Pavlovian Society Annual Meeting, 2023

September 21 – 23, 2023
Thompson Hotel, Austin, TX

Overview

Thur	6:00- 8:00 pm	Opening Reception Bass Ballroom Hors d'Oeuvres & Cash Bar
Fri	7:30–8:20 am	Breakfast
	8:20–12:00	Morning Sessions
	12:00–1:30 pm	Lunch (Exec. Committee Meeting)
	1:30–5:30	Afternoon Sessions
	5:30–7:30	Posters & Cash Bar
Sat	7:30–8:20 am	Breakfast
	8:20–12:00	Morning Sessions
	12:00–2:00 pm	Lunch (WIL Luncheon)
	2:00–5:45	Afternoon Sessions
	5:45–7:30	Posters & Cash Bar
	7:30–9:00	Banquet

Program

Friday (September 22)

7:30–8:20 **Breakfast** (outside Red River Ballroom)
All talks in Red River Ballroom

8:20–8:30 **Michael Drew** (*UT Austin*) Welcome

8:30–9:00 **Karyn Frick†** (*UW Milwaukee*) Past President Lecture: Adventures in translational research: How studying estrogen receptors and memory led to my alter-ego in Pharma and novel compounds to alleviate memory loss and hot flashes in menopause

The Society thanks the following for their support:

9:00–10:20 **Symposium 1: Neural mechanisms for learning about significant stimuli (Brian Wiltgen** (*UC Davis*), **Chair)**

* **Brian Wiltgen** (*UC Davis*) Phasic locus coeruleus activity enhances trace fear conditioning by increasing dopamine release in the hippocampus
* **Paul Frankland** (*U Toronto*) Microglial regulation of extinction of conditioned fear
* **Erin Calipari** (*Vanderbilt*) The role of dopamine release in the nucleus accumbens in novelty and salience processing
* **Mihaela Iordanova** (*Concordia U*) Cortico-amygdala regulation of fear propagation

10:20–10:40 **Coffee Break**

10:40–12:00 **Symposium 2: Stress-induced blunting of motivational processes across the lifespan (Puja Parekh** (*Weill Cornell*), **Chair)**

* **Puja Parekh** (*Weill Cornell*) Frontocortical circuits encode reward- and effort-related information and are sensitive to chronic stress
* **Millie Rincón-Cortés** (*UT Dallas*) Postpartum stress effects on maternal behavior and mesolimbic dopamine activity
* **Meghan Gallo** (*Brown*) Dopaminergic and behavioral adaptations to early life adversity

12:00–1:30 **Lunch (on your own)**
Executive Committee Meeting (Singer Boardroom)

1:30–2:00 **Marie Monfils** (*UT Austin*) Women In Learning Lecture: Rats are us

2:00–3:20 **Symposium 3: The role of cortico-limbic dysfunction following ELS in disrupted developmental learning, emotional, and psychosocial systems (Patrese Robinson-Drummer** (*Haverford*, **Chair)**

* **Patrese Robinson-Drummer** (*Haverford*) Exploring the lifetime

impact of early-life stress on learning systems
* **Laura DeNardo** (*UCLA*) Developmentally distinct architectures in top-down circuits
* **Nicole Ferrara** (*Rosalind Franklin*) Social engagement is regulated by the basomedial amygdala in adolescents and adults
* **Maya Opendak** (*Kennedy Krieger Inst*) Mesolimbic circuit control of social transitions in typical and perturbed development

3:20–3:40 **Coffee Break**

3:40–5:00 **Symposium 4: Hierarchical regulation of reinforcement and reinstatement (Kurt Fraser** (*UC Berkeley*), **Chair)**
* **Kurt Fraser** (*UC Berkeley*) Occasion setters control value encoding in the mesolimbic dopamine system
* **Rutsuko Ito** (*U Toronto*) Ventral hippocampal engagement in contextual control over adaptive responding signaled by sucrose reward and punishment cues
* **Céline Drieu** (*Johns Hopkins*) Rapid emergence of latent knowledge in the sensory cortex drives learning
* **Benjamin Saunders** (*U Minnesota*) Basolateral amygdala controls drug-related state representations

5:00–5:30 **Michael Mauk** (*UT Austin*) On stimulus trace explanations for the Inter-Stimulus-Interval function

5:30–7:30 **Posters & Cash Bar** (Bass Ballroom / Red River Pre-Function Room)

7:30 **Dinner (on your own)**

Saturday (September 23)

7:30–8:20 **Breakfast** (outside Red River Ballroom) **All talks in Red River Ballroom**

8:20–8:30 **Michael Drew** (*UT Austin*) Welcome

8:30–9:00 **Michael Domjan†** (*UT Austin*) Pavlovian sensitization

9:00–10:20 **Symposium 5: Neural circuits underlying stress and emotional memories (Denise Cai** (*Mt. Sinai*), **Chair)**
* **Janine Kwapis** (*Penn State*) Diurnal regulation of fear memory by the clock gene *Per1*
* **Mazen Kheirbek** (*UCSF*) Neural dynamics underlying associative learning in the ventral hippocampus
* **Kirstie Cummings** (*UA Birmingham*) The mouse dorsal peduncular cortex encodes fear memory
* **Zachary Pennington** (*Mt. Sinai*) Dissociable amygdala and hippocampal circuits support stress-induced changes in defensive behavior

10:20–10:40 **Coffee Break**

10:40–12:00 **Symposium 6: Neural processing of rewarding and aversive information across species (Melissa Sharpe** (*U Sydney*)**, Chair)**
* **Melissa Sharpe** (*U Sydney*) Dopamine release in nucleus accumbens during backward conditioning
* **Daniela Schiller** (*Mt. Sinai*) Assessing real life traumatic memories in the human brain
* **JJ O'Malley** (*NIMH*) A thalamo-striatal circuit that drives safety-related reinforcement of an active avoidance response
* **Thorsten Kahnt** (*NIH*) Characterizing sensory prediction errors and their role in stimulus-outcome learning

12:00–2:00 **Lunch/ Women in Learning Luncheon** **RSVP was required; at capacity**

2:00–2:30 **Mark Bouton†** (*U Vermont*) Habit, goal direction, and behavior change

2:30–3:50 **Symposium 7: Brain substrates for learning about safety (Roger Clem** (*Mt. Sinai*), **Chair)**
* **Anthony Lacagnina** (*Mt. Sinai*) Ventral hippocampal interneurons govern extinction and relapse of contextual associations
* **Ekaterina Likhtik** (*CUNY Hunter*) Chronic stress reorganizes prefrontal-amygdala circuit activity during safety processing
* **Scott Waddell** (*Oxford*) Lessons learned from Pavlovian conditioning in *Drosophila*

3:50–4:10 **Coffee Break**

4:10–5:10 **Symposium 8: Factors governing defensive decisions: from threat imminence to reinforcement mechanism (Robert Sears** (*Nathan Kline Inst*), **Chair)**
* **Robert Sears** (*Nathan Kline Inst*) Neural substrates underlying reinforcement of active avoidance
* **Justin Moscarello** *(TAMU*) Fear, anxiety, and the neural circuitry of avoidance
* **Miguel Á. Luján** (*U Maryland*) Dopamine neurons mobilize 2-AG to

encode predictive warning signals during active avoidance

5:10–5:40 **Peter Balsam†** (*Barnard/ Columbia*) Scotomas, Necker Cubes and Contingencies

5:45–7:30 **Posters & Cash Bar** (Bass Ballroom / Red River Pre-Function Room)

7:30–9:00 **Banquet, Awards, & Closing** (Red River Ballroom)

* **Caitlin M. Casey** (*UT Austin*) Guest Lecture–Department of Astronomy–The First Year of the James Webb Space Telescope

† indicates a Past President of the Society.

Posters

In the Bass Ballroom/ Red River Pre-Function Room. Generally alphabetical by first author's last name, with A–J on Friday and K–Z on Saturday, except in the case of special lab grouping requests by authors.

Friday (September 22)

1. Abramenko AP, Nygren R, May A, Murrell C, Robinson PK, Met Hoxha E, Mackey AP, Trask S, Robinson-Drummer PA (*Haverford/ Purdue U/ U Penn*) Preliminary evidence suggesting early decline in associative recognition learning following early-life stress

2. Agee LA, Drew MR (*UT Austin*) Fear Extinction Causes Delta FosB Buildup in the Dentate Gyrus of Male but not Female Mice

3. Nishimura KJ, Paredes D, Nocera NA, Drew MR (*UT Austin)* Role of the paraventricular thalamus in stress-induced fear sensitization

4. Paredes D, Drew MR (*UT Austin*) Effects of stress on formation and retrieval of hippocampal ensemble representations of contextual fear memory

5. Alwood MR, Moscarello JM (*TAMU*) Dorsal hippocampus underpins dissociable patterns of defensive behavior in male and female rats

6. Guerra DP, Benesch J, Gorman JC, Ho DT, Karam YE, Zhang A, Moscarello JM (*TAMU*) Sex-specific role for the bed nucleus of the stria terminalis in avoidant behavior

7. Anderson ZC, Parsons RG (*SUNY Stony Brook*) The effect of the number of trials on sex differences in contextual fear

8. Cole KE, Parsons RG (*SUNY Stony Broo*k) Sex difference in the facilitation of fear learning by prior fear conditioning

9. He W, Parsons RG (*SUNY Stony Brook*) Novel context induced activation of the claustrum in rats

10. Vazquez KE, Parsons RG (*SUNY Stony Brook*) Contextual fear expression activates frontal cortex projections to the ventral periaqueductal gray

11. Arellano Perez AD, Hassell Jr. JE, Maren S (*TAMU*) Chemogenetic reactivation of a hippocampal extinction ensemble mitigates circuit-induced relapse of extinguished fear in rats

12. Bayer H, Oleksiak C, Hassel Jr. J, Juliano V, Maren S (*TAMU*) Pharmacological stimulation of the infralimbic cortex during fear memory consolidation impairs fear retrieval

13. Crayton KL, Binette AN, Bayer H, Melissari L, Sweck SO, Maren S (*TAMU*) Chemogenetic activation of infralimbic parvalbumin interneurons impairs extinction in male and female rats

14. Garcia GM, Hassell JE, Vasudevan K, Vierkant V, Pham N, Tercilla C, Parr M, Maren S (*TAMU*) Context fear memories are not necessary for renewal of extinguished fear in rats

15. Hassell JE, Parr MA, Perez MA, Tercilla C, Maren S (*TAMU*) Does the bed nucleus of the stria terminalis mediate circuit-induced relapse of extinguished fear?

16. Oleksiak CR, Plas SL, Carriaga D, Vasudevan K, Maren S, Moscarello JM (*TAMU*) Ventral hippocampus regulates inter-trial responding in a two-way signaled active avoidance task in male and female rats

17. Plas SL, Oleksiak CR, Moscarello JM, Maren S (*TAMU*) Acute Stress Facilitates Two-Way Signaled Active Avoidance in Male and Female Rats

18. Sweck SO, Binette AN, Maren S (*TAMU*) Chemogenetic activation of the locus coeruleus mimics stress-impaired fear extinction in male and female rats

19. Tuna T, Totty MS, Peters S, Maren S (*TAMU*) Neuronal activity in the thalamic nucleus reuniens during the conditioning and extinction of fear in male and female rats

20. Bacharach SZ, Schneps HM, Wahba JI, Alhadeff AL (*Monell*) AgRP neurons that project to the lateral hypothalamus influence dopamine signaling and food intake

21. Bai Y, Grier B, Geron E (*NYU School of Med*) Motor cortex inactivity, enabled by anti-Hebbian plasticity, is key for threat expression

22. Bellfy L, Smies CW, Bernhardt AR, Sebastian A, von Abo MJ, Murakami S, Boyd HM, Albert I, Kwapis JL (*Penn State*) Hippocampal *Per1* contributes to time-of-day effects on memory consolidation

23. Boyd HM, Urban MW, Kwapis JL (*Penn State*) Sex-Specific Epigenetic Regulation of Context Fear Memory

24. Brunswick CA, Baldwin DJ, McKenna AR, Fleischer AW, Smies CW, Brockway DF, Kwapis JL (*Penn State/ UW Milwaukee*) Impaired Neuronal Ensemble Dynamics May Underlie Age-related Deficits in Memory Updating

25. Pifer GC, Bellfy L, Kwapis JL (*Penn State*) Examining sex differences in memory performance across the day-night cycle

26. Smies CW, Bellfy L, Wright DS, Bennetts SS, Urban MW, Brunswick CA, Kwapis JL (*Penn State*) The role of histone deacetylation in memory competition

27. Bennett EB, Prandy J, Patel D, Perez A, Abdel-Nabi H, Millo, E, Shors TJ (*Rutgers*) Mental and Physical (MAP) Training with Meditation and Aerobic Exercise Reduces PTSD in Women Who Experienced Sexual Violence During College

28. Blair RS, Nagaya N (*TAMU*) Intra-BNST androstanediol modulates Pavlovian fear conditioning in male rats

29. Bonanno GR, Met Hoxha E, Robinson P, Trask S, Swithers SE (*Purdue U*) Impaired contextual fear extinction and UCS deflation in aged rats

30. Bond SR, Nerz J, Solorzano-Restrepo J, Lasater M, Leising KJ (*Texas Christian U*) The effect of temporal proximity on learning a dual-response feature-positive discrimination with rats

31. Nerz JH, Bond SR, Miranda A, Gillespie C, Leising KJ (*Texas Christian U*) Reinforcer Value Affects the Emergence of the Differential Outcomes Effect in Rats

32. Brandel-Ankrapp KL, Arey RA (*Baylor College of Med*) Uncovering EtOH-mediated GLR-1 regulation and memory deficits across aging in *C. elegans*

33. Hayden AN, Brandel K, Merlau P, Pietryk E, Arey RN (*Baylor College of Med*) CEY-1/YBX RNA binding protein dysfunction causes impairments in memory and cognition

34. Leptich EJ, Arey RA (*Baylor College of Med*) Uncovering novel neuropeptide regulators of associative behaviors

35. Calderon D, Rodriguez de Souza R, Tseng C, Sandoval A, Ploski J, Thorn C, McIntyre C (*UT Dallas*) Role of the locus coeruleus in vagus nerve stimulation-induced enhancement of conditioned fear in rats

36. Campos-Cardoso R, Desa ZR, Fitzgerald BL, Cummings KA (*UA Birmingham*) Role of infralimbic cortex inhibitory neurons in the extinction of auditory fear memory

37. Desa ZD, Campos-Cardoso R, Fitzgerald BL, Moore A, Duhon J, Landar VA, Clem RL, Cummings KA (*UA Birmingham*) The mouse dorsal peduncular cortex encodes fear memory

38. Fitzgerald BL, Duhon JL, Landar VA, Cummings KA (*UA Birmingham*) Analysis of brain-wide projections to the dorsal peduncular cortex

39. Chalkia A, Craske MG, Beckers T (*KU Leuven/ UCLA*) Disrupting emotional associative memories through instructions to forget

40. Cheng HY, Li L, Todd TP (*U Vermont*) A context-dependent cue-evoked neural code in the retrosplenial cortex

41. Moriarty SK, Schoenberg HL, Winterbauer NE, Hammack SE, Toufexis DJ, Todd TP *(U Vermont*) ABA and AAB fear renewal in male and female rats

42. Chu A, Anzellotti S, Russell EL, McDannald MA (*Boston College*) Ventral tegmental area dopamine and orchestration of diverse fear conditioned behaviors

43. Russell EL, McDannald MA (*Boston College*) Ventral pallidum neurons are necessary to generalize and express fear-related responding in a minimal threat setting

44. Denholtz LE, Kolaric R, Surrence K, Grunfeld IS, Likhtik E (*CUNY Hunter*) Explicit Safety Learning Engages Parvalbumin Interneurons and Neuro-Glial Interactions in the Prefrontal Cortex

45. Fernandes-Henriques C, Sclar M, Guetta Y, Miura Y, Friedman A, Likhtik E (*CUNY Hunter*) The

contribution of Infralimbic-Basal Forebrain communication to fear extinction

46. Dinckol O, Zachry JE, Kutlu MG (*Rowan-Virtua*) Nucleus accumbens core single cell ensembles bidirectionally respond to experienced versus observed aversive events

47. Driskill C, Jalivand S, Salazar F, Khan A, Vu L, Tata S, Nuna R, Kanwal Z, Molin N, Kroener S (*UT Dallas*) Vagus nerve stimulation (VNS) alters activity in networks that regulate extinction from drug-seeking

