR5 CS-). Participants were also surveyed on valuation of task elements, inferences and personality traits. In both rat and human tasks, punishment avoidance was bimodally distributed, with a large proportion of individuals failing to avoid the punished response (punishment-insensitive cluster). In both studies, punishment-insensitive individuals exhibited intact Pavlovian fear and reward-seeking, suggesting these individuals had normal appetitive and aversive motivation. Insensitivity was also not attributable to increased impulsivity. Rather, effects were attributable to failures in

encoding the response-punisher association, revealing a critical and overlooked mechanism for punishment insensitivity. -

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Kreitlow MR, Moscarello JM, Keppler LJ - *A systems consolidation-like process recruits the retrosplenial cortex to the long-term maintenance of signaled avoidance* - Texas A&M University - The acquisition and expression of two-way signaled active avoidance (SAA) involves a multistage learning process supported by a shifting neural substrate. Though recent work has begun to explore the circuitry underpinning the early stages of SAA the neural mechanisms underlying the long-term maintenance of the avoidance response are unknown. Because of its established role in the long-lasting forms of aversive memory, we hypothesized that the retrosplenial cortex (RSC) would be recruited to support SAA with extensive training post-acquisition. In our first experiment, male rats received intra-RSC injections of an AAV5 containing the gene construct for either the inhibitory hM4Di DREADD or GFP on a CamKII promoter. Following recovery, animals underwent an SAA training paradigm in which they learned to shuttle across a divided chamber during a tone in order to avoid foot shock. Animals were trained for 4 days to achieve asymptotic levels of avoidance. Subjects then received two SAA test sessions on days 5 and 6 that were preceded by counterbalanced IP injections of either CNO (3mg/kg) or VEH (saline + 10% DMSO). Animals received 2 more days of training, followed by another pair of test sessions on days 9 and 10. Though CNO inactivation of RSC had no effect on avoidance behavior after 4 days of training, RSC inhibition caused a robust decrement at the latter timepoint, demonstrating that long-term maintenance of the avoidance response requires RSC. We then set out to determine whether recruitment of the RSC was brought about by extensive training or whether time following initial acquisition would result in the RSC-dependence of SAA, suggesting a role for a systems consolidation-like process. hM4Di or GFP was expressed in RSC pyramidal neurons, as previously described. After recovery, subjects received either 4 days of training immediately followed by 2 SAA test sessions preceded by CNO or VEH, or 4 days of training followed by 4 days off in the homecage prior to 2 SAA test sessions. CNO inactivation of RSC attenuated avoidance behavior only in animals that received 4 days off in the homecage, suggesting that time following acquisition is sufficient to recruit RSC to SAA. These data demonstrate that RSC is involved in the long-term maintenance of the avoidance response, and demonstrate that continued training beyond initial acquisition is not necessary for recruitment of RSC. To confirm the anatomical specificity of our results, we performed a control experiment in which we chemogenetically inactivated a neighboring region highly interconnected with RSC, the dorsal hippocampus, after 4 or 8 days of SAA. These manipulations had no effect on SAA. Collectively, our data demonstrate that RSC is a key substrate underlying the long-term maintenance of SAA and is recruited via a systems consolidation-like mechanism. -

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Laine MA, Mitchell JR, Clark R, Keith J, & Shansky RM - *Ultrasonic alarm calls of rats during cued fear conditioning and extinction.* - Department of Psychology, Northeastern