48. Drupka CV, Somers MK, Bonsib AG, Totis JE, Petersen AR, Cramer EC, Williams JT, Taibl EG, Kochli DE (*Washington College*) Social Isolation Differentially Reduces Goal-Directed Behavior in Male and Female Rats

49. Fleischer AW, Abdelazim FH, Mitter K, Rotter J, Chaudhury S, Ford SD, Donaldson WA, Sem DS, Frick KM (*UW Milwaukee/ Marquette U/ Concordia U*) Effects of two highly selective estrogen receptor β agonists on memory and other behaviors in a mouse model of menopause

50. Gabriel DBK, Sangha S (*Indiana U School of Med*) A mixed Pavlovian/operant safety task to assess adaptive versus maladaptive avoidant responses

51. Lyvers DP, Mangrum JS, Sheehan T, Privratsky MA, Sangha S (*Indiana U School of Med*) Influence of prior stress and alcohol on safety behaviors, shock sensitivity, and subsequent alcohol consumption

52. Privratsky MA, Lyvers DP, Gabriel DBK, Lapish CC, Sangha S (*Indiana U School of Med*) Neural Correlates of Safety Signaling and Conditioned Inhibition of Fear in the Infralimbic Prefrontal Cortex

53. Garcia-Castañeda BI, Kirchner ZS, Stelly CE, Wanat MJ (*UT San Antonio*) The role of midbrain astrocytes during aversive situations

54. Johnston MP, Garcia-Castaneda BI, Vargas V, Alexandre KF, Patel SK, Wanat MJ (*UT San Antonio*) Estrous-dependent effects of single and repeated stress on Pavlovian conditioning and dopamine release

55. Giovanniello J, Paredes N, Weiner A, Oregwam C, Uwadia H, Nnamdi G, Seghal M, Reis FMCV, Sias AC, Malvaez M, Adhikari A, Silva A, Wassum KM (*UCLA*) Opposing amygdala-striatal pathways enable chronic stress to promote habit formation

56. Lamparelli AC, Vilchez GE, Patel K, Wassum KM (*UCLA*) Projections from the basolateral amygdala to the anterior cingulate cortex enable stimulus-generated reward value expectations

57. Goodpaster CM, Klune CB, Gongwer M, Jones N, DeNardo LA (*UCLA*) Increased basolateral amygdala activity linked to enhanced learning in adolescent mice exposed to early life adversity

58. Jin B, DeNardo LA *(UCLA)* Brain-wide mapping of fear memory circuits throughout development

59. Zeidler ZE, Moonkyu P, DeNardo LW (*UCLA*) Reorganization of cortical fear memories across time

60. Greiner EM, Adel-Zaheh B, Laine MA, Ravaglia I, Shansky RM (*Northeastern U*) Distinct behavioral & neural profiles for pregnant and unmated female rats during fear conditioning

61. Mitchell JR, Ziane L, Bergeron E, Shansky RM (*Northeastern U*) Chemogenic manipulation of prefrontal-periaqueductal gray circuits in female and male rats during Pavlovian Fear Conditioning

62. Sheppard VM, Mitchell JR, Shansky RM (*Northeastern U*) Sexual dimorphism in the anterior cingulate cortex-ventrolateral periaqueductal gray pathway

63. Hagen CW, Brice KN, Braden-Kuhle PN, Papini MR (*Texas Christian U*) Acute inflammation disrupts behavior in a Pavlovian, but not a consummatory, model of reward loss in rats

64. Halcomb CJ, Vanderhoof SO, Mott DD, Jasnow AM (*UofSC School of Med*) An amygdala-cortical circuit for encoding generalized fear memories

65. Hasenhundl VT, Qureshi OA, Gostolupce D, Lozzi M, Williams T, Iordanova MD (*Concordia U*) The role of the orbitofrontal cortex in learning about associatively evoked stimuli

66. Qureshi OA, Gostolupce D, Iordanova MD (*Concordia U*) Sensory preconditioned fear requires the pathway between the orbitofrontal cortex and the perirhinal cortex

67. Iyer ES, Muir J, Bagot RC (*McGill*) Nucleus accumbens glutamatergic afferents integrate outcomes across time

68. Schuler H, Mandel Weinbaum A, Siemonsmeier G, Iyer E, Bagot RC (*McGill*) A novel protocol for

simultaneous appetitive and aversive associative learning in male and female mice

69. Kim J, Bade I, Brenneman E, Erdley B, Hougham A, Keiss P, Leder J, Micek L, Miler J, Powell K, Vicknair E, Wang C, Pickens C (*Kansas State U*) Extremely high adolescent consumption of a sucrose solution, but not high consumption of an alcohol-sucrose cocktail, impairs devaluation in rats

70. Reichert A, Gogusoglu SA, Buck AL, Seipel KA, Foor EB, Leslie TG, Quinn JJ (*Miami U/ Purdue U*) Role of the paraventricular nucleus of the thalamus in the expression of threat and safety

Saturday (September 23)

1. Kaplan K, Toennies L, Hunsberger H (*Rosalind Franklin*) Alprazolam impairs fear memory and alters dorsoventral CA1 neuronal ensembles in female mice

2. Verma M, Toennies L, Kaplan K, Ferrara N, Hunsberger H (*Lake Forest/ Rosalind Franklin*) Sex differences in cognitive decline, affective behaviors, and PV interneuron activation in AD mice after isolation

3. Kim J, Waren O, Leder J, Bade I, Pickens C (*Kansas State U*) Behavioral training in a nicotine-paired environment impairs devaluation in male rats

4. King C, Pahua A, Warnes E, Neyhard J, Plakke B (*Kansas State U*) Effects of exercise on cognition and BDNF levels in the cortex of a rodent model of autism

5. Kinsky NR, Haddad J, Diba K (*U Michigan Med*) Combined Electrophysiology and Imaging to Investigate Hippocampal-Cortical Interactions During Trace Fear Memory Consolidation

6. Knox D, David N, Hause S, Biddle M, Mohammadmirzaei N, Sudheimer K, Jeun C, Barth M (*U Delaware/ S Illinois U School of Med*) Emotional properties of city and suburban landscapes

7. Kokan N, Steidl S, Yee S, Boldt A, Rankin C (*U British Columbia*) Behavioral and genetic evidence that habituation at different interstimulus intervals involves dissociable processes

8. Lacagnina AF, Dong TN, Iyer R, Khan S, Mohammed M, Clem RL (*Mt. Sinai*) Hippocampal somatostatin interneurons govern switching between memories of threat and safety

9. Laing PAF, Dunsmoor JE (*UT Austin*) Pattern separation of fear extinction memory in humans

10. Le QE, Hereford D, Borkar CD, Aldaco Z, Klar J, Alam T, Resendez A, Fadok JP (*Tulane*) Investigating contributions of the central amygdala to dynamic defensive behaviors during fear extinction

11. Lefner MJ, Moghaddam B (*OHSU*) Valence-specific gating of behavioral flexibility by medial prefrontal cortex projections to the ventral tegmental area

12. LeVasseur GW, Timothy SC, Perrine SA, Norrholm SD (*Wayne State U School of Med*) Development of robust fear potentiated startle protocol optimized for observation of within-session extinction learning in rats

13. Lozano-Ortiz K, Felix-Ortiz AC, Ramos AR, Velazquez-Hernandez G, Rodriguez-Romaguera J, Burgos-Robles A (*UT San Antonio*) Contribution of medial prefrontal cortical areas to the development of social phobia

14. Msengi HD, Felix-Ortiz AC, Magalhães G, Diehl MM, Burgos-Robles A (*UT San Antonio*) New insights on the mechanisms of active avoidance behavior during signaled threat: Prefrontal contributions and behavioral differences across sex and age groups

15. Felix-Ortiz AC, Terrell JM, Gonzalez C, Msengi HD, Ramos AR, Boggan MB, Lopez-Pesina SM, Magalhães G, Burgos-Robles A (*UT San Antonio*) The prelimbic and infralimbic cortical areas bidirectionally modulate safety learning during naturalistic thermal threat

16. Ly A, Root DH (*UC Boulder*) Bed nucleus of the stria terminalis (BNST) GABA signaling in backward conditioned suppression

17. Macdonald EE, Ma J, Authement ME, Alvarez VA, Penzo MA (*NIMH*) A thalamostriatal circuit that shapes dopamine-mediated safety signaling during avoidance learning

18. O'Malley JJ, Ma J, Kreiker M, Penzo MA (*NIMH*) Non-canonical cortico-thalamic dynamics gate avoidance decisions

19. Brockway ET, Simon S, Drew MR (*UT Austin*) Ventral hippocampal activity differences in contextual fear and extinction recall

20. Matsumura K, Nicot A, Choi IB, Asokan M, Le N, Natividad L, Dobbs LK (*UT Austin*) Endogenous opioid system modulates cocaine reward in a sex-dependent manner

21. Remmers BC, Nicot A, Choi IB, Matsumura K, Alvarez V, Dobbs LK (*UT Austin*) Mu Opioid Receptors have Divergent Effects on Cocaine and Opiate Behaviors

22. Moaddab M, Qian S, Boyce JB, Gordon NT, DuBois AM, Fitzpatrick AC, McDannald MA (*Boston College*) Ventral pallidum-defined pathways modulate fear-related behavior during threat discrimination

23. Mohammadmirzaei N, Biddle M, Hekmatyar K, Cai X, Kulkarni P, Knox D (*U Delaware*) Sex differences in the traumatic stress effects on functional connectivity, brain volume, and mu-opioid receptor levels within the reward circuit

24. Moore E, Harris H, Slover W, Simpson J, Meyer W, Campolattaro MM (*Christopher Newport U*) Contextual Control of Classically Conditioned Eyeblink Responses in Rats

25. Moran KM, Enstrom AE, Jarrell L, Khashchuluun M, Tran A, Delville Y (*UT Austin*) Hamsters as an animal model for stress and obesity

26. Mousset M, Trombetti K, Ruben E, Ruben M, David J, Chu P, Huynh TN (*Midwestern U*) Chronic mild stress leads to anxiety-like behavior and decreased p70 S6K1 activity in the hippocampus of male mice

27. Murray JA, Ke-Lind PL, Schuh KM, Alltop KW, Tronson NC (*U Michigan*) Partial reinforcement prevents blocking: considering a role for affect in Pavlovian conditioning

28. Oak SS, Lauraine E, Nguyen C, Rincón-Cortés M (*UT Dallas*) Effects of early life scarcity-adversity on developmental milestones in male and female rats

29. Porras A, Rodney-Hernández P, Rincón-Cortés M (*UT Dallas*) That's stimulating! Assessing the effects of early life sensory overstimulation on later life behavioral function in rodents

30. Rodney-Hernández P, Porras A, Rincón-Cortés M (*UT Dallas*) Effects of sensory overstimulation in postpartum rodents

31. Penna SR, Ng Q, Watts ME, Zhao A, Cazares VA (*Williams*) Severe stress produces maladaptive cognitive and emotional function via non-associative sensitization

32. Gonzalez SE, Fukunaga Y, Watts M, Locke E, Schulman E, Cazares VA (*Williams*) Investigating the neuromodulatory role of dopamine in strengthening fear extinction memories formed in multiple contexts

33. Pyon WS, Blaes SL, Orsini CA, Faraji M, Viera OA, Singhal SM, Frazier CJ, Bizon JL, Setlow B (*U Florida*) Investigating the functional role of ventral tegmental area dopamine neurons in decision making under risk of punishment

34. DiFazio LE, Reis FMCV, Adhikari A (*UCLA*) Role of periaqueductal gray GABAergic cells in instrumental food seeking

35. Hoang IB, Taira M, Wikenheiser AM, Sharpe MJ (*UCLA*) Unexpected rewards are signaled by dopamine release into the lateral hypothalamus

36. Markowitz SM, Fanselow MS, Sharpe MJ (*UCLA/ U Sydney*) The Role of Lateral Periaqueductal Grey During Associative and Non-Associative Fear Learning

37. Ortega ME, Hoang IB, Sharpe MJ (*UCLA*) The motivational properties of drug-paired cues on model-free and model-based associations

38. Reyes VR, Sharpe MJ (*UCLA*) Role of VTA GABA neurons in encoding sensory specific expectations of rewards

39. Robinson PK, Met Hoxha E, Williams D, Kinzig KP, Trask S (*Purdue U*) Extinction learning is impaired by normal aging

40. Met Hoxha E, Robinson PK, Trask S (*Purdue U*) Generalization and discrimination of inhibitory avoidance differentially engage retrosplenial subregions

41. Ruble S, Kramer C, Kettler C, West L, Auletti I, Diehl MM (*Kansas State U*) Active avoidance is enhanced when learned indirectly through observation

42. Kramer C, Ruble S, West L, Payne K, Auletti I, Diehl M (*Kansas State U*) Active avoidance under social conditions enhances fear responses and recruits the anterior cingulate cortex in male and female rats

43. Sattler KP, Hedges A, Miller R, Zelikowsky M (*U Utah*) The Ventral Hippocampus is necessary for trauma-altered social behavior

44. Schamber G, LaViola M, Grisales K, Awad A, Herbst MR, Twining RC, Gilmartin MR (*Marquette U*) Prefrontal cortical output to the mediodorsal thalamus encodes trace fear conditioning

45. Sheynin J, Baidya S, Shrestha G, Liberzon I (*TAMU/ U Michigan*) Learning and generalization of avoidance: A mismatch between expectancy and behavior and associations with anxiety vulnerability

46. Vogt GS, Sheynin J, Nickelsen T, Lokshina Y, Abelson JL, Liberzon I (*TAMU*) Effects of context processing on physiological fear responses during learning and recall of fear and extinction memories

47. Shilyansky C, Kochalka J, Raffiee M, Cordero A, Ramakrishnan C, Quirin S, Deisseroth K (*Stanford/ HHMI*) Prefrontal cortex controls large-scale neural dynamics coupled to fear during memory recall

48. Siller-Perez C, Andrade EC, Smiley J, Cain CK, Sears RM (*EBI/ NKI/ NYU Langone*) Orexin promotes active avoidance via amygdalar and midbrain targets

49. Sosa R, Saavedra P, Jiménez S, Lago G, Buenrostro-Jáuregui M (*UP/ UNAM/ ITESM/ UIA-México*) Dissociation between response restraint and response cancellation: Evidence from a Pavlovian protocol

50. Spring MG, Nautiyal KM (*Dartmouth*) Serotonin is released in the dorsal striatum in anticipation of a reward

51. Stevanovic KD,Wilson LR, Letsinger AC, Cushman JD (*NIEHS*) Systematic comparison of three *in vivo* fiber photometry recording methods

52. Wilson LR, Plummer NW, Evsyukova IY, Patino D, Stewart CL, Smith KG, Konrad KS, Fry SA, Deal AL, Kilonzo VW, Panda S, Sciolino NR, Cushman JD, Jensen P (*NIEHS/ NIH/ Social and Scientific Systems, Inc./ UConn*) Partial or complete loss of norepinephrine differentially alters contextual fear and catecholamine release dynamics in hippocampal CA1

53. Torres JB, Williams E, Martin MJ, Bishop CA, Saddoris MP (*UC Boulder*) Cocaine self-administration experience alters phasic responses of prefrontal cortex in response to controllable stressors

54. Truckenbrod L, Garner M, Carlos N, Gore AC, Orsini CA (*UT Austin*) Contributions of estradiol and progesterone to female risk aversion

55. Wheeler AW, Truckenbrod LM, Garner M, Orsini CA (*UT Austin*) Relationships between fentanyl self-administration and risk-taking behavior in rats

56. Vasquez A, Kim G, Dominguez JM, Monfils M-H, Lee HJ (*UT Austin*) Oral hormonal contraceptives affect female rat gonadal function and lead to reduced amphetamine-preference during extinction

57. Olvera ME, Raskin M, Cofresi RU, Gonzales RA, Monfils MH, Lee HJ (*UT Austin/ U Missouri*) Extinction after memory retrieval reduces conditioned response to alcohol cues in male and female rats with a history of alcohol dependence

58. Raskin M, Keller NE, Agee LA, Shumake J, Lee HJ, Monfils M-H (*UT Austin*) CO2 reactivity predicts fear expression after extinction and retrieval-extinction

59. Simmons TA, Gonzales RA, Monfils M-H, Lee HJ (*UT Austin*) Renewal of alcohol-seeking in males and female rats

60. Vasquez LS, Stack SM, Taylor WW, Dias BG (*CHLA/ USC*) Learning, memory, and motivation in Prader-Willi Syndrome through the lens of *snord116* in catecholaminergic cells

61. Parker RK, Padival M, Selby D, Ferrara NC (*Rosalind Franklin*) Social instability interferes with fear elevation

62. Welch HF, Thorn CA (*UT Dallas*) Exploring right versus left VNS effects on motor performance and cortical plasticity

63. West L, Davis I, Smith TR, Southern R, Panfil K, Kirkpatrick K (*Kansas State U*) Chemogenetic inactivation of the pre-limbic cortex increases impulsive decision-making in rats

64. Williams BL, Demaestri C, Berry GC, Darling AM, Bath KG (*Columbia/ NYSPI*) Exploring the impact of early life adversity on stress-enhanced fear learning and fear expression in mice

65. Piantadosi P, Perry S, Coden K, Sandon Veliz R, Coden K, Choi H, Spitz N, Schwab N, Authement M, Alvarez V, Schaffer J, Da Silva D, Goff J, Halfeld M, Holmes A (*NIAAA*) Amygdalar regulation of punished decision-making

Abstracts

Both for invited talks and for posters. In alphabetical order by first author's last name.

Abramenko AP, Nygren R, May A, Murrell C, Robinson PK, Met Hoxha E, Mackey AP, Trask S, Robinson-Drummer PA (*Haverford/ Purdue U/ U Penn*) Preliminary evidence suggesting early decline in associative recognition learning following early-life stress ABSTRACT: To survive to adulthood, it is critical for developing animals to learn

about changing environmental elements. However, early-life trauma can cause alterations in neurobiological learning systems that persist into adulthood. Indeed, reports in humans suggest early-life trauma accelerates cognitive decline in later life, though the cause of this decline is poorly understood. The low bedding-nesting (LBN) model, a paradigm that alters maternal behavior and elicits stress in pups, has been used to study the effects of early-life stress (ELS) in rodents and lends supporting evidence that ELS may accelerate aging of memory systems. In this study we sought to extend literature examining how ELS affects learning between adolescence and young adulthood by assessing performance of rats on non-reinforced recognition tasks: object-in-place (OiP) and object recognition (OR) tasks. Preliminary data suggests that PD8-12 LBN ELS may result in the early decline of associative spatial recognition (OiP performance) in young adulthood. Furthermore, there may be an early spike preceding a modest decline in basic recognition memory following LBN, however further investigation is necessary to confirm these results. Additional analyses are ongoing to elucidate the neural components of this cognitive decline; notably whether dysfunction in the ubiquitin proteasome (UPS) system (typically associated with age-related memory decline) is detected in subregions critical for associative learning in young adults. This work was supported by: Scialog grant #29105 from Research Corporation for Science Advancement and Frederick Gardner Cottrell Foundation.

Agee LA, Drew MR (*UT Austin*) Fear Extinction Causes Delta FosB Buildup in the Dentate Gyrus of Male but not Female Mice ABSTRACT: Acquisition of a learned fear association results in the emergence of a stable set of neurons whose reactivation is necessary and sufficient to evoke a fear response. Our lab has previously demonstrated that extinction of a fear response through repeated re-exposure to the feared stimuli suppresses this reactivation in the dentate gyrus of the hippocampus. However, the molecular processes which support this suppression of activity remain unclear. Here, we examined delta FosB - a long lasting transcription factor whose buildup has been demonstrated to decrease excitability in hippocampal neurons - as a potential mediator of extinction-based activity suppression in fear encoding neurons. We used an activity dependent tagging strategy to indelibly label fear activated neurons in transgenic mice and compared expression of delta FosB in the dentate gyrus of mice that had undergone extinction and those that had not. We found that delta FosB levels were indeed upregulated in the dentate gyrus of some mice that had undergone extinction, both in the dentate gyrus at large and in fear tagged cells within the dentate gyrus. Surprisingly, however, this effect was sex-dependent and only observed in our male subjects. These results suggest that delta FosB buildup might serve a sex-specific role in mediating the extinction-based suppression of fear retrieval.

Alwood MR, Moscarello JM (*TAMU*) Dorsal hippocampus underpins dissociable patterns of defensive behavior in male and female rats ABSTRACT: Unsignaled active avoidance (USAA) is a complex associative conditioning paradigm in which subjects acquire escape and avoidance responses to earn and prolong, respectively, periods of safety from a shock US delivered at regular intervals. Unlike other active avoidance tasks, responses in USAA are not triggered by a conditioned cue, suggesting the hypothesis that this ‘free operant’ form of defensive behavior may indeed be under the control of hippocampally mediated contextual processes. To test this idea, we expressed the excitatory hM3Dq DREADD in Dlx+ inhibitory interneurons in the dorsal hippocampus (dHPC) of male and female rats with the goal of broad dHPC inhibition. DREADD-expressing interneurons were then activated with systemic CNO on days two and eight of training to explore the role of dHPC during acquisition and expression of USAA behavior. dHPC inhibition was found to facilitate the acquisition of avoidance in female, but not male rats. In contrast, dHPC inactivation in male rats produced a permanent increase in escape latency, though the overall number of escapes was unchanged. These data suggest sex-specific roles for the hippocampus in both reactive and proactive defensive behaviors. In a separate, ongoing experiment, we quantified cfos expression following dHPC inactivation during USAA acquisition, in order to confirm the effect of DREADD activation of interneurons on neural activity in dHPC itself, as well as to explore the downstream effects in the broader system of avoidance-relevant brain regions (i.e. medial frontal cortex, midline thalamic nuclei, amygdala, nucleus accumbens). Preliminary data from this ongoing experiment will be presented at the conference.

Anderson ZC, Parsons RG (*SUNY Stony Brook*) The effect of the number of trials on sex differences in contextual fear ABSTRACT: Contextual fear conditioning occurs when a relationship forms between a novel environment and an aversive stimulus so that when the individual is re-exposed to the environment there is a conditioned fear response. This form of learning has been used extensively in the laboratory as it holds special relevance to fear and anxiety based disorders, which often involve the expression of fear in contexts where the aversive stimulus was not encountered. Females show a higher incidence of anxiety disorders making the study of how sex influences contextual fear in rodent models of high importance. Prior research comparing males and females is mixed, with some studies showing that males exhibit higher levels of contextual fear than females, others reporting the opposite pattern, and others that report no difference. These discordant findings are often suggested to be the result of parametric differences between studies, but the determinative factors are not well understood. Our prior work has shown that males exhibit higher levels of freezing to an aversive context when trained with 3 trials.

Here we tested whether or not varying the number of trials would mitigate sex differences in contextual fear. In the experiment, male and female rats received either a single shock or 6 shocks (with an ITI of 20 sec) during training. A day later rats were tested in the context in which shock occurred, followed the next day in a novel context to measure the generalization of context fear. Preliminary results suggest that the observation of higher levels of freezing to an aversive context in males was maintained across groups trained with different numbers of shock and that males also showed higher levels of freezing behavior in a novel context. Experiments are ongoing testing whether or not these factors influence sex differences in contextual fear across others measures of fear.

Arellano Perez AD, Hassell Jr. JE, Maren S (*TAMU*) Chemogenetic reactivation of a hippocampal extinction ensemble mitigates circuit-induced relapse of extinguished fear in rats ABSTRACT: Fear relapse is a major challenge for extinction-based behavioral interventions for fear and anxiety disorders, including PTSD. We have recently discovered that the thalamic nucleus reuniens (RE) has a critical role in suppressing conditional fear responses, including freezing behavior, after extinction. Specifically, pharmacological inactivation of RE produces a robust "circuit-induced relapse" of extinguished fear. A number of convergent pieces of evidence lead to the hypothesis that the RE inhibits extinguished fear by suppressing hippocampal fear memories. If true, chemogenetically activating hippocampal extinction engrams should oppose circuit-induced fear relapse. To explore this question, we used a Tet-tag system (AAV-Fos-tTA and AAV-TRE-hM3Dq-mCherry) to achieve activity-dependent expression of "designer receptors exclusively activated by designer drugs" (DREADDs) in the dorsal hippocampus (DH). Adult male and female Long-Evans rats first underwent auditory fear conditioning (context A), followed by extinction of the auditory CS in a novel context (context B). Two days later, they were taken off the doxycycline (DOX) diet and returned to the extinction context to tag hippocampal ensembles active during an extinction retrieval test. Demonstrating the efficacy of the tagging procedure, chemogenetic reactivation of captured extinction ensembles reduced contextual freezing in the conditioning context. To test whether reactivation of DH extinction ensembles would attenuate circuit-induced relapse, rats received intra-RE infusions of either saline (SAL) or muscimol (0.1 μg/μl) and systemic injections of the clozapine-N-oxide (CNO, 3 mg/kg) or SAL (counterbalanced order) prior to an extinction retrieval test. We found that CNO administration reduced the relapse of conditioned freezing induced by intra-RE muscimol infusions. These results suggest that circuit-induced relapse of extinguished fear may be due to a suppression of hippocampal extinction engrams; chemogenetic activation of these ensembles overcomes the relapse of fear produced by RE inactivation. These results increase our understanding of thalamo-hippocampal interactions involved in regulating the retrieval of fear and extinction memories.

Bacharach SZ, Schneps HM, Wahba JI, Alhadeff AL (*Monell*) AgRP neurons that project to the lateral hypothalamus influence dopamine signaling and food intake ABSTRACT: Feeding behavior is mediated by homeostatic (e.g., hypothalamic) and hedonic [e.g., mesolimbic dopamine (DA)] neural circuits, but how these systems interact to drive feeding behavior remain unclear. We recently demonstrated that stimulation of arcuate nucleus (Arc) agouti-related protein (AgRP) neurons, which robustly drives food intake, also increases nucleus accumbens (NAc) DA release in response to food. However, AgRP neurons do not project directly to mesolimbic centers, and little is known about how AgRP circuits regulate the dopamine system. Because the lateral hypothalamus (LH) serves as an established relay point between AgRP neurons and DA circuits through synapses within the ventral tegmental area, we predicted that discrete AgRP projections to the LH modulate food-evoked NAc DA activity and feeding behavior. To test this hypothesis, we optogenetically stimulated ArcAgRP axons in the LH while recording DA release within the NAc using fiber photometry. We first confirmed that LH terminal stimulation significantly increased food intake. We found that stimulation of these axons increased DA release in response to food presentation. Interestingly, stimulation of this pathway alone produced increases in tonic DA release over tens of minutes. In contrast, stimulation of the ArcAgRP-to-paraventricular hypothalamus pathway increased feeding behavior but did not increase tonic dopamine release. Current studies are combining optogenetic stimulation of ArcAgRP-to-LH projections with pharmacological blockade of DA receptor subtypes to assess the necessity of this DA signaling for the motivation to find and consume food. Together, these studies demonstrate that the ArcAgRP-to-LH pathway increases both tonic DA and food-evoked DA, suggesting this canonical homeostatic pathway plays a role in food reward.

Bai Y, Grier B, Geron E (*NYU School of Med*) Motor cortex inactivity, enabled by anti-Hebbian plasticity, is key for threat expression ABSTRACT: The primary motor cortex (M1) is generally considered to be primarily involved in executing movement, rather than participating in higher emotional processes. Previous research however did show that freezing behavior is associated with reduced activity in M1. Several unresolved questions remain: Is the reduced activity in M1 a cause or a consequence of freezing? Does M1 contribute to threat learning, and if so, how? To explore these questions, we adapted the threat conditioning paradigm to be compatible with two-photon microscopy—a method sensitive enough to capture the activity of individual dendritic spines during shock application. Our findings

revealed that: (1) layer 5 pyramidal neurons (L5 PNs) developed negative tone responses within an hour after conditioning; (2) freezing behavior was not a prerequisite for these negative responses; and (3) these responses were not due to an overt increase in inhibition. We discovered that these negative responses depended on the weakening of dendritic spines that were active during training. When we blocked this form of anti-Hebbian plasticity through optogenetic manipulation of CaMKII, both negative tone responses and freezing behavior were disrupted. Therefore, the weakening of spines active during memory encoding renders L5 PNs insensitive to training-related inputs, leading to an activity drop from baseline levels. This reduction in activity, in turn, curbs M1's ability to facilitate movement, thereby contributing to freezing. We thank B. Rudy and members of his lab for fruitful discussions and support. We also thank W. Gan and members of his lab. We thank O. Issler and R. Machold for comments on the manuscript, and D. Schiller. Funding: This work was supported by the National Institutes of Health grant: R01NS110079 to B. Rudy.

Balsam PD (*Barnard/ Columbia*) Scotomas, Necker Cubes and Contingencies ABSTRACT: Contingency theory posits: (1) When the probability of an unconditioned stimulus (US) in the presence of a conditioned stimulus (CS) is greater than the probability of the US in its absence, the CS will be excitatory; (2) When the probability of an unconditioned stimulus (US) in the presence of a conditioned stimulus (CS) is less than the probability of the US in its absence, the CS will be inhibitory. (3) When the US is equally likely in the presence and absence of the CS the cue provides no reduction in uncertainty about when the US will occur and the CS is neutral in value. We exposed groups of rodents to perfect negative contingencies in which reinforcers were never delivered during the CS but were delivered in the absence of the CS at different rates. We found that during training the CS did not become inhibitory. Instead, the offset of the CS became a conditioned excitor. The degree of excitation was determined by the ratio of the rate of reinforcement in the absence of the CS to the overall reinforcement rate in the context. Dopamine release as measured by photometry using the dLite sensor showed a similar pattern for both positive and negative cues. There is a transient increase when a change in stimuli signals reward availability and transient decrease when it signals the absence of reward. In this way positive and negative contingencies are two sides of the same coin.

Bayer H, Oleksiak C, Hassel Jr J, Juliano V, Maren S (*TAMU*) Pharmacological stimulation of the infralimbic cortex during fear memory consolidation impairs fear retrieval ABSTRACT: Recent work suggests that the role of the infralimbic (IL) cortex in extinction learning may be established soon after fear conditioning, during the initial consolidation of fear. To test this hypothesis, we examined whether post-conditioning pharmacological stimulation of the IL would affect expression and/or facilitate extinction of fear. In experiment 1, rats received intra-IL infusions of picrotoxin (PIC) or vehicle (VEH) immediately after auditory fear conditioning. The next day, PIC-treated animals exhibited less freezing during a retrieval test, suggesting that IL stimulation during consolidation produced retrograde amnesia. In experiment 2, we observed a similar effect when intra-IL PIC infusions were performed 24 hours after fear conditioning. This suggests that stimulation of the IL outside of the classical consolidation window also yields retrograde amnesia (or "pharmacological extinction") of the fear memory. We hypothesized that these effects may depend on conditioning-induced plasticity in the IL. To test this in experiment 3, animals received intra-IL VEH or anisomycin (ANI, a protein synthesis inhibitor) immediately after conditioning and VEH or PIC into the IL 24h later. Interestingly, ANI prevented the subsequent amnestic effect of PIC (the VEH-PIC group presented lower levels of freezing than the ANI-PIC). Furthermore, since in all the previous experiments extinction was conducted no longer than 4 days after conditioning and treatments, we conducted an additional experiment assessing the effects of PIC on remote memory extinction. Rats were treated with PIC or VEH 24h or 13 days after conditioning and memory extinction was conducted 14 days after conditioning. Animals that received PIC in either (or both) time-point froze less than controls. Together, these results indicate that stimulation of the IL after learning (up to two weeks after consolidation is over) can serve as a form of “pharmacological extinction” of the fear memory, this effect is long lasting and relies on protein synthesis during early stages of memory consolidation in the IL. This is in line with the counterintuitive notion that IL’s role in fear memory extinction is set during fear memory consolidation.

Bellfy L, Smies CW, Bernhardt AR, Sebastian A, von Abo MJ, Murakami S, Boyd HM, Albert I, Kwapis JL (*Penn State*) Hippocampal *Per1* contributes to time-of-day effects on memory consolidation ABSTRACT: Many biological processes are affected by the circadian system, including memory. Behavioral paradigms, such as the dorsal hippocampus (DH)-dependent Object Location Memory (OLM), show oscillating memory performance across the diurnal cycle, specifically better memory during the day than at night. Here, we used OLM to gain an understanding of potential molecular mechanisms that underlie this time-of-day memory effect. First, we needed to determine which phase of memory is impacted by the time of day. To assess acquisition, we tested short-term memory and found that mice showed normal short-term memory for OLM during both day and night, suggesting that acquisition is not regulated across the diurnal cycle. Next, to assess memory retrieval, we trained mice at the peak (ZT5/12p) and trough (ZT17/12a) of memory performance, but tested them 36

hours later, so mice trained during the day were tested at night and vice versa. We found the time of memory acquisition, not the time of retrieval, was the driving factor behind whether memory was intact; day-trained mice were able to retrieve the memory at night whereas night-trained mice showed poor retrieval when tested during either the day or night. Together, these results suggest that nighttime memory deficits are due to impaired consolidation. As memory consolidation is transcription-dependent, we performed RNA-seq on DH tissue to identify learning-induced gene changes over the day/night cycle. Notably, the circadian rhythm gene *Period1* (*Per1*) was upregulated in response to learning during the day but not night, oscillating in tandem with memory performance. This suggests hippocampal *Per1* may regulate memory across the day/night cycle. To determine if hippocampal *Per1* contributes to memory, we knocked down *Per1* using CRISPR interference (CRISPRi) locally in the dorsal hippocampus prior to training at ZT5 which resulted in the mice being unable to learn the training. As *Per1* modulates CREB activity, we also looked at memory allocation and found that more neurons are allocated to the memory trace during the day (when *Per1* and memory peak) than at night. In conclusion, diurnal oscillations in memory consolidation may be regulated in part by hippocampal *Per1* expression by gatekeeping CREB activity that drives neuronal allocation.