University, Boston, MA - Fear conditioning is a powerful tool for investigating factors involved in fear learning, a key component of stress-related and anxiety disorders. Historically preclinical research using this model has utilized primarily male rodents. To gain a broader, more translational view we and others include female rodents, allowing the study of both shared and divergent mechanism by biological sex. We have previously shown sex differences in the behavioral manifestation of fear learning; while the large majority of male rats freeze in response to a conditioned stimulus, nearly half of female rats express a flight-like darting response instead. Here we explore another behavioral readout; ultrasonic vocalisations (USVs) during cued fear conditioning, extinction learning, and extinction recall. Adult rats emit distinct "alarm calls" in the 22 kHz range to signal threat. Our preliminary data shows that male rats exposed to mild (0.3 mA) tone-paired foot shocks make significantly fewer alarm calls than those exposed to medium (0.6 mA) or strong (1 mA) shocks. This effect was not observed in females. Rats exposed only to the context and tone, without foot shocks, do not emit alarm calls, validating their relationship with fear learning. The number of alarm calls attenuates through extinction learning, but some individuals of both sexes continue to make alarm calls even during extinction retention. Future work will analyse how the call frequency and length changes throughout fear conditioning and extinction. Using our custom Python-based tool for detecting bouts of darting and freezing (ScaredyRat, Mitchell et al, under review) we will also analyse vocalisations co-occurring with specific behaviors. These analyses will enrich our understanding of individual and sex differences in aversive learning. -

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Lee R, Engelhard B, Witten I, Daw N - *Explaining dopaminergic response heterogeneity as a reflection of cortical state representation* - Phasic responses from midbrain dopamine (DA) neurons have been argued to report a reward prediction error (RPE) signal from reinforcement learning (RL) models. In the classic formulation, RPE is defined as a scalar signal, consistent with early work suggesting homogeneous responses across neurons. However, recent work has challenged this view by clearly demonstrating that DA can exhibit heterogeneous responses across neurons and regions. We examine one dataset reporting cell body responses during a complex visual evidence accumulation task. Two key features emerge: neurons respond heterogeneously to a variety of features (some seemingly RPE-irrelevant) during the cue period (ie the navigation and decision making portion of the task), but the population behaves largely uniformly with RPE-like responses at trial outcome. We introduce a new model that explains both aspects of the data by positing that DA neurons report individual RPEs for each dimension of a population vector code for the task's state. Given any such representation, this scheme reproduces both cue period heterogeneity (driven by the feature-dependent future value term in the RPE) and outcome period homogeneity (driven by a scalar reward). To investigate this claim, we train a deep RL model on the evidence accumulation task to extract a feature representation of task state from raw video input. The vector of RPEs derived from the features of the deep RL model exhibit similar qualitative behavior as the DA neurons: specifically, the vector RPEs respond to various sensory variables of the task during cue period but are still uniformly modulated by RPE at reward

time. Taken together, our work provides a path to reconcile new observations of DA neuron heterogeneity with classic ideas about RPE coding, while also providing a new perspective on how the brain performs reinforcement learning in high dimensional environments.

Liang A, Malvaez M, Murphy MD, Wassum KM - *Contribution of Dorsomedial Striatal Direct and Indirect Pathway Projections to Goal-directed and Habit Learning* - UCLA - In our daily lives, habits are essential methods that allow us to efficiently perform basic and repetitive tasks. Habitual actions are automated actions without outcome expectancy and when used, require less cognition and critical thinking. However, their disruption contributes to the symptoms that underlie many psychiatric diseases. The striatum is a major input station of the basal ganglia with subregion functional specificity, in which the dorsomedial striatum (DMS) is responsible for goal directed actions, specifically. This project investigated the contribution of the two unique projection pathways within the DMS, the direct (dMSNs) and indirect (iMSNs) pathways, to the acquisition of goal driven actions. We used a chemogenetic approach and cre-driver transgenic mice to specifically manipulate direct (D1-cre) or indirect (A2A-cre) DMS projections by using either an activation or inactivation DREADD. After, we either activated or inhibited the targeted pathways during instrumental learning and then used a devaluation procedure to probe goal-directed versus habit strategy to determine the individual contributions of the DMS projections. Our data suggest that inhibiting both the dMSNs and iMSNs produced insensitivity to outcome devaluation, indicating the mice were using habitual strategy, while activating either the dMSNs or iMSNs projections in the DMS, produced sensitivity to outcome devaluation. Our data further indicated that both the dMSNs and iMSNs are necessary in promoting goal directed strategies. -