Bennett EB, Prandy J, Patel D, Perez A, Abdel-Nabi H, Millo E, Shors TJ (*Rutgers*) Mental and Physical (MAP) Training with Meditation and Aerobic Exercise Reduces PTSD in Women Who Experienced Sexual Violence During College ABSTRACT: Women on college campuses are at high risk for sexual violence (Cantor et al., 2020; Sabina & Ho, 2014). Those who do experience sexual trauma are often diagnosed with post-traumatic stress disorder (Abebe et al., 2018; PTSD). But many do not seek help or do not have access to evidence-based treatments (Murn & Schultz, 2020; Sabina & Ho, 2014). Mental And Physical (MAP) Training is a novel clinical intervention that combines mental training through meditation and physical training through aerobic exercise (Shors et al., 2016; 2018; Shors 2021). The intervention was translated from preclinical studies on adult neurogenesis and has been tested in numerous populations, many with trauma history (Millon & Shors, 2019). Our goal here was to determine whether an online presentation of MAP Train My Brain would reduce PTSD symptoms, based on DSM-5 criteria, as well as lessen ruminative thoughts in college sexual violence survivors. Twenty cis-gender women were recruited from a large, northeast university, all of whom reported experiencing sexual violence while in college. Participants completed mental health outcome measures before and after the intervention. MAP Training combines 30 minutes of silent meditation (20 min sitting and 10 min slow walking) followed by 30 minutes of aerobic exercise with guided routines to music. Participants were supervised by research assistants while engaging in two sessions per week for six weeks. The sessions were pre-recorded and presented online. Prior to training, all but one participant met criterion for a provisional PTSD diagnosis, whereas more than half no longer met criterion afterwards. Symptoms of PTSD decreased by 40% ($t(19) = 5.49$, $p < 0.001$). We further detected a decrease in intrusion symptoms ($t(19) = 3.10$, $p < 0.05$), avoidance symptoms ($t(19) = 3.51$, $p < 0.01$), negative alterations in cognition and mood ($t(19) = 4.524$, $p < 0.001$), and arousal symptoms ($t(19) = 5.82$, $p < 0.001$). The brain training intervention reduced rumination by 23% ($t(19) = 3.99$, $p < 0.001$), including decreases in both depressive ($t(19) = 3.76$, $p < 0.001$) and reflective subtypes ($t(19) = 6.64$, $p < 0.001$). Sexual violence and the trauma it inflicts upon students on college campuses is a serious problem across the country. These results indicate that a combination of mental and physical training is an effective intervention for survivors. Indeed, the changes were substantial, and enough to eliminate PTSD for most participants. MAP Train My Brain is a low-cost, efficient, and effective way to reduce suffering and improve mental health for survivors on college campuses, and elsewhere. This work was funded by: Center for Advanced Human Brain Imaging Research--Brain Health Institute Pilot Award.

Blair RS, Nagaya N (*TAMU*) Intra-BNST androstanediol modulates Pavlovian fear conditioning in male rats ABSTRACT: Sex steroid hormones and their neuroactive metabolites can modulate fear and anxiety in both humans and rodents. We have previously shown that the progesterone metabolite, allopregnanolone (ALLO), confers state dependence to contextual fear when infused into the bed nucleus of the stria terminalis (BNST) of male rats. Like ALLO, the testosterone metabolite, 3a-androstanediol (3a-diol), is also an endogenous neurosteroid and has been implicated in fear and anxiety. Here, we explore the role of intra-BNST infusion of 3a-diol on the acquisition and expression of Pavlovian fear conditioning in adult male rats. We infused 3a-diol (7.3 mg/mL) or vehicle (VEH; 30% 2-hydroxypropyl-B-cyclodextrin) into the BNST 10 min prior to a conditioning session consisting of 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) after infusion of either the same or different drug (3a-diol or VEH). Pretraining infusion of 3a-diol did not affect acquisition of conditioned fear and expression of cued fear was similar regardless of drug infusion. Expression of contextual fear, however, was reduced in animals trained and tested after different drug infusions, suggesting that 3a-diol confers state dependence. This work further supports a role for intra-BNST neurosteroids in defining an interoceptive context that can contribute to state-dependent learning.

Bonanno GR, Met Hoxha E, Robinson P, Trask S, Swithers SE (*Purdue U*) Impaired contextual fear extinction and UCS deflation in aged rats ABSTRACT: Recent work in adult rats has demonstrated that fear memories established by pairing a context with a footshock can be reduced by subsequent pairings of that context with multiple weaker footshocks. This unconditional stimulus (UCS) deflation procedure is similar to extinction in that both include additional exposure to the training context and both reduce freezing to that context. However, unlike extinction, UCS deflation is not context-dependent; USC deflation reduces freezing to the training context whether the weak shock exposure is delivered in the training context or in a novel context. Consistent with this context dependency, extinction but not weak shock-exposed animals showed increased cellular activity in the dorsal hippocampus, an area important for encoding contextual information. Further, only UCS deflation animals showed changes in protein degradation in the basolateral amygdala following testing suggesting that the USC deflation procedure may work to alter the original representation of the shock to reduce fear responding rather than creating a new inhibitory memory, like that acquired in extinction. The UCS deflation procedure may thus provide a viable alternative method for altering memories, especially in older populations, as studies over the past four decades have demonstrated that performance on a wide variety of hippocampal-dependent tasks is impaired in aged rats. To better understand how aging impacts the ability to flexibly change behavioral responding to an aversive stimulus, we directly compared the UCS deflation procedure to extinction following contextual fear conditioning in aged (19-21-month-old) male and female Sprague Dawley rats. We found that while freezing decreased across the extinction and weak shock sessions, neither extinction- nor weak shock-exposed animals froze less during testing when compared to animals that received no subsequent exposure to the training context prior to testing. In addition, while extinction animals showed a decrease in freezing between the extinction session and testing, weak shock-exposed animals showed increased context freezing during testing compared to the weak shock session. We speculate that unlike adult animals who used the weaker shocks to modify the predicted value of the context, in aged animals the original value of the UCS remained persistent. Future work will focus on investigating how aging has modified cellular activity in the hippocampus and basolateral amygdala and the role that protein degradation plays in contributing to this impairment in behavioral flexibility.

Bond SR, Nerz J, Solorzano-Restrepo J, Lasater M, Leising KJ (*Texas Christian U*) The effect of temporal proximity on learning a dual-response feature-positive discrimination with rats ABSTRACT: In an operant feature-positive discrimination, responses are reinforced (+) only when the target stimulus (A) is presented with the feature stimulus (X), but not during trials where the target stimulus is presented alone (A-). Typically, when X and A are presented simultaneously, direct control over responding by X is observed, but when they are presented serially, X sets the occasion for responding to A. However, Holland and colleagues found that decreasing the salience of X relative to A resulted in occasion setting with simultaneous pairings of X and A. The current experiment utilized rats and a dual-response feature-positive procedure. One response (e.g., left lever press) was reinforced during trials with both X and A stimuli (XA+), but a different response (e.g., right lever press) was reinforced during target-alone trials (A). The goal of the current experiment was to examine whether the relative salience of X and A could be altered by changing the temporal proximity of X from reinforcement. The more proximal X is to reinforcement, the more salient it should be. Rats received either simultaneous (X:A+) presentations of the feature-target compound, serial (X→A+) presentations with a 5-s interval between X and A+ (responses were reinforced during A), or serial (X→A+) presentations with a 15-s interval between X and A+. All groups also received target-alone trials (A+) with reinforcement of the other response. Nonreinforced test trials of X, XA, and A were delivered before and after extinction of X. Results revealed direct control by X in all groups, regardless of the temporal proximity of X. The role of stimulus salience and temporal proximity will be discussed. Additional research from our lab has investigated behavior after longer feature-target intervals, as well as direct manipulation of the salience of X.

Boyd HM, Urban MW, Kwapis JL (*Penn State*) Sex-Specific Epigenetic Regulation of Context Fear Memory ABSTRACT: Post-traumatic stress disorder (PTSD) is known to be more prevalent among women than men, but the mechanisms that underlie this sex difference are unknown. In PTSD, exposure to trauma persistently changes the brain's response to subsequent stress, leading to lasting fear sensitization. Currently, the molecular mechanisms which support the persistence of trauma are unclear, with even less known about sex-specific mechanisms. One possibility is that epigenetic modifications support the lasting effects of trauma. Epigenetic mechanisms, which change gene expression by modifying chromatin structure, are known to drive long-lasting changes to cellular function that may support persistent changes in behavior. In particular, histone acetylation may play a key role, as this epigenetic mechanism is critical for normal fear memory formation. Blocking the repressive enzyme histone deacetylase 3 (HDAC3) in the hippocampus or amygdala, for example, enhances both histone acetylation and fear memory formation. It is less clear whether HDAC3 is also involved in the exaggeration of fear memories acquired following trauma exposure in male or female mice. Here we tested for sex differences in the stress-enhanced

fear learning (SEFL) paradigm, in which an acute "trauma" event (10 unsignaled shocks) reliably potentiates subsequent fear learning. We found that two shocks are sufficient to drive SEFL in females, but not males, implying that females may be predisposed to encoding traumatic stimuli more robustly. We then used a dominant-negative, deacetylase-dead point mutant virus to block HDAC3 activity and in turn enhance histone acetylation during this weak trauma event in the amygdala of male and female mice. We found that amygdalar HDAC3 inhibition transformed a weak trauma event into one that established persistent fear sensitization in males. In females, the same manipulation had little effect, likely because the female mice already showed robust stress-enhanced fear learning even with the weak trauma event. Together, this suggests that HDAC3 activity may contribute to stress-enhanced fear learning, possibly by setting a molecular threshold that enables the severity of fear sensitization observed following acute stress sex-specifically.

Brandel-Ankrapp KL, Arey RA (*Baylor College of Med*) Uncovering EtOH-mediated GLR-1 regulation and memory deficits across aging in *C. elegans* ABSTRACT: Chronic ethanol (EtOH) consumption is linked to memory deficits and worsened cognitive aging trajectories. Thus, it is critical to identify molecular pathways disrupted by chronic alcohol that contribute to persistent memory deficits. EtOH targets conserved memory regulators, including the ionotropic glutamate receptor a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR). *In vivo* and in cell culture, chronic EtOH exposure and withdrawal (WD) enhances AMPAR-mediated signaling and expression. However, it remains unknown which AMPAR regulatory pathway is impacted by chronic ethanol exposure that leads to memory deficits across aging. Due to the complexity of the mammalian brain, it is difficult to unravel how EtOH regulates AMPARs in mammals to modify behavior, let alone across aging. In *C. elegans*, GLR-1, an AMPAR ortholog, has tightly defined expression in relatively few neurons and is linked to molecularly conserved memories including those disrupted by EtOH. Previous studies demonstrated exposing worms to 400mM EtOH is sufficient to induce 40-50mM internal concentrations that correspond to .2% BAC in humans and induce WD behaviors upon 1h removal from EtOH. Using this exposure protocol in *C. elegans*, we assessed how EtOH affects associative olfactory memory. Memory was measured using a positive olfactory associative memory assay, in which pairing a neutral odorant with a food source yields long-lasting and measurable attraction towards that odor. This assay can delineate distinct forms of memory (learning, short-term associative memory/STM, intermediate-term memory/ITM, forgetting), each of which requires conserved molecular processes. We find that chronic EtOH exposure and WD during early adulthood causes significant ITM defects and negatively impacts learning in aged worms without compromising basic sensorimotor function. Chronic EtOH also increases surface area of GLR-1 expression at timepoints corresponding to behavioral defects in young adult animals. Overall, these results suggest that 1) Adult chronic EtOH exposure and WD disrupts ITM, 2) Adult chronic EtOH and WD alters AMPA-type receptor expression, which has been previously associated with associative memory deficits, and 3) Adult chronic EtOH and WD disrupts learning across aging. Future experiments will examine the role of multiple pathways regulating GLR-1 in the observed behavioral defects following EtOH treatment, and will determine if there are windows of susceptibility to exacerbation of cognitive aging by ethanol exposure.

Brockway ET, Simon S, Drew MR (*UT Austin*) Ventral hippocampal activity differences in contextual fear and extinction recall ABSTRACT: Fear and extinction memory are separate learning experiences that are distinctly represented in the hippocampus. How these memory representations are conveyed from the hippocampus to other regions to initiate opposing behavioral responses is less clear. In these studies we investigated how activity in the ventral hippocampus contributes to fear and extinction recall. First, we used cholera toxin B subunit to retrolabel ventral hippocampal (vHPC) neurons projecting to the infralimbic cortex (IL) and basolateral amygdala (BLA) and then quantified cfos immediate early gene activity within these populations following expression of either contextual fear recall or contextual fear extinction recall. Fear recall was associated with increased c-Fos expression in vHPC projections to the BLA, whereas extinction recall was associated with increased activity in vHPC projections to IL. A control experiment confirmed that the apparent shift in projection neuron activity was associated with extinction learning rather than mere context exposure. Overall, results indicate that hippocampal contextual fear and extinction memory representations differentially activate vHPC projections to IL and BLA. Additionally, we used channelrhodopsin2 to stimulate somatostatin (SST) interneurons in vHPC during context fear retrieval and extinction. We show that SST stimulation during fear retrieval reduced freezing to the conditioning context. SST stimulation over four consecutive days of extinction showed a similar initial decrease in freezing behavior, however, both groups froze similar amounts during later days of extinction. Notably, stimulated mice froze more than the control mice during a final light-free retrieval test, showing that SST stimulation during extinction disrupted extinction learning. We additionally performed SST interneuron stimulation in the PFC in a separate group of mice. In this group we did not see any effects of stimulation during context fear or extinction retrieval, however we were able to demonstrate an inhibition of extinction learning in a cued fear conditioning paradigm. These results show a unique role for

vHPC in modulating contextual fear and extinction recall behavior through specific projection activity.

Brunswick CA, Baldwin DJ, McKenna AR, Fleischer AW, Smies CW, Brockway DF, Kwapis JL (*Penn State/ UW Milwaukee*) Impaired Neuronal Ensemble Dynamics May Underlie Age-related Deficits in Memory Updating ABSTRACT: Memories are not rigid records of experience but rather malleable entities that can be updated in response to new information. Notably, memory updating deficits are a common symptom of age-related cognitive decline and various dementias. However, the neuronal mechanisms underlying the process of memory updating are poorly understood. One proposed explanation is that, during a memory update, some proportion of the original memory engram is engaged and linked to additional cells that represent the update information (Mau et al., 2020). That is, the original training engram and the update training engram are coallocated—or comprised of overlapping neuronal ensembles. We hypothesized that during memory updating, old mice do not properly coallocate the update information with the original training information, preventing them from reconsolidating the update into long-term storage. Here, we used the Objects in Updated Locations (OUL) task to investigate memory updating and the underlying neuronal ensemble dynamics in young adult (3-m.o.) and old (18-m.o.) C57BL/6J mice. First, we show that, in a short-term test (testing 75m after updating), old mice exhibit no apparent deficits in memory updating, suggesting age-related memory updating deficits are specific to the process of reconsolidation rather than acquisition of the update. Next, using Compartment Analysis of Temporal activity by Fluorescence *In Situ* Hybridization (catFISH) for the immediate early gene Arc, we observed that old mice engage less overlapping neuronal ensembles for training and updating compared to what has previously been observed in young mice. As these results suggest old mice cannot properly coallocate the update ensemble with the training ensemble, we next tested whether experimentally increasing coallocation would restore memory updating. To do so, we trained old mice with a modified version of the OUL task in which the update session was presented shortly (2h) after the final training session, as prior work (Cai et al., 2016) has demonstrated that learning events presented close together in time are prone to being coallocated. Our preliminary results suggest that this behavioral manipulation might restore memory updating in old mice, and we are actively investigating whether this is mediated by an increase in coallocation. Future experiments will track neuronal ensemble dynamics in OUL with a viral TetTag system that will permit persistent expression of a fluorescent reporter in the original training ensemble. Additionally, we are actively investigating if increasing coallocation in old mice by engram-specific manipulations of gene expression or cell excitability can also restore memory updating.

Calderon D, Rodriguez de Souza R, Tseng C, Sandoval A, Ploski J, Thorn C, McIntyre C (*UT Dallas*) Role of the locus coeruleus in vagus nerve stimulation-induced enhancement of conditioned fear in rats ABSTRACT:Vagus nerve stimulation (VNS) is FDA approved for use in the treatment of epilepsy, depression, and stroke and it is currently being tested as an adjuvant for exposure therapy in the treatment of post-traumatic stress disorder (PTSD) in humans. VNS enhances extinction of conditioned fear and reverses extinction impairments in rat models of PTSD. However, the mechanisms of VNS enhancement of fear extinction remain largely unknown. VNS increases neuronal activity in the locus coeruleus (LC) and leads to increased levels of NE in limbic system regions involved in consolidation of fear and extinction memories. Here, we investigated whether the extinction-enhancing effects of VNS are mediated by LC activity. Three month-old male and female tyrosine hydroxylase-Cre+ Long Evans rats received bilateral intra-LC infusions of adeno-associated viral vectors containing the inhibitory opsin ArchT3.0 (AAV8-Ef1a-DIO-ArchT3.0-eYFP) or control virus (AAV8-Ef1a-DIO-eYFP). Three weeks later, a VNS cuff electrode was implanted around the left cervical vagus nerve and optic fibers were implanted directly above the LC. After a one-week recovery period, rats were subjected to two days of auditory fear conditioning followed, 24 hours later, by a pre-extinction test to quantify fear of the auditory conditioned stimulus (9 kHz tone, 30 sec). On the next day, an extinction session was given where VNS (4 x 2 sec trains, 0.8 mA, 30 Hz) overlapped with laser emission (4 x 4 sec pulses, 593 nm) during each of four exposures to the conditioned stimulus. An extinction retention test was given 24 hours later. Fourteen days later, all rats were retested for spontaneous recovery of fear. On all test days, freezing was used as a measure of conditioned fear during four presentations of the conditioned stimulus in the absence of the unconditioned stimulus. Results indicate that inhibition of the LC during VNS presentations blocked the enhancing effects of VNS on consolidation of extinction learning 14 days after extinction training paired with VNS. Interestingly, inhibition of the LC in the absence of VNS significantly enhanced fear extinction 24 hours but not 14 days after extinction training. These findings support the hypothesis that the extinction-enhancing effects of VNS are mediated by LC activity during extinction and they suggest that VNS-enhanced extinction memory and natural extinction memory depend on different mechanisms.

Campos-Cardoso R, Desa ZR, Fitzgerald BL, Cummings KA (*UA Birmingham*) Role of infralimbic cortex inhibitory neurons in the extinction of auditory fear memory ABSTRACT: The rodent medial prefrontal cortex (mPFC) plays a crucial role in fear modulation, with the prelimbic (PL) and infralimbic (IL) subregions linked to fear promotion and suppression, respectively. While the role of PL inhibitory microcircuit plasticity in fear memory

processing has been described recently, the plasticity mechanisms supporting a role for the IL in fear suppression have been less explored. Recent studies have demonstrated that both IL PV- and SST-INs are potentially involved in fear suppression post-extinction training. However, the mechanisms underlying their involvement are not fully elucidated. Moreover, how these IN populations are organized, their *in vivo* activity dynamics, and learning-related plasticity following extinction training remain unknown. By using whole-cell brain slice electrophysiology, we show that both IL PV- and SST-INs undergo layer-specific experience-dependent synaptic plasticity after auditory fear conditioning and/or extinction training. To begin to reveal the role that each of these IN classes has in shaping behavior, we performed *in vivo* optogenetic manipulations of IL PV-INs and SST-INs. We observed that the activity of IL PV-INs seems to control fear-related behaviors, where their optogenetic activation and silencing during extinction training impaired and promoted extinction memory expression on the next day, respectively. These results suggest that the dynamic activity of IL INs is required for the extinction of learned fear. Current work is focused on revealing the dynamics of in vivo SST-IN and PV-IN activity during fear memory encoding and extinction to reveal a circuit model describing their involvement.

Chalkia A, Craske MG, Beckers T (*KU Leuven/ UCLA*) Disrupting emotional associative memories through instructions to forget ABSTRACT: Empirical work spanning over 50 years has repeatedly demonstrated that declarative memory encoding is sensitive to disruption, as subjects can be cued to intentionally “forget” information before it is stored using a directed forgetting (DF) manipulation. Recently, original research from our lab has revealed that DF may also be successfully applied to interfere with the encoding of emotional associative memories acquired through fear conditioning. To that end, we developed a novel, trial-unique, fear conditioning procedure where neutral visual stimuli were displayed one at a time, and half of them were paired with a mild electric shock US (CS+), while the other half were not (CS-). An acoustic forget cue was presented after half of all the CS+ and CS- trials, indicating that those trials were to be forgotten. Skin conductance responding (SCR) was included as an index of conditioned fear responding and declarative memory retention was probed with free recall and recognition tasks. Across a series of studies, subjects recalled and recognized fewer of the items that were followed by the forget cue (referred to as the DF effect). Most importantly, subjects developed weaker conditioned fear responding (SCR) in response to CS+ items that were instructed to be forgotten. Here, I will report on a series of follow-up, control experiments, where we manipulated certain aspects of our procedure, aiming to replicate and confirm the DF effect in emotional associative memories. Further, in order to gauge possible mechanisms driving DF of emotional memories, we also investigated the influence of subjects' working memory capacity on the magnitude of the DF effect and explored the relationship between this effect and certain personality traits. Taken together, our findings provide robust evidence in favor of a disruption of the encoding of emotional associative memories by DF, yet we were unable to decisively identify the individual difference factors that may influence DF of emotional memories.

Cheng HY, Li L, Todd TP (*U Vermont*) A context-dependent cue-evoked neural code in the retrosplenial cortex ABSTRACT: The retrosplenial cortex, a dorsally located cortical structure in rats, receives inputs from sensory cortices including visual and auditory cortex, and has been implicated in a range of spatial and cognitive processes. Particularly, the role of the retrosplenial cortex in visual encoding and visually guided spatial behaviors is well-documented. In contrast, less is known about the role of the retrosplenial cortex in auditory as well as multi-sensory encoding. Here, we sought to further understand sensory processing in the retrosplenial cortex by recording single units in freely moving rats as they experienced three auditory (noise, high and low frequency tone) cues and a visual cue. These cues were presented in two operant chambers that served as distinct contexts; chambers differed with respect to odor, tactile, color and geometric cues. Consistent with the inputs retrosplenial cortex receives from both visual and auditory cortex, we observed cue-evoked responses to both visual and auditory cues. However, we did not observe any multi-sensory cued responses in retrosplenial neurons, suggesting parallel streams of information processing for visual and individual auditory cues. Surprisingly, cue-evoked responses also did not translate across contexts, indicating modulation of cue-evoked neural responses by context. Thus, our preliminary data suggests a context-dependent cue-evoked neural code in the retrosplenial cortex.

Chu A, Anzellotti S, Russell EL, McDannald MA (*Boston College*) Ventral tegmental area dopamine and orchestration of diverse fear conditioned behaviors ABSTRACT: In Pavlovian fear conditioning, a neutral cue is paired with an aversive stimulus, such as foot shock. One result of this predictive relationship is the fear-conditioned cue will elicit defensive behaviors. The most commonly measured behavior is freezing, a ‘passive’ behavior defined by the absence of movement. Recently our laboratory has shown that a fear-conditioned cue can elicit ‘active’ defensive behaviors, characterized by rearing, jumping, and increased movement (Chu et al. 2022, in progress). The goal of the present study was to examine a role for ventral tegmental area dopamine neurons in organizing these diverse fear behaviors. To do so, we deleted ventral tegmental area (VTA) dopamine neurons and tested rats in a Pavlovian fear

discrimination procedure. Subjects were 32, Th-cre rats (16 female). A Casp3 group received VTA dopamine neuron deletion via bilateral infusion of cre-dependent Caspase 3 (n=16, 8 female). A Control group received bilateral infusion of cre-dependent EYFP, leaving VTA dopamine neurons intact. Following recovery, rats received fear discrimination in a conditioned suppression setting. Rats were trained to nose poke for a food reward, then received 12 sessions in which a danger cue predicted foot shock while a safety cue did not. Poke-reward and cue-shock contingencies were independent. A TTL-triggered GiGE camera captured frames at sub-second resolution around cue presentation (5 s pre-cue, 10 s during cue, 5 s post-cue). Histological and nose poke suppression data will be presented. Ultimately, we aim to use a convolutional neural network to construct complete ethograms of cue-elicited behaviors spanning passive defensive, active defensive, and reward to reveal VTA dopamine's role in orchestrating diverse fear conditioned behaviors.

Cole KE, Parsons RG (*SUNY Stony Brook*) Sex difference in the facilitation of fear learning by prior fear conditioning ABSTRACT: There is now ample evidence that the strength and underlying mechanisms of memory formation can be drastically altered by prior experience. However, the prior work using rodent models on this topic has used only males as subjects, and as a result, there is little data testing whether or not the effects of prior experience on subsequent learning are similar in both sexes. As a first step towards addressing this shortcoming, in the experiments reported here, rats of both sexes were given auditory fear conditioning, or fear conditioning with unsignaled shocks, followed an hour or a day later by a single pairing of light and shock. Fear memory for each experience was assessed by measuring freezing behavior to the auditory cue and fear-potentiated startle to the light. Results showed that males trained with auditory fear conditioning showed facilitated learning to the subsequent visual fear conditioning session when the two training sessions were separated by one hour or one day. Females showed evidence of facilitation in rats given auditory conditioning when they were spaced by an hour but not when they were spaced by one day. Contextual fear conditioning did not support the facilitation of subsequent learning under any conditions. These results indicate that the mechanism by which prior fear conditioning facilitates subsequent learning differs between sexes, and they set the stage for mechanistic studies to understand the neurobiological basis of this sex difference. This work was supported by: NIH R21 MH121772 (to RGP) and the Stony Brook Foundation.

Crayton KL, Binette AN, Bayer H, Melissari L, Sweck SO, Maren S (*TAMU*) Chemogenetic activation of infralimbic parvalbumin interneurons impairs extinction in male and female rats ABSTRACT: Stress is a major contributor to many psychiatric disorders, particularly trauma- and anxiety-related disorders such as post-traumatic stress disorder (PTSD). Studies from our lab and others have shown that stress activates the basolateral amygdala (BLA), which in turn dampens activity in the infralimbic cortex (IL), a region of the medial prefrontal cortex (mPFC) that is critical for the reduction of learned fear (i.e., fear extinction). Although fear memory is durable and adaptive, fear extinction is easily disturbed. We propose that under high-stress conditions, the BLA has an inhibitory effect on the activity of IL principal neurons via inhibitory parvalbumin (PV) interneurons. Prior work in our lab has shown that footshock stress induces Fos expression in mPFC PV interneurons in IL. Here, we test the hypothesis that chemogenetic excitation of IL PV neurons will impair extinction. Male and female Long-Evans rats were injected with a viral vector in the IL to selectively drive the expression of an excitatory designer receptor exclusively activated by a designer drug (DREADD; AAV-S5E2-Gq-dTomato) in PV interneurons. After recovery, animals underwent a standard auditory fear conditioning procedure (5 tone-footshock trials). Twenty-four hours later, animals received systemic injections of either vehicle (VEH) or clozapine-N-oxide (CNO, 5 mg/kg, i.p.) followed by extinction training (45 tone-alone trials) in a novel context. All animals acquired extinction similarly, with an initial increase in CS-evoked freezing followed by a reduction in freezing across the session. However, CNO-treated male and female rats exhibited higher levels of freezing (normalized to baseline) compared to VEH- treated controls in an extinction retrieval test conducted 24 hours after extinction. These results demonstrate that chemogenetic excitation of IL PV interneurons impairs the encoding of long-term extinction memories in male and female rats. These data suggest that IL PV interneurons may be particularly sensitive to stress and contribute to stress-induced psychopathology and dysregulation of the mPFC.

Cummings KA (*UA Birmingham*) The mouse dorsal peduncular cortex encodes fear memory ABSTRACT: The rodent medial prefrontal cortex (mPFC) is a locus for both the promotion and suppression (e.g. extinction) of fear and is composed of four anatomically distinct subregions, including anterior cingulate 1 (Cg1), prelimbic (PL), infralimbic (IL), and the dorsal peduncular (DP) cortex. A vast majority of studies have focused on Cg1, PL, and IL. The Cg1 and PL have been implicated in the promotion of fear, while the IL has been linked to a role in the suppression, or extinction, of fear. Due to its anatomical location ventral to IL, the DP has been hypothesized to function as a fear-suppressing brain region; however, no studies have explicitly tested its role in this function or in the regulation of memory generally. Here, we provide evidence that the DP paradoxically functions as a cued fear-encoding brain region and plays little to no role in fear memory extinction. By using a combination of cFos

immunohistochemistry, whole-cell brain slice electrophysiology, fiber photometry, and activity-dependent neural tagging, we demonstrate that DP neurons exhibit learning-related plasticity, acquire cue-associated activity across learning and memory retrieval, and that DP neurons activated by learning are preferentially reactivated upon fear memory retrieval. Further, optogenetic activation and silencing of fear learning-related DP neural ensembles drives the promotion and suppression of freezing, respectively. Overall, these data suggest that the DP plays a novel and unexpected role in fear memory encoding. More broadly, our results reveal new principles of organization across the dorsoventral axis of the mPFC.

Denholtz LE, Kolaric R, Surrence K, Grunfeld IS, Likhtik E (*CUNY Hunter*) Explicit Safety Learning Engages Parvalbumin Interneurons and Neuro-Glial Interactions in the Prefrontal Cortex ABSTRACT: Stress- and anxiety-disorders such as post-traumatic stress disorder (PTSD) are characterized by fear generalization. Poor discrimination of non-threat is linked to lower medial prefrontal cortex (mPFC) volume, and white matter loss in tracts connecting the mPFC with other regions. One promising approach to mitigate excessive fear expression is safety learning, which establishes an explicit association between a cue and safety and decreases overgeneralized fear. However, the circuit-level changes exerted by safety training for this therapeutic effect are not known. We investigate whether safety training modulates neuro-glial dynamics in the mPFC, with the potential to restore mPFC communication with regions that are important for discrimination of non-threat, such as the amygdala and auditory cortex. We show that 1) male ($F(3,7)= 3.146, p<0.01$), and female ($F(3,19)=5.112$, $p<0.01$) mice learn to suppress fear during safety cues, 2) immunolabeling for cFos shows that parvalbumin-expressing interneurons (PV IN) of the prelimbic (PL) mPFC are more active during cued safety memory retrieval than during cued- or contextual- fear memory retrieval ($F(1.234, 4.937)= 31.00$, $p<0.01$), 3) Single unit recordings in the mPFC show that safety cues evoke spiking in fast-firing, putative PV INs ($p<0.05$), and 4) 3-weeks after safety learning, there is increased co-localization of satellite oligodendrocytes with non-PV cells in the mPFC ($F(2, 10) = 14.99$, $p<0.01$). We are currently assessing the effects of optogenetic inhibition of PV IN in the PL on safety learning and on neuroglial interactions. We hypothesize that PV INs engagement in the PL for safety learning, leads to myelin remodeling in the mPFC, thereby altering the dynamics of local mPFC activity and its long-range communication with crucial regions that partake in threat-safety discrimination. This work was supported by: NIH R01MH118441, PSC-CUNY, ASRC Seed Grant.