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Mallea J, Schulhof A, Le L, Gallistel CR, Balsam PD - *Probability of reinforcement affects CR strength independently of reinforcement rate* - Columbia University, Barnard College, Rutgers University - Manipulating the probability of the unconditioned stimulus (US) affects the level of conditioned responding in Pavlovian learning. Changes in probability are often confounded with changes in the rate of reinforcement (total reinforcers/total CS time). Two experiments in mice aimed to unconfound the probability and rate of reinforcement. In Experiment 1, five groups of mice were exposed to a Pavlovian appetitive conditioning procedure, in which groups differed in their probability of reinforcement (.25, .50, .50x2, .75, and 1.0). Two groups (.50 and .50x2) shared the same probability of reinforcement but group .50x2 received twice the number of reward presentations on every reinforced trial. Thus, group .50x2 received the same rate of reinforcement as group 1.0 but differed in the probability of reinforcement. After this phase, all subjects went through 4 sessions of extinction with no reinforcement delivery. The probability of reinforcement did not affect the speed of acquisition but did affect the probability and rate of responding, while latencies were influenced by the reward rate. During extinction, subjects with higher probability of reinforcement showed quicker extinction (i.e., Partial Reinforcement Extinction Effect; PREE).

Experiment 2 further assessed the role of probability on these measures of behavior while holding the rate of reinforcement constant. The experiment employed a split trial design, in which the total CS time per reward was held constant but presented in different numbers of discrete trials. Two groups received Pavlovian training with a CS of average duration of 12s with probabilities of reinforcement of .50, .50x2. Two other groups were reinforced with probability of 1.0, one of them with an average CS duration of 12s as while the other group received trials of an average of 24s. Thus the .5-12s group and the 1.0-24 s group had equivalent reward rates but different probabilities of reinforcement. Similarly, the .5X2-12s group and the 1.0-12s also had equivalent rates of reinforcement but different probabilities. The results showed, again, that the probability of reinforcement did not affect acquisition speed but did influence the probability and rate of responding. Together, these results suggest that even when the rate of reward is held constant, the probability of reinforcement affects multiple aspects of behavior, while having little effect on the speed of acquisition. Future accounts of Pavlovian learning will need to consider the encoding of the probability independently of rate of reinforcement and consider the possibility that different types of information may differentially affect different aspects of anticipatory behavior. -

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Mitchell JR, Potgieter L, Shansky RM - *Neural Modulators of Conditioned Fear Responses in Male and Female Rats* - Northeastern University - Darting is a sex-dependent conditioned fear response characterized by a quick movement across a fear conditioning chamber in response to a conditioned stimulus (CS). Forty percent of females dart while only ten percent of males do. The underlying circuitry involved in darting is unknown. The dorsal periaqueductal gray (dPAG), known to control active threat responses, receives inputs from the caudal portion of the infralimbic cortex (cIL). If the cIL regulates threat responses in the dPAG, activation of this circuit could increase an animal's likelihood to engage in active threat responses such as darting. In this study we used intersectional, excitatory DREADDs to activate the cIL-dPAG pathway, hypothesizing that excitation of this pathway would increase the number of darters across the sexes. We injected a cre-dependent, excitatory Gq virus into the cIL, and a retrograde-cre virus into the dPAG. After 6 weeks the animals received an injection of clozapine-n-oxide (CNO), to activate the cIL-dPAG circuit, before undergoing classic Pavlovian Fear Conditioning. Using ScaredyRat, a custom Python tool designed in our lab to analyze raw Ethovision Data files, we were able to quantity the number of darters, as well as the animals' response (in cm/s) to the shock and their post-shock maximum velocities reached. Although we did not see an increase in darters, we observed a significant decrease in freezing for females with an excited cIL-dPAG circuit compared to controls. There was no difference between males with the excited circuit and their control group, and no difference in shock and post-shock response between groups in either females or males. These data indicate that excitation of the cIL-dPAG circuit, although not apparently involved in darting, might influence an animals' propensity to freeze in response to a CS in a sex-dependent manner. Future studies will investigate the IL-PAG circuit involved in freezing as well as the organization of the projections from the IL to the dorsal and ventral PAG. - Funding was provided by NIH grant 1R01MH123803-01