Desa ZD, Campos-Cardoso R, Fitzgerald BL, Moore A, Duhon J, Landar VA, Clem RL, Cummings KA (*UA Birmingham*) The mouse dorsal peduncular cortex encodes fear memory ABSTRACT: The rodent medial prefrontal cortex (mPFC) is composed of four anatomically distinct subregions, including anterior cingulate 1 (Cg1), prelimbic (PL), infralimbic (IL), and dorsal peduncular (DP) cortex. The mPFC has been thought to possess a functional dichotomy of dorsal fear-promoting and ventral fear-suppressing brain regions, where Cg1 and PL comprise the dorsal mPFC, and IL and DP comprise the ventral mPFC. While the IL has been linked to a role in the suppression of fear, the DP has been vastly understudied for its role in fear memory. Because the mPFC has been thought to possess a clear contrast in function between dorsal and ventral areas and because of its anatomical position ventral to IL, the DP has also been hypothesized to function as a fear-suppressing region. Yet, no studies have explicitly demonstrated its function in fear or in memory mechanisms generally. Our findings show evidence that the DP contradictorily functions as a fear-encoding brain region and does not appear to play a role in fear extinction. We employ a combination of techniques, including cFos immunohistochemistry, whole-cell brain slice electrophysiology, fiber photometry, and activity-dependent neural tagging to show that the DP exhibits cue-dependent activity during both learning and memory retrieval and to show evidence of learning-related plasticity in DP neurons. By activating and silencing fear-tagged DP neural ensembles using optogenetics, we were able to both drive promotion and suppression of fear, respectively. Overall, these data support that the DP plays a novel and unexpected role in fear memory encoding, fundamentally changing our previous understanding of the organization of the rodent mPFC.

DiFazio LE, Reis FMCV, Adhikari A (*UCLA*) Role of periaqueductal gray GABAergic cells in instrumental food seeking ABSTRACT: The periaqueductal gray supports exploratory, foraging and hunting behaviors. Specifically, the lateral and ventrolateral periaqueductal gray (l/vlPAG) have been described as downstream targets of different circuits involved in novelty exploration, aggression and hunting. Prior evidence from our lab has demonstrated that GABAergic cells in the l/vlPAG of mice encode food approach and consumption, and activation of these cells is necessary and sufficient to induce food-seeking. Given their role in food seeking, we decided to explore how l/vlPAG GABAergic cells might influence learning and motivational processes. We will optogenetically stimulate l/vlPAG during Pavlovian and Instrumental learning tasks. Mice will either learn to lever press for or associate a neutral cue with a reward. Following training, they will receive optogenetic stimulation in l/vlPAG GABAergic cells to assess if their motivation to lever press or food seek changes. We hypothesize that activation of l/vlPAG GABAergic cells will increase motivation for performing instrumental lever pressing.

Dinckol O, Zachry JE, Kutlu MG (*Rowan-Virtua*) Nucleus accumbens core single cell ensembles bidirectionally respond to experienced versus observed aversive events ABSTRACT: Empathy is the ability of adopting others' sensory and emotional states and is an evolutionary conserved trait among mammalians. In rodents, empathy manifests itself as social modulation of aversive stimuli such as acknowledging and acting on conspecifics' distress. The neuronal network underlying social transmission of information is known to overlap with the brain regions that mediate behavioral responses to aversive and rewarding stimuli. In this study, we aimed to identify single cell ensembles within the nucleus accumbens (NAc) core that respond to experienced and/or observed aversive stimuli using *in vivo* optical imaging of calcium activity via miniature scopes. Our results showed that experienced and observed aversive stimuli evoke NAc core ensemble activity that is largely positive, with a smaller subset of negative responses. The size of the NAc single cell ensemble response was greater for experienced aversive stimuli as compared to observed aversive events. Our results also revealed a subpopulation within the NAc core single cell ensembles that show a bidirectional response to experienced versus observed aversive stimuli (i.e., negative response to experienced and positive response to observed aversive stimuli). These results suggest that the NAc plays a role in differentiating somatosensory experience from social observation of aversion at a single cell level. This has important implications for psychopathologies where social information processing is maladaptive, such as autism spectrum disorders.

Domjan M (*UT Austin*) Pavlovian Sensitization ABSTRACT: Investigators typically take great care to distinguish Pavlovian conditioning from sensitization, which is appropriate in studies that focus on the development of a conditioned response as the primary outcome of conditioning. But, what about cases in which an association is learned between a CS and a US but there is no evidence of a conditioned response elicited directly by the CS? In such instances, investigators have resorted to introducing a probe stimulus during the CS to see if the CS increases reactivity to the probe stimulus. Fear potentiated startle is the most extensively investigated instance of this type of effect but analogous phenomena have been identified in appetitive conditioning with food and sexual reinforcement. The term "Pavlovian sensitization" is intended to highlight these phenomena. In Pavlovian sensitization, the focus is not on a CR directly elicited by the CS but on the state of activation that occurs when a CS is presented that increases reactivity to other cues that were not directly involved in the conditioning of the CS. Pavlovian sensitization raises interesting questions about the range of such sensitization effects, the similarities and differences between Pavlovian sensitization and more conventional conceptions of sensitization, and how our neural models of sensitization may have to be modified in light of these ideas.

Driskill C, Jalivand S, Salazar F, Khan A, Vu L, Tata S, Nuna R, Kanwal Z, Molin N, Kroener S (*UT Dallas*) Vagus nerve stimulation (VNS) alters activity in networks that regulate extinction from drug-seeking ABSTRACT: Substance use disorder is a chronic relapsing condition often marked by the inability to cease drug use despite negative outcomes. Environmental stimuli presented during drug taking can become hyper-salient cues that trigger craving and make abstaining from drugs difficult. Extinction is a learning process that can reduce the salience of these cues by creating new neutral associations. Unfortunately, extinction-based therapies have had limited success in long term prevention of relapse. Our lab has previously shown that pairing extinction training with vagus nerve stimulation (VNS) reduces drug seeking during cue-induced reinstatement. Additionally, we found changes between rats given VNS versus Sham stimulation in the expression of immediate early genes in regions that control drug seeking. The Infralimbic cortex (IL) is implicated in the consolidation and expression of extinction learning, but little is known about the networks that drive IL activity during cue-induced reinstatement and how VNS could modulate them to reduce drug seeking. Here we tested how pairing extinction learning with VNS alters expression of the immediate early gene cFos during reinstatement in networks that converge on the mPFC. We infused a GFP-expressing retrograde AAV into the infralimbic cortex (IL) to label cells that project to the mPFC. Rats self-administered cocaine for 15 days and then underwent 10 days of extinction training with VNS or sham-stimulation, followed by cue-induced reinstatement. Rats were sacrificed after reinstatement and tissue from regions associated with reinstatement (including the basolateral amygdala, paraventricular nucleus of the thalamus, and hippocampus) were stained for cFos as a marker of neuronal activity. We then quantified how VNS altered the total number of cFos-positive cells, as well as the co-localization of cFos with GFP cells that project to the IL. We hypothesize that these changes in neuronal activity in IL-projecting neurons contribute to the VNS-induced suppression of drug seeking during cued reinstatement. These results help us gain a better understanding of the mechanisms of how VNS facilitates extinction learning from drug seeking behavior.

Drupka CV, Somers MK, Bonsib AG, Totis JE, Petersen AR, Cramer EC, Williams JT, Taibl EG, Kochli DE (*Washington College*) Social Isolation Differentially Reduces Goal-Directed Behavior in Male and Female Rats ABSTRACT: Pavlovian Lever Autoshaping (PLA) is a common method for assessing individual differences in addiction vulnerability. In this procedure, brief lever

insertion predicts non-contingent delivery of a food pellet. Sign-trackers preferentially interact with the lever, while goal-trackers preferentially interact with the foodcup. Sign-trackers display a variety of "addiction-vulnerable" behaviors such as poor behavioral flexibility, while goal-trackers are sensitive to outcome value. To promote motivation during PLA, rats commonly undergo mild food restriction that requires isolation housing—stressful living conditions for social species. The present work examines the contribution of housing conditions to PLA and a Morris Water Maze (MWM) dual solution task. Male and female rats are assigned to one of three housing conditions at weaning: Enriched (four rats to a large cage with enrichment objects), Single (singly-housed shoebox cage with no enrichment), and Raised Enriched (raised under Enriched conditions, but transitioned to Single conditions at eight weeks). The Raised Enriched condition models a "typical" experiment in which rats are raised in groups but isolated seven days prior to the start of experimentation. We found that housing conditions influence PLA performance in a sex-dependent manner. Overall, enriched rats engage in more goal-directed behavior while Single and Raised Enriched rats engage in more cue-directed behavior; this pattern is more pronounced in male rats. Additionally, Enriched rats favor a flexible "place" strategy in the MWM. Results suggest that measures of individual differences in goal- vs. cue-directed behavior are highly sensitive to stressors such as housing conditions and this sensitivity is greater for males.

Felix-Ortiz AC, Terrell JM, Gonzalez C, Msengi HD, Ramos AR, Boggan MB, Lopez-Pesina SM, Magalhães G, Burgos-Robles A (*UT San Antonio*) The prelimbic and infralimbic cortical areas bidirectionally modulate safety learning during naturalistic thermal threat ABSTRACT: Optimal differentiation between threat and safety is critical for environmental fitness and survival. While animal studies have implicated the prelimbic (PL) and infralimbic (IL) subregions of the medial prefrontal cortex (mPFC) in threat and safety learning, it remains poorly understood how these cortical areas contribute to these functions during naturalistic situations involving environmental thermal signals, and how such contributions become affected during stress-induced disease-like states. We evaluated these issues using a novel semi-naturalistic model in which mice learned that specific zones within an acrylic arena were associated with either noxious cold temperatures ("threat zones") or pleasant warm temperatures ("safety zones"). Optogenetic manipulations and social isolation stress revealed that PL and IL play critical roles to differentially modulate thermal safety learning, but not thermal threat learning. Interestingly, thermal safety learning was highly susceptible to stress pre-exposure, and IL inhibition mimicked the deficits produced by stress. In contrast, PL inhibition fully rescued thermal safety learning in stressed mice. Collectively, these findings indicate that while IL activity promotes safety learning during thermal threat, PL activity impairs this function especially during stress-induced disease. A model of balanced activity between PL and IL is proposed as a fundamental mechanism for controlling safety learning during naturalistic environmental conditions.

Fernandes-Henriques C, Sclar M, Guetta Y, Miura Y, Friedman A, Likhtik E (*CUNY Hunter*) The contribution of Infralimbic-Basal Forebrain communication to fear extinction ABSTRACT: The infralimbic (IL) region of the medial prefrontal cortex is an important region for extinction learning, with activity in IL projections to the basolateral amygdala (BLA) serving an important role in suppressing defensive fear responses during extinction. However, how or whether other major outputs of the IL contribute to extinction is not well-understood. The basal forebrain (BF) is one of the major targets of IL output, is highly interconnected with the BLA, and is an important area for attention and fear learning. Thus, we investigated whether IL-BF communication contributes to extinction. Using *in vitro* patch clamp recordings, we found that IL-BF projectors become progressively less excitable as animals undergo extinction learning and recall. To better understand the patterns of communication of IL-BF-BLA during extinction, we used multisite local field potential recordings. We show that during extinction learning and recall, cue-evoked IL and BF theta power (4-8Hz) is high at the beginning of extinction learning and recall, and then decreased to pre-tone levels with training. Similar but slower cue-evoked theta dynamics were also observed in the BLA. Additionally, percent freezing in the first two trials of extinction recall correlated with evoked theta power only in IL and BF. We are currently investigating how optogenetic inhibition of IL inputs to the BF affects extinction learning and recall. Our preliminary data show that inhibiting IL->BF decreases defensive freezing. Taken together, these data open the possibility that IL activation of the BF is involved in retaining the fear association of the extinguished cue, possibly via activation of cholinergic neurons in the BF, and that this projection becomes less excitable as a function of extinction. This work was supported by NIMH R01MH118441 (EL).

Ferrara, N (*Rosalind Franklin*) Social engagement is regulated by the basomedial amygdala in adolescents and adults ABSTRACT: Social behaviors dynamically change throughout the lifespan, and age-specific social engagement during development plays an important role in neural and behavioral maturation. Adolescent age-specific social engagement is primarily comprised of play behaviors that transitions towards a greater proportion of investigation-related behaviors towards same-sex partners in adulthood. The adult basomedial amygdala (BMA) is socially sensitive, differentially responding to same- and opposite-sex partners and regulating physiological responses to social novelty. Our recent work together with prior work shows that unlike

other amygdala subregions, BMA activity mirrors social behavior following social stressor exposure. However, responsiveness to and regulation of social behavior by the BMA during developmental periods characterized by a high sociability is unclear. We use a combination of approaches to understand how the BMA regulates and responds to age-specific social behaviors in adolescents and adults. We found that BMA activity increases during and is essential for both adolescent and adult age-specific social interaction. The BMA is a major target of the ventral medial prefrontal cortex (vmPFC), and vmPFC inputs can increase adult BMA activity. Social transitions coincide with and are postulated to be guided by maturation of cortical inputs within the amygdala. The vmPFC→BMA pathway may therefore undergo maturational changes regulating transitions in social engagement. To understand how the vmPFC→BMA pathway changes in adolescents and adults, we used single unit *in vivo* recordings in anesthetized rats and found greater neuronal firing within the BMA in response to vmPFC stimulation in adolescents relative to adults. Together, these results provide insight to the maturation of BMA circuitry contributing to social development.

Fitzgerald BL, Duhon JL, Landar VA, Cummings KA (*UA Birmingham*) Analysis of brain-wide projections to the dorsal peduncular cortex ABSTRACT: The rodent medial prefrontal cortex (mPFC) is important for cognitive activity including working memory, emotions, and behaviors as they relate to environmental conditions. The dorsal areas, anterior cingulate 1 (Cg1) and prelimbic (PL) cortex, have been implicated in fear promotion while the ventral areas, infralimbic (IL) and dorsal peduncular (DP) cortex, have been implicated in fear suppression (extinction). We recently discovered that, in contrast to its hypothesized role, the DP paradoxically participates in fear memory encoding and not extinction. However, the long-range projections that may engage the DP during fear memory encoding and retrieval are largely unknown. Moreover, whether the DP and PL might be engaged by the same or distinct brain regions/cell populations to cooperatively encode fear memory is also unknown. Here, we performed circuit tracing experiments to investigate how the DP is interconnected with other fear-related brain regions. Our results generate several hypotheses regarding the engagement of DP in fear memory encoding as well as potential differences in PL and DP circuitry. Future work will test the contributions of projections to the DP in mediating the behavioral and physiological correlates of fear memory encoding in the region.

Fleischer AW, Abdelazim FH, Mitter K, Rotter J, Chaudhury S, Ford SD, Donaldson WA, Sem DS, Frick KM (*UW Milwaukee/ Marquette U/ Concordia U*) Effects of two highly selective estrogen receptor β agonists on memory and other behaviors in a mouse model of menopause ABSTRACT: The menopausal transition is accompanied by a plethora of negative mental and physical health outcomes. Memory fog, hot flashes, anxiety and depression, and weight gain are highlighted as being exceptionally disruptive to women's lives, prompting the need for more effective treatments for middle-aged women afflicted by these symptoms. Estrogen-based treatments have been effective in negating many menopausal symptoms but are also associated with heightened risks of breast cancer and other health concerns. These estrogenic side effects appear to be driven by activation of the alpha (ERα), but not beta (ERβ), estrogen receptor isoform, indicating a potential therapeutic avenue via ERβ agonism while avoiding ERα activation. Here, we aimed to test the efficacy of two novel, highly selective ERβ agonists, EGX358 and EGX854, in reducing menopause-related symptoms in a rodent model. Young, ovariectomized female mice were orally gavaged daily for 8 weeks. During this time, the following was assessed: 1) memory for object identity and spatial locations were tested via object recognition (OR) and object placement (OP) tasks, respectively, 2) hot flash-like symptoms via measurements in the change in tail skin temperature (ΔT) following injection of the tachykinin 3 receptor agonist, senktide, 3) anxiety-like behaviors in the open field (OF) and elevated plus maze (EPM) tasks, and 4) depression-like behaviors in the forced swim (FST) and tail suspension tests (TST). Furthermore, mice were weighed weekly to determine the effects of treatment on weight gain following ovariectomy. Preliminary data show that EGX358 and EGX854 treatments increased time spent with the moved object in OP , but did not enhance time spent with the novel object in OR, suggestive of greater spatial, but not object identity, memory than vehicle treatment. Neither compound seems to affect OF or EPM measures relative to vehicle treatment, indicating that these treatments did not influence anxiety-like behaviors. Importantly, neither agonist affected ovariectomy-related weight gain compared to vehicle, corroborating previous findings with EGX358. Analyses of depression-like behaviors and drug-induced hot flashes are ongoing. Nevertheless, initial findings suggest that EGX358 and EGX854 positively modulate spatial memory without altering anxiety-like behaviors or ovariectomy-induced weight gain in young female mice.

Frick KM (*UW Milwaukee*) Adventures in translational research: How studying estrogen receptors and memory led to my alter-ego in Pharma and novel compounds to alleviate memory loss and hot flashes in menopause ABSTRACT: The menopausal loss of circulating estrogens is associated with many negative symptoms, including hot flashes, cognitive decline, anxiety, and depression. Although estrogen therapy (ET) can alleviate these symptoms in many women, treatment has also been associated with increased risks of cancer and heart disease, due in part to activation of estrogen receptor alpha (ERα). Although preclinical studies

demonstrate that activation of ERα or estrogen receptor beta (ERβ) can improve memory, ERβ activation reduces cell proliferation, drug-induced hot flashes, and affective symptoms in rodents, so therapies that target ERβ may provide benefits of ET without the negative side effects associated with ER activation. Since 2015, our lab has been part of a collaborative effort to design potent and highly selective ERβ agonists to safely relieve the symptoms of menopause in women. This talk will describe our preclinical research in female mice illustrating the beneficial effects of 17β-estradiol and ERβ agonism on memory, as well as our studies evaluating the efficacy of novel selective ERβ agonists to facilitate memory formation, mitigate drug-induced hot flashes, and reduced anxiety- and depression-like behaviors in mouse models of menopause and Alzheimer's disease. This work is supported by: Supported by R01MH107886, R15GM118304, a University of Wisconsin System Regent Scholar Award, a UW Milwaukee Research Foundation (UWMRF) Bradley Catalyst Grants, and UWMR Research Growth Initiative Grant 101x240.

Gabriel DBK, Sangha S (*Indiana U School of Med*) A mixed Pavlovian/operant safety task to assess adaptive versus maladaptive avoidant responses ABSTRACT: Quick, precise discrimination between motivational stimuli is critical for maintaining healthy, adaptive patterns of behavior. Our lab has previously demonstrated that rats accurately discriminate between reward, fear, and safety cues and that fear evoked by a conditioned fear cue is downregulated in the presence of a co-occurring safety cue that signals the absence of threat. Optimal behavioral responses often involve direct, proactive interaction with threat-adjacent stimuli, which requires individuals to suppress instinctive responses such as freezing/fleeing. Active avoidance, for example, is an evolutionarily beneficial behavior characterized by deliberate, threat-mitigating efforts to prevent aversive outcomes in actively dangerous situations. Here, we built upon our lab's well-established Pavlovian safety discrimination task to measure the effect of safety cues on fear cue-evoked active avoidance. We used an operant task in which male and female Long-Evans rats were trained to 1) earn sucrose reward by pressing a reward lever, 2) prevent a mild (0.3 mA, 0.5s) footshock by pressing a separate avoidance lever, and 3) suppress active avoidance behavior in the absence of threat (signaled by co-occurring fear and safety cues). After acquiring this task, rats received 2 sequential probe sessions. In the first, both the reward and avoidance levers were extended during fear+safety and safety alone cues. Due to the lack of any shock in these trials, responding on the avoidance lever rather than the reward lever to earn sucrose indicates a maladaptive bias towards perseverative avoidant behavior, reducing rewarding outcomes. The second probe expanded this by also presenting both levers during active avoidance trials, i.e., those containing the shock-predicting fear cue by itself. In these trials, avoidance lever presses prevented foot shock and reward lever presses delivered sucrose. This measured the rat's ability to maintain distinct goal-oriented behaviors simultaneously, i.e. avoid punishers and pursue rewards. This mixed Pavlovian/operant safety task set against a background of fear and reward conditioning offers the opportunity to assess the development of persistent, maladaptive patterns of avoidant responding similar to those seen in stress disorders that can be resistant to treatment.

Garcia GM, Hassell JE, Vasudevan K, Vierkant V, Pham N, Tercilla C, Parr M, Maren S (*TAMU*) Context fear memories are not necessary for renewal of extinguished fear in rats ABSTRACT: Extinction learning is central to behavioral therapies for treatment of stressor- and trauma-related disorders including PTSD. We recently discovered that mPFC projections to the thalamic nucleus reuniens (RE), which projects strongly to the hippocampus (HPC), play a critical role in extinction learning and retrieval. After extinction, fear to the extinguished conditioned stimulus (CS) can relapse under a variety of conditions, including after an animal experiences the CS outside the extinction context—a phenomenon termed renewal. Additionally, pharmacological inactivation of the RE results in a relapse of extinguished fear after extinction. Behavioral studies suggest that renewal is not due to aversive properties of the test context, because it occurs in contexts that have never been paired with shock (e.g., ABC or ABB renewal). In experiment (Exp 1), we hypothesize that the RE promotes extinction retrieval by suppressing the retrieval of HPC-dependent fear memories. We sought to determine whether preventing the formation of hippocampal context fear memories would reduce the relapse of extinguished fear associated with RE inactivation. To further explore this, in Exp 2 we examined whether antagonism of hippocampal NMDA receptors, which prevents the formation of context fear memories, would attenuate renewal of fear in the conditioning context. Animals received an intra-HPC infusion of saline or the NMDA receptor antagonist, AP5 (10 ug/ul, 0.3 µl per side) (n = 8 per group) and immediately underwent auditory fear conditioning (Exp 1 & Exp 2). The next day rats underwent fear extinction in a novel context, this was followed by an extinction retrieval test 24 hours later. In Exp 1, prior to the retrieval test, rats received intra-RE infusions of saline or muscimol. Consistent with prior findings, inactivation of the RE increased freezing to the CS in the extinction context. Additionally, prior hippocampal NMDA receptor antagonism attenuated the RE-inactivated induced increase in freezing. In Exp 2, animals treated with AP5 exhibited a robust deficit in freezing in the conditioning context, revealing that they failed to acquire a context-shock memory. Nevertheless, these rats showed robust renewal of extinguished responding to the CS in the conditioning context. These data are consistent with the hypothesis that

RE is involved in suppressing inappropriate contextual fear memories (Exp 1) and that renewal of fear does not require contextual fear memories (Exp 2). These results, consistent with other literature, suggest that only forms of relapse that involve direct context-shock associations (e.g., reinstatement) would be attenuated by hippocampal NMDA receptor antagonism.

Garcia-Castañeda BI, Kirchner ZS, Stelly CE, Wanat MJ (*UT San Antonio*) The role of midbrain astrocytes during aversive situations ABSTRACTS: Learning to avoid aversive outcomes is an adaptive strategy to limit one's future exposure to stressful events. The first step in active avoidance learning is to actively respond during the aversive encounter, a process that is controlled by ventral tegmental area (VTA) dopamine neurons. Increasing evidence highlights learning is not solely driven by neurons but rather by astrocyte-neuron interactions. Here, we examined how VTA astrocytes regulate behavioral responding during aversive events and active avoidance learning. To address this, we use viral approaches to manipulate astrocyte function. For gain of function experiments we used a chemogenetic approach where we expressed Gq-DREADD receptors in VTA astrocytes of male and female rats. For loss of function experiments we expressed plasma membrane calcium pump (CalEx) in VTA astrocytes. We find that chemogenetic activation of VTA astrocytes promotes active responding in animals trained on an inescapable foot shock task. Gq-DREADD activation of VTA astrocytes blunted the decrease in VMS dopamine levels in response to aversive cues. In contrast, CalEx expression in VTA astrocytes suppressed active responding during inescapable foot shock stress. Preliminary results indicate that chemogenetic activation at a mid-point during a training does not affect active avoidance learning. However, CalEx expression in VTA astrocytes suppresses active avoidance learning. Collectively, these data suggest VTA astrocytes may potently regulate active avoidance learning during early training sessions.

Giovanniello J, Paredes N, Weiner A, Oregwam C, Uwadia H, Nnamdi G, Seghal M, Reis FMCV, Sias AC, Malvaez M, Adhikari A, Silva A, Wassum KM (*UCLA*) Opposing amygdala-striatal pathways enable chronic stress to promote habit formation ABSTRACT: When making decisions, we prospectively evaluate our potential actions and their predicted outcomes to choose an appropriate behavior. This goal-directed strategy allows us to adapt when situations change but it is cognitively taxing. Habits are a more efficient but less flexible strategy whereby routine behaviors are executed without forethought of their consequences, based on past success. Balance between these strategies allows behavior to be adaptive when needed, but efficient when appropriate. Overreliance on habit is characteristic of many psychiatric conditions, including addictions. Stress is a major contributing factor to these conditions. It also promotes habits. Thus, we sought to uncover the neuronal circuits that permit stress to promote habits. We first established a model of stress-potentiated habit formation in mice using chronic mild unpredictable stress and subsequent lever press – food reward instrumental conditioning. Mice with a history of chronic stress formed habits prematurely, as evidenced by insensitivity to outcome devaluation or omission contingency. The dorsomedial striatum (DMS) mediates goal-directed behavior and the amygdala is a stress hub. Therefore, we next used a multifaceted approach to expose the contribution of amygdala-striatal projections to goal-directed learning and stress-potentiated habit formation. The basolateral amygdala (BLA) sends a direct excitatory projection to the DMS. We found that these neurons are activated during instrumental learning and that this activity is critical for the action-outcome learning that supports goal-directed behavioral control. Stress dampens this to disrupt action-outcome learning and promote habit formation. Activating this pathway during post-stress learning restores normal goal-directed behavioral control. A direct central amygdala (CeA) projection to the dorsal striatum was recently identified. We found that CeA neurons target DMS and inhibit striatal projection neurons. CeA-DMS projections are not typically robustly activated during goal-directed learning, but stress recruits activity in this pathway to promote habit formation. Inhibition of the CeA-DMS pathway during post-stress learning prevents habit formation. This establishes the first functional role for the CeA-DMS pathway in modulating behavior. Together, these data reveal that stress disrupts the balance between two opposing amygdala-striatal projections to prevent the learning that underlies adaptive decision making and promote the formation of premature inflexible habits. This work was supported by: NIH R01DA046679 (KW), NIH T32DA024635 (JG), NIH F32DA056201 (JG), A.P. Giannini Fellowship (JG), and NIH TL4GM118977 (NP).

Gonzalez SE, Fukunaga Y, Watts M, Locke E, Schulman E, Cazares VA (*Williams*) Investigating the neuromodulatory role of dopamine in strengthening fear extinction memories formed in multiple contexts ABSTRACT: Novelty has been shown to fortify learning and memory due to its ability to promote hippocampal reactivation and plasticity, yet specifically hinders recall of fear extinction memories. Fear extinction is context-specific; thus if a (extinguished) conditioned stimulus (CS), such as a tone, is experienced in a novel context where it had not been extinguished, it will re-elicit the fear response, a phenomenon termed fear renewal. However, we've found that fear extinction conducted in serial novel environments actually strengthens the fear extinction memory by overcoming fear renewal and preventing spontaneous recovery of fear at recent (48 hours) and remote (30 days) timepoints post-extinction. To elucidate the neurobiological mechanism that makes fear

extinction memories more resilient via multiple context fear extinction (MCFE), we chemogenetically inhibited hippocampal neurons and found that performance of mice that underwent MCFE was impaired, but not of those that underwent single context fear extinction (SCFE). This suggests that hippocampal involvement is necessary for MCFE to be successful. Due to the role that dopamine (DA) release in the hippocampus plays in consolidation and long-term plasticity, we hypothesize that DA mediates the effects of MCFE. To test this, we enhanced dopamine transmission during fear extinction memory acquisition or consolidation by administering (20 mg/kg, ip) of L-dopa 30 minutes prior to or immediately after day 2 of extinction training – when MCFE groups are first exposed to a novel extinction context. We found that L-dopa enhanced the effects of MCFE, but not SCFE, during memory acquisition, and that it had no effect on either group during memory consolidation. We also tested our hypothesis by administering the D1/D5 receptor antagonist SCH23390 (0.1 mg/kg, ip) during the same acquisition stage, with preliminary data suggesting that inhibiting dopamine transmission leads to fear renewal in both the MCFE and SCFE groups. Overall, our data indicate that MCFE supports stronger, longer-lasting extinction memories and provides preliminary support to the idea that context generalizable extinction is mediated by the hippocampus and DA neuromodulation. This work is supported by: NIH R15-MH129947.

Goodpaster CM, Klune CB, Gongwer M, Jones N, DeNardo LA (*UCLA*) Increased basolateral amygdala activity linked to enhanced learning in adolescent mice exposed to early life adversity ABSTRACT: Early life adversity (ELA), such as neglect or maltreatment in childhood, is a major risk factor for the development of psychiatric disorders later in life. ELA heightens responses to threats, usually at the expense of rewarding, goal-directed behaviors. Excessive threat avoidance is a hallmark of anxiety, phobias, and depression. Circuits that process both rewards and threats, including the basolateral amygdala (BLA), are known to be dysregulated by ELA and have been implicated in the pathophysiology of these conditions. Symptoms often manifest during adolescence, when interactions with the environment have significant influence over brain development and the refinement of adaptive behavioral strategies. Yet most studies focus on adult outcomes following ELA. As a result, the biological mechanisms connecting ELA to the development of threat sensitization remain largely unknown. To investigate this, I employ limited nesting and bedding (LBN), a model of resource scarcity, to investigate how ELA impacts threat avoidance behavior and BLA activity in adolescent mice. To assess differences in threat encoding and retrieval I use a platform-mediated avoidance (PMA) assay, during which a fear conditioned tone prompts mice to navigate to a safety platform. While both standard reared (SR) controls and LBN mice learn to successfully avoid foot shocks during training, LBN mice display elevated avoidance during retrieval. Using fiber photometry to record bulk activity in the BLA, I found that during training the LBN mice have heightened and prolonged activity during and after foot shocks. During retrieval the following day, LBN mice have elevated cue-evoked activity in the BLA when the threat requires action (i.e. when the animals are off the safety platform). Additionally, BLA activity is increased in LBN animals when they exit the safety platform compared to SR mice. These data indicate that in mice with a history of ELA, elevated BLA responses to aversive stimuli drive stronger associative learning and enhance avoidance of threatening cues in adolescence. Thus far, most ELA research has focused on behaviors and cellular-level outcomes in adults. Here we are uncovering new insights into how early experiences impact developmental circuit dynamics that are necessary for refined avoidance behaviors.

Greiner EM, Adel-Zaheh B, Laine MA, Ravaglia I, Shansky RM (*Northeastern U*) Distinct behavioral & neural profiles for pregnant and unmated female rats during fear conditioning ABSTRACT: Women who experience anxiety and stress-related disorders during pregnancy are at an increased risk of experiencing adverse birth outcomes and postpartum psychopathology. Despite this, fear conditioning research has largely overlooked pregnant animal models. To address this gap, we investigated whether pregnancy impacts fear behaviors and cortical neural activity in response to a conditioned tone. Rats at different stages of pregnancy—day 8 and day 17—alongside unmated females, underwent a singular fear conditioning session involving 7 tone-shock pairings. Following each tone presentation, we measured time spent freezing and the number of darts, defined as a quick movement across a fear conditioning chamber, for each animal. We additionally examined ultrasonic vocalizations, particularly “alarm calls”, distinct calls in the 22 kHz range that signal threat, and “shock calls”, calls emitted during shock presentation. Unmated females froze more than both pregnant female groups throughout conditioning and showed a higher proportion of animals displaying darting behavior. For ultrasonic vocalizations, while there was no difference in the number of alarm calls, there was a difference in the number of animals that engaged in alarm calling within each group. Only half of the unmated females made alarm calls, compared to 88% females on day 8 of pregnancy and 72% of females on day 17. Additionally, females on day 8 of pregnancy made significantly more shock calls on average than unmated females. On a subsequent day, rats underwent a recall test, where the tone was presented three times in the absence of shock and were perfused 90 minutes later for their brains to be immunohistochemically processed for c-fos. We focused our analysis on the anterior cingulate (ACC), prelimbic (PL), and infralimbic cortices (IL).

During the recall test, unmated females froze more than both pregnant groups, with females on day 8 of pregnancy freezing the least. Our neural analysis revealed that unmated females had greater Fos expression in the ACC and IL than both pregnant groups and greater Fos expression in the PL than rats on day 17 of pregnancy. Overall, our findings underscore the emergence of distinct behavioral fear responses during pregnancy, with these patterns evolving across gestational stages. This work was funded by NIH R01MH123803-2023 to Rebecca Shansky.