Mooney-Leber, S. M. 2, Zeid, D 1, Garcia-Trevizo, P. 1, Seemiller, L. R.1, Bogue, M. A. 4, Grubb S.C. 4, Peltz, G. 3, & Gould, T.J. 1* - *Acetylcholinesterase activity varies across genetic background and correlates with fear learning* - 1 Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA 16802. 2 Department of Psychology, University of Wisconsin-Stevens Point, Stevens Point, WI, 54481 3 Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University, Palo Alto, CA 94305. 4 The Jackson Laboratory, Bar Harbor, ME 04609 - Learning and memory processes in the brain are carefully orchestrated by different neurotransmitters. Acetylcholine, a major neurotransmitter, is involved in key processes for memory encoding in the hippocampus. Studying the enzyme that metabolizes acetylcholine, acetylcholinesterase (AChE), can inform understanding of acetylcholine dynamics and provide insight into how learning and memory processes are regulated. Previous research in our laboratory found that fear learning (a hippocampus-dependent process) varies by genetic background. To assess the relationship of AChE activity levels in the hippocampus with learning, we collected brain tissue of male mice from 20 inbred mouse strains (129S1/SvlmJ, 129S4/SvJaeJ, 129S8/SvEvNimrJ, A/J, AKR/J, BALB/cJ, BTBRT¡+¿ltpr3¡tf¿/J, C3H/HeJ, C57BL/6J, CBA/J, DBA/1J, DBA/2J, FVB/NJ, LP/J, MA/MyJ, NZB/BINJ, SJL/J, SM/J, SWR/J and 129S2/SvPasCrl) after fear conditioning. Dorsal and ventral hippocampus as well as cerebellum were collected, and AChE activity levels were measured. Furthermore, we correlated AChE activity levels with publicly available data from the Mouse Phenome Database (MPD) and we conducted a SNP query with AChE-relevant genes to assess possible polymorphisms contributing to the changes in AChE activity. We found that AChE activity was negatively correlated with contextual and cued fear learning in the dorsal hippocampus but not correlated in the ventral hippocampus or cerebellum. Furthermore, AChE activity varied by genetic background in all three brain regions. Mouse Phenome Database correlations revealed that errors in three different cognitive assessments were correlated with AChE activity in the dorsal hippocampus. SNP query results for the inbred strain panel indicated that the AChE gene as well as other relevant cholinergic signaling genes had several polymorphisms. In conclusion, this study indicates that AChE activity in dorsal hippocampus, but not ventral hippocampus, is a modulator of fear learning, supporting a functional distinction between the dorsal and ventral hippocampus. Genetic variants between inbred mouse strains in relevant cholinergic signaling genes may be related to strain differences in AChE activity and MPD correlations support the role of dorsal hippocampal AChE activity in learning. -

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Navis TM, Kim EA, Lattal KM - *A dissociation of enhanced drug-seeking and sensitized fear following inactivation of basolateral amygdala during acute stress.* - Oregon Health & Science University - Objective: Earlier we've shown increased reinstatement of lever-pressing to a previously methamphetamine (METH)-paired cue (cue-induced reinstatement, cueIR) in rats with a history of repeated unsignaled footshock (SHK) in another context. SHK

rats show increased freezing to one footshock in a new context (stress-enhanced fear learning). The basolateral amygdala (BLA) is necessary for cueIR and this fear response. This study determines if BLA inactivation using muscimol (Musc) during SHK eliminates enhanced cueIR compared to rats receiving vehicle (Veh) and/or context exposure without shock (NoSHK). Methods: Male Long-Evans rats with jugular vein catheters for METH self-administration were infused with Musc or Veh into the BLA, then exposed to SHK or NoSHK. In another context rats could lever-press for METH with cue light. Following several days, lever-presses ceased to produce an effect. Reinstatement of lever-pressing by presentation of cue, sensitized fear response to a third context after a single shock, and test of fear to SHK context were assessed. Results: NoSHK groups had no differences and were combined. Muscimol during shock (Musc+SHK) blocked sensitized fear compared to Vehicle (Veh+SHK) $F(2,43)=12.8$, p¡0.001, but not NoSHK. Musc+SHK showed significantly less freezing behavior than Veh+SHK in SHK context, indicating impaired expression of original fear memory, $F(2,43)=13.7$, p ¡0.001. There's a trend toward enhanced lever pressing to meth-paired visual cue, $F(2,41)=3.06$, $p=0.058$. Conclusion: Expression of fear and sensitized fear response are unnecessary for enhanced lever-pressing for a meth-paired cue after SHK, implying long-term nonassociative effects on reward processing after stress independent of direct memory of stress. - R01 DA047981-03S1

Olekanma DI, Lee J, Cho B, Herrera Charpentier AN, Arguello AA, Johnson AW - *Attenuation of cocaine-seeking following representation mediated devaluation* - Psychology, Michigan State Univ., East Lansing, MI - Mediated learning reflects the capacity of an environmental conditioned stimulus (CS) to substitute for an unconditioned stimulus (US). In doing so, mediated learning allows for the establishment of new learning to the biologically meaningful US. In this study, we examined whether mediated devaluation via LiCl could be used to disrupt features of cocaine reward and subsequently attenuate cocaine-seeking, as well as cue- and cocaine-induced reinstatement. Male, Sprague Dawley rats underwent jugular catheterization surgery followed by behavioral training. Cocaine self-administration (Coc-SA) training occurred during daily 2h sessions, in a distinct contextual environment (FR1 schedule of reinforcement, minimum of 10 days). During Coc-SA, an active lever press resulted in an infusion of cocaine paired with presentation of a complex CS (tone + cue light above active lever). On completion, rats were placed into a novel context and received multiple presentations of the complex CS alone followed by injections of either saline or LiCl. In the next phase of the experiment, all groups were returned to the cocaine-associated context for extinction training (2h, 1x/d, 8d). CS reinstatement test occurred 24 hr after the last extinction session and consisted of presentation of CS without drug. Additional extinction sessions were given, before another cocaine prime induced reinstatement test was administered with ip cocaine given before test. Rats that received CS—LiCl pairings displayed a tendency for reduced cocaine seeking during the initial extinction session, though no differences in the groups were noted for either CS reinstatement or cocaine prime sessions. These findings suggest that mediated devaluation procedures might be a useful strategy for attenuating cocaine-seeking in rats. -

Olvera ME, Cervantes CM, Cofresi R, Monfils MH, Lee HJ - *Attenuation of Alcohol Seeking Behavior via Retrieval + Extinction in a Rat Model of Alcohol Dependence* - Department of Psychology, University of Texas at Austin, Austin TX, The College of Pharmacy, University of Texas at Austin, Austin TX, Department of Psychological Sciences, University of Missouri, Columbia, MO - Cues surrounding alcohol consumption create a conditioned behavior over time. In the extreme case of alcohol use disorders (AUD), reactivity to such cues can lead to alcohol consumption or relapse. Extinction based therapies aim to reduce cue-reactivity but do not fully extinguish return of relapse-like behavior in the form of spontaneous recovery, reinstatement or renewal. Recently, we tested the effectiveness of the retrieval-extinction paradigm, in which extinction occurs after cue-induced memory retrieval, as a modified alternative to standard extinction in preventing relapse-like behavior. We showed that this paradigm effectively attenuated spontaneous recovery and reinstatement in rats with a moderate drinking history. In the current study, we examined whether the retrieval-extinction paradigm continues to prevent relapse-like behavior in a model of alcohol dependence, in which rats are exposed to chronic ethanol vapor. Following ethanol vapor or control air exposure, rats underwent visual cue conditioning in which a light cue predicts the presentation of a sipper containing 15% ethanol. After acquisition, rats were further divided into retrieval+extinction or no-retrieval+extinction groups. Rats in the retrieval+extinction group received a single presentation of the light cue an hour before extinction training, while rats in the no-retrieval+extinction group were only exposed to the context an hour before extinction training. Preliminary results suggest that the retrieval-extinction paradigm retains its efficacy in preventing relapse-like behaviors even in a rat model of alcohol dependence induced via chronic ethanol vapor exposure. -