Guerra DP, Benesch J, Gorman JC, Ho DT, Karam YE, Zhang A, Moscarello JM (*TAMU*) Sex-specific role for the bed nucleus of the stria terminalis in avoidant behavior ABSTRACT: Although avoidant behavior is a unifying symptom of many anxiety disorders, the neural basis of avoidance has yet to be fully characterized. Signaled active avoidance (SAA), an associative learning paradigm, models avoidant behavior in rats. This learning paradigm is composed of at least two phases: 1) The acquisition of an association between a tone (conditioned stimulus) and a shock (unconditioned stimulus), and 2) the performance of an avoidance response during the tone to prevent shock delivery. These distinct phases allow for a shift in behavior that is akin to a shift of fear to anxiety. The bed nucleus of the stria terminalis (BNST) is a brain area that has been implicated in anxiety disorders in humans and uncertain threats in rodent models. However, its role in SAA is yet to be elucidated. Here, we report on a series of experiments that used a chemogenetic (DREADD) approach to explore the role of the BNST in the SAA procedure in both male and female rats. First, we inactivated the BNST with an inhibitory hM4Di DREADD to test the hypothesis that the BNST is necessary for the expression of the avoidance response. Animals received four days of SAA training to establish the avoidance response. Then, to test the role of the BNST, animals received two additional days of SAA training preceded by counterbalanced intraperitoneal (IP) injections of either the DREADD ligand CNO or vehicle. In males, CNO decreased avoidance responses in hM4Di subjects but not in GFP controls, demonstrating that the BNST is necessary for the avoidance response. However, there was no effect of CNO on hM4Di- and GFP-expressing females. Thus, the BNST is necessary for avoidance in male but not female rats. An ongoing follow-up experiment uses cfos expression induced by a master-yoke variant of the SAA paradigm to compare neural activity in male and female masters (subjects allowed to acquire avoidance) to yoked controls (subjects that have a stimulus history identical to masters but that are not allowed to acquire the response). This design will allow us to confirm the hypothesis that the BNST is recruited to avoidant behavior in males but not females, while also allowing us to elucidate the distinct neural substrates of avoidance in female subjects.

Hagen CW, Brice KN, Braden-Kuhle PN, Papini MR (*Texas Christian U*) Acute inflammation disrupts behavior in a Pavlovian, but not a consummatory, model of reward loss in rats ABSTRACT: Frustration, an emotional reaction arising from missing or devalued rewards, induces various behavioral and biological changes in mammals including inhibiting approach to the reward location, switching to search behavior, activation of the sympathetic nervous system and HPA axis and subsequent release of stress hormones. For animals, this response may be evolutionarily advantageous because it helps break the emotional attachment with a specific location or environment that is no longer providing adequate reward so that the animal can move on to forage elsewhere. Two reward loss paradigms with rats involve (1) direct consumption of various concentrations of sucrose (consummatory successive negative contrast, cSNC) and (2) Pavlovian associations between lever presentations and food pellet delivery (Pavlovian successive negative contrast; pSNC). Both paradigms involve reward loss through training with a high value reward (32% sucrose or 12 pellets) and then unexpectedly downshifting to a lower value reward (2% sucrose or 2 pellets). The goal of these studies was to investigate whether disruption of neuronal signaling via acute inflammation affects these two paradigms of reward loss. Peripheral injections of lipopolysaccharide (LPS), which induces the release of inflammatory signaling molecules, before key episodes of reward loss were shown to eliminate signs of frustration in pSNC, but not in cSNC. We also determined that the same LPS regime induced cytokine (TNF-alpha) expression preferentially in the dorsal hippocampus. One hypothesis is that the dorsal region of the hippocampus plays a larger role in regulating Pavlovian/instrumental behavior (e.g., lever pressing) during a reward loss episode than influencing consummatory behavior (e.g., licking).

Halcomb CJ, Vanderhoof SO, Mott DD, Jasnow AM (*UofSC School of Med*) An amygdala-cortical circuit for encoding generalized fear memories ABSTRACT: A common symptom of anxiety disorders and trauma- and stress-related disorders is overgeneralizing fear. This is characterized by the expression of fear in new or ambiguous environments and can disrupt normative functioning. The anterior cingulate cortex (ACC) is a critical node in a larger circuit that regulates fear and adaptive generalization to contextual cues. Our work demonstrated that the ACC and its projections to the basolateral amygdala (BLA) control generalized fear when rodents experience new contexts in which threat is uncertain. However, the role of the ACC in context fear learning is not well understood. In this study, we used neuron activity analysis, pharmacological inactivation, NMDA receptor blockade, and circuit-specific manipulations of the ACC to determine its role in context fear learning. We found that pharmacological inactivation of the ACC and NMDA receptor blockade during context fear

learning eliminates generalized fear when mice are tested in a novel context but do not alter specific fear to the training context. We then investigated the role of BLA inputs to the ACC during fear learning to determine circuit mechanisms involved in encoding generalized fear. We used a chemogenetic, intersectional approach to inactivate BLA-to-ACC projections during context fear learning. We used a similar chemogenetic approach to drive this circuit during weak fear training. Our data show that chemogenetic silencing of BLA-to-ACC projections during context fear learning eliminates fear in a novel context but leaves specific fear in the training context fully intact. Further, we found that driving the BLA-to-ACC inputs was sufficient to produce fear generalization under conditions that normally produce specific fear. These results suggest 1) a critical role for NMDAR-dependent plasticity in the ACC in encoding generalized memories and 2) that a BLA-ACC circuit is both necessary and sufficient for learning generalized fear. Thus, the role of the ACC may be to encode highly salient experiences that shape subsequent behavior in new similar environments or situations. This work was supported by: 180800-23-62693 to CJH, R15MH118705 to AMJ, R01MH131808 to AMJ & DDM.

Hasenhundl VT, Qureshi OA, Gostolupce D, Lozzi M, Williams T, Iordanova MD (*Concordia U*) The role of the orbitofrontal cortex in learning about associatively evoked stimuli ABSTRACT: A first order cue (S1) that has become associated with a fearful outcome can link this fear to a secondary cue (S2), regardless of whether the two innocuous cues (S2 and S1) were associated before or after the initial presentation of the fearful outcome. In sensory preconditioning, the innocuous stimuli become associated beforehand (Experiment 1). In second order conditioning, the innocuous stimuli become linked after the fearful outcome is experienced (Experiment 2). Here, we sought to investigate the role of the orbitofrontal cortex (OFC) in these procedures by inactivating the region during the session where the first order cue is paired with the fearful outcome (S1+). We show that the OFC is required at the time of S1+ pairings in order to achieve sensory preconditioning (Experiment 3) but not second order conditioning (Experiment 4). Additionally, this inactivation did not affect fear to S1 at test in either experiment. Further, we sought to investigate whether the OFC is required during S2-S1 pairings in second order conditioning (Experiment 5). We show that inactivation of the OFC impaired responding to S2 at test in the second order conditioning protocol. Taken together, these findings reveal that the OFC is essential in endowing stimuli with value as new information becomes available from the environment.

Hassell JE, Parr MA, Perez MA, Tercilla C, Maren S (*TAMU*) Does the bed nucleus of the stria terminalis mediate circuit-induced relapse of extinguished fear? ABSTRACT: Extinction learning is central to behavioral therapies for treatment of stressor- and trauma-related disorders including PTSD. We have recently discovered that the hippocampus (HPC) plays a critical role in the relapse of extinguished fear that occurs after pharmacological inactivation of the thalamic nucleus reuniens (RE) in rats. In particular, the HPC appears to encode contextual fear memories that are important for the “circuit-induced” relapse that occurs after RE inactivation. How these contextual memories drive increases in conditioned freezing behavior is unclear. However, there are abundant projections from the HPC to the bed nucleus of the stria terminalis (BNST) that may underlie relapse-induced freezing. Because the BNST has a prominent role in the expression of contextual fear memories, we hypothesize that it plays an important role in the circuit-induced relapse. To test this idea, we explored whether pharmacological inactivation of the BNST would reduce the relapse of extinguished fear associated with RE inactivation. Adult male and female Long-Evans rats first underwent auditory fear conditioning followed twenty-four hours later by extinction. The next day, animals received intra-BNST infusions of either vehicle (saline) or the AMPA receptor antagonist, NBQX (10 ug/ul, 0.3 µl per side) (n = 6-8 per group) and intra-RE infusions of either vehicle (saline) or muscimol in a factorial design. Consistent with prior findings, RE inactivation caused a relapse of fear and increased freezing to the extinguished CS in the extinction context. However, preliminary data suggest that concurrent BNST inactivation does not attenuate this circuit-induced relapse. Further work will explore alternate neural pathways by which RE inactivation drives relapse-induced increases in freezing behavior.

Hayden AN, Brandel K, Merlau P, Pietryk E, Arey RN (*Baylor College of Med*) CEY-1/YBX RNA binding protein dysfunction causes impairments in memory and cognition ABSTRACT: Cognitive processes, such as learning and memory, require the precise control of mRNA translation and new protein synthesis. One class of proteins that regulates mRNA translation are RNA binding proteins, the dysfunction of which are becoming increasingly associated with neurological disorders. However, despite their biological significance, many RNA binding proteins expressed within the nervous system remain uncharacterized. We have identified a family of conserved RNA binding proteins, the CEY-1/YBX RNA binding proteins, that are broadly expressed in the nervous systems of both worms and humans and have uncovered a previously unappreciated role of these proteins in memory and cognitive function. First, we identified CEY-1 as the primary *C. elegans* ortholog to the mammalian YBX’s. We found that CEY-1 is required for memory, as both truncated and full loss of CEY-1 cause learning and memory deficits in positive associative olfactory assays. To identify if this was due to a tissue-autonomous role of CEY-1 in the adult nervous system, we next tested whether adult-only, neuron-specific knockdown of CEY-1 using RNAi

decreased associative memory. Loss of CEY-1 specifically in the adult nervous system decreased both short-term and intermediate-term associative memory. To test whether CEY-1 is sufficient to increase memory, we overexpressed a single copy of CEY-1 specifically in neurons. Overexpression of neuronal CEY-1 enhanced short term and intermediate term associative memory, suggesting that CEY-1 is sufficient to increase memory. To determine the importance of mammalian YBX RNA binding proteins for cognition, we examined whether human variants in YBX1, YBX2, and YBX3 are associated with neurological deficits. Using a combination of publicly available and institute-specific human variant datasets, we were surprised to discover over 80% of patients with single nucleotide variants in any YBX RNA binding protein have severe neurological symptoms. Importantly, the most common symptom is intellectual disability, mirroring our findings in *C. elegans*. In summary, we have uncovered a new conserved role for the CEY-1/YBX RNA-binding proteins in memory and cognition. In ongoing work, we are investigating which biological pathways are altered when CEY-1/YBX function is disrupted as well as the mechanisms of specific human variants using humanized *C. elegans*. Overall, our studies suggest that mechanisms in *C. elegans* can inform the molecular underpinnings of YBX-related neurological symptoms in human patients and underscore the importance of neuronal RNA binding proteins in cognition.

He W, Parsons RG (*SUNY Stony Brook*) Novel context induced activation of the claustrum in rats ABSTRACT: The claustrum (CLA) is a subcortical structure between the insular cortex and striatum. It has been found to participate in a variety of fundamental brain functions including processing sensory information, attention, sleep, and memory. Prior work from our lab showed that the claustrum was activated during the expression of auditory fear in rats. However, this study was not able to discern whether the activation was related to the expression of auditory fear or exposure to the novel context in which testing occurred. Here, we examined freezing behavior and cFos expression of claustrum in male and female rats using Pavlovian fear conditioning. In Experiment 1, male rats were conditioned in Context A and received 2 tone-shock pairings. On testing day, both groups were exposed to Context B (novel context), while one group of animals received 4 presentations of the tone and the other were only exposed to the context. We found that the tone-exposed group froze more than the novel group during testing, yet there was no significant difference between groups in cFos expression; and both groups showed elevated cFos expression in CLA relative to naïve rats. In Experiment 2, male and female rats were conditioned in Context A using the same parameters as Experiment 1. One group of rats (familiar) was exposed to Context B at 24 hours and again at 48 hours, while the other group (novelty) received a single context exposure at 48 hours. We found that rats in the novelty group showed significantly higher cFos expression in CLA than rats in the familiar group and that this effect did not differ between sexes. Our data indicate that the claustrum is activated by exposure to a novel environment, but not by the expression of fear. Ongoing experiments will determine if the CLA is necessary for attentional and mnemonic processes supporting fear learning.

Hoang IB, Taira M, Wikenheiser AM, Sharpe MJ (*UCLA*) Unexpected rewards are signaled by dopamine release into the lateral hypothalamus ABSTRACT: We have previously revealed that the lateral hypothalamus (LH) is critical for learning about food-paired cues (Sharpe et al., 2017, Current Biology), which is facilitated by dopaminergic input from the ventral tegmental area (VTA; Hoang et al., 2023, bioRxiv). However, how dopaminergic activity in this region responds to rewarding events is unknown. Using fiber photometry, we measured dopamine release in the LH of freely-moving rats with the dopamine biosensor, GRABDA, during receipt of reward. These data show that dopamine release in the LH increases in response to unexpected rewards and is sustained across reward consumption before returning to baseline activity. Given the importance of dopamine for associative learning and evidence from our lab showing this process takes place in the LH, we are now investigating how the dopamine response in LH changes during learning about reward-paired cues, with a view to eventually examine the impact of drug exposure (i.e., methamphetamine) on dopamine release during learning. This line of work is clinically relevant for psychopathologies such as substance use disorder as it could provide insight into how dopaminergic activity is disrupted with a history of drug abuse to produce aberrant reward learning in individuals with the disorder. This work was supported by: NSF CAREER 243910 (awarded to MJS).

Iyer ES, Muir J, Bagot RC (*McGill*) Nucleus accumbens glutamatergic afferents integrate outcomes across time ABSTRACT: The ability to integrate information about reward over time is essential to adaptive behavior in dynamic environments. While the role of dopaminergic inputs to the nucleus accumbens (NAc) is widely studied, there is increasing evidence that accumbens glutamatergic afferents also contribute to processing of reward-associated information. Using *in vivo* fiber photometry, we simultaneously recorded population-level activity of medial prefrontal cortex (mPFC) and ventral hippocampus (vHIP) projections to NAc in male and female adult mice performing a two-armed bandit task. We find that mPFC-NAc and vHIP-NAc track the recent history of reward with subtle pathway-specific differences. While mPFC-NAc consistently encodes outcome, vHIP-NAc preferentially encodes surprising reward, with outcome no longer encoded after repeated reward. To determine the precise information encoded in these neural signals, we

manipulated task design to systematically degrade behavioral requirements. This revealed that outcome history is tracked preferentially when linking a behavioral response with an outcome. However, when action-outcome pairing is not relevant, mPFC-NAc ceases to encode outcome history, preferentially encoding only immediate outcomes while vHIP-NAc fails to encode any outcome information. Together, these findings establish that mPFC-NAc and vHIP-NAc integrate outcomes over time when these outcomes are contingent upon behavior and reveal a neural mechanism for the propagation of reward-associated information over time.

Jin B, DeNardo LA *(UCLA)* Brain-wide mapping of fear memory circuits throughout development ABSTRACT: Infantile amnesia describes a process wherein episodic memories formed early in life are rapidly forgotten. On the other hand, memories formed in older animals can last a lifetime. Although the neural substrates of memory have been well studied in adults, how memories are stored and retrieved during development remains poorly understood. Here, we performed a brain-wide screen to identify developmental changes in memory networks. We used TRAP2 mice (Targeted Recombination in Active Populations) in combination with brain clearing and light sheet fluorescence microscopy to compare neuronal populations and networks activated by recent (1 day) fear memory retrieval at infant (P17), juvenile (P25) or adult (P60) stages. While adults had more activated neurons in the prefrontal cortex, and anterior thalamic nuclei, juveniles had more activated cells in the hypothalamus and midbrain during fear memory retrieval. Network analyses revealed that the functional organization of memory networks was also developmentally regulated. Adult memory networks included a highly interconnected set of cortical regions. Of these, the retrosplenial cortex (RSP), a key memory center, was absent from juvenile networks. To further understand how activity in these regions related to memory retrieval, we examined correlations between TRAPed cell numbers and freezing levels of individual animals. Regions that were highly correlated with freezing differed by age. Notably, RSP became increasingly salient for freezing with developmental age. To further examine how RSP memory functions change with age, we looked at the extent to which neurons activated during recent memory retrieval (1d) are reactivated during later memory retrieval (7d) at different ages. Adults had higher reactivation rates in RSP compared to younger groups. To determine whether we could enhance memory retrieval, we injected cre-dependent excitatory DREADDs to label and manipulate TRAPed RSC ensembles between 1d and 7d retrieval sessions