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Packard K., Hu S., Wang J., Opendak M., - *Developmental regulation of lateral habenula structure and function.* - Kennedy Krieger Institute, Department of Neuroscience-Johns Hopkins School of Medicine, New York University, Nathan Kline Institute - Infants rely on the mother to provide them with the sensory stimulation needed for normal brain development. However, harsh or neglectful care can perturb the mother's ability to regulate the infant brain. Recent work has highlighted the mesolimbic dopamine system as a critical interface between early quality care and maternal regulation of the infant, but specific mechanisms remain unclear. Here, we assessed how care quality impacted maternal regulation of core nodes of the mesolimbic dopamine circuit, including the lateral/medial habenula and ventral tegmental area (VTA). To modulate maternal care quality, we employed the Scarcity-Adversity model of low bedding (SAM-LB). Following control or SAM-LB rearing from PN8-12, rat pups underwent odor-shock conditioning with or without maternal presence at postnatal days 18-20 (PN18, before weaning) and postnatal days 26-28 (PN28, after weaning). In control-reared pups, we observed that maternal presence suppressed VTA and lateral habenula engagement, as measured through 2-deoxyglucose metabolism. The effect of

maternal presence was abolished in adversity-reared pups, with early rearing significantly altering patterns of functional connectivity within this circuit. Altogether, these data suggest that lasting social impairments following early adversity may be due to impaired maternal regulation of the mesolimbic dopamine circuit. -

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Simmons TS, Lee HJ - *Orexin Response to Amphetamine in Female Rats with Different Cue-Directed Orienting Behavior* - University of Mary Hardin-Baylor, University of Texas at Austin - Orexin peptides are synthesized in small quantities in the lateral hypothalamus (LH) and identified by their key roles in diverse functions such as wakefulness and arousal, appetitive and motivated behaviors, and extinction learning. We recently showed that female rats with high orienting behaviors towards a light cue predicting food show more amphetamine seeking behavior as measured by a conditioned place preference (CPP). We propose that orexin (ORX) peptides in the LH might play an important role in the expression of the individual differences in orienting behavior and subsequent preference to amphetamine. The rats were first screened for their conditioned orienting behavior in a light-food conditioning paradigm. Then, they were conditioned and tested for amphetamine preference and reinstatement. Brains were collected and processed to detect amphetamine-induced FOS+ cells and orexin+ cells in the LH. Immunohistochemical staining and microscopic imaging were utilized to capture activated orexin cells (double labeled with ORX and FOS), non-active orexin cells (single labeled with ORX), and activated non-orexin cells (single labeled with FOS). Cell count using Image J was done on three separate regions (medial, perifornical, and lateral) of LH since distinct ORX subsystems are suggested to mediate different functions. Our results suggest that the medial region of LH might show greater response to amphetamine. We saw higher number of FOS+ cells and ORX+ cells that were double labeled with FOS in the medial region of LH. However, we did not see differences in ORX+ and/or FOS+ cell counts based on the orienting phenotype or amphetamine preference of the rats. Thus, it is likely that orexin is not directly involved in amphetamine preference predicted by an orienting phenotype. Keywords: Amphetamine; CPP; Orexin; Lateral Hypothalamus; Orienting. - Thank you Dr. Lee and Emily Hilz for your hard work and support!

Smith KA, Donovan MH, Raskin MR, Shumake J, Mansoorshahi S, Telch MJ, Noble-Haeusslein LJ, Monfils MH - *Predicting the effects of traumatic brain Injury (TBI) on fear extinction learning and retention in rats* - University of Texas at Austin - We have previously shown that CO2 reactivity is predictive of extinction phenotype in rats [1]. While studies have reported mixed results in extinction of fear after TBI, none have examined the long-term durability of this phenotype in the more chronically injured brain[2-5]. Here we tested the hypothesis that TBI would results in a long-term deficit in fear extinction, and that CO2 reactivity would be predictive of extinction phenotype. Isoflurane-anesthetized adult male rats received TBI (produced by a controlled cortical impactor) or sham surgery. One-month post injury or sham surgery, rats underwent a CO2 challenge, followed by fear

conditioning, extinction, and long-term memory (LTM) testing. Injured rats showed no difference during extinction-LTM relative to shams. We did, however, find that rats that received TBI and were exposed to CO2, showed significantly better extinction-LTM than rats that received TBI and were exposed to air. In contrast to our previous findings, we observed no relationship between CO2 reactivity and extinction-LTM in either the sham or the injured rats. However, compared to the previously observed naïve sample [1], we observed more variability in extinction-LTM but a very similar distribution of CO2 reactivity in the current sample. We hypothesize that isoflurane anesthesia may lead to interoceptive threat habituation, possibly via action on orexin receptors in the lateral hypothalamus, and interacts with CO2 exposure to lead to enhanced extinction. Future work will directly test this possibility.

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Walsh EN, Shetty M, Abel T - *cAMP mediates the impact of sleep deprivation on synaptic plasticity.* - Department of Neuroscience and Pharmacology, Iowa Neuroscience Institute, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA - Sleep facilitates memory storage and sleep loss leads to impairments in memory. Memory for tasks that involve the hippocampus, a brain region that mediates memory for facts and events, is particularly sensitive to sleep loss. Our lab has found that within the hippocampus, sleep loss leads to deficits in forms of synaptic plasticity that depend on cyclic AMP (cAMP) signaling. Previous work in our lab has also found that sleep deprivation reduces levels of cAMP in the hippocampus, and that the reduction of cAMP mediates the memory loss that accompanies sleep deprivation. We hypothesized that elevating cAMP levels in the hippocampus during the course of sleep deprivation, would convey resilience to hippocampal synaptic plasticity. We used a chemogenetic strategy to selectively increase cAMP levels in excitatory neurons of the hippocampus, where we expressed the Drosophila melanogaster G-protein coupled octopamine receptor (DmOctβ1R), and activated it by injecting the ligand (octopamine) during specific time points during the sleep deprivation period. Immediately following sleep deprivation, hippocampal slices were prepared for electrophysiological recordings. We found that cAMP elevation during the five-hour deprivation period prevents deficits in spaced 4-train long-term potentiation (LTP), a persistent form of LTP. We are currently working to investigate the importance of cAMP signaling in other forms of synaptic plasticity impacted

by sleep-deprivation to understand the mechanism by which the dysfunctions in memory and plasticity occur. -

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Zeid, D, Seemiller, LS, and Gould, TJ - *Behavioral and genetic architecture of fear conditioning and related phenotypes* - Penn State University - Contextual fear conditioning is a form of Pavlovian learning during which an organism learns to fear a previously neutral stimulus assembly (context) following its close temporal presentation with an aversive stimulus. In mouse models, freezing behavior is typically used to quantify learned fear response. This dependent variable is the sum of multiple processes including, but not limited to associative learning, configural learning, fear and anxiety, and general activity. To better understand latent constructs impacting performance in contextual fear conditioning and correlated behaviors, we tested 4 BXD RI strains previously found to show extreme contextual fear conditioning phenotypes (highest/lowest freezing among 31 strains) and intermediate freezing BXD parental strains, C57BL/6J and DBA/2J, in a battery including locomotor, anxiety, contextual/cued fear conditioning and non-associative hippocampus-dependent learning behaviors. Hippocampi were dissected at the conclusion of the battery, and expression of two previously identified candidate genes for contextual fear conditioning was quantified. Resulting behavioral and gene expression data were analyzed using an exploratory factor analysis (EFA), which extracted five unique latent constructs (factors) representing activity/anxiety/exploration, associative fear learning, anxiety, post-shock freezing, and open field activity phenotypes. Associative learning and expression of one candidate gene for contextual fear conditioning emerged as a unique construct within the factor analysis. Post-shock freezing during the fear conditioning training trial and expression of a second candidate gene for contextual fear conditioning emerged as an additional unique construct, highlighting the independence of this measure within the fear conditioning paradigm. These data additionally support a link between adaptive prey behaviors expressed in anxiety, activity, and exploratory phenotypes. In sum, these findings inform understanding of fear conditioning in terms of its secondary measures, underlying biological mechanisms, and interaction with other mouse behaviors. -

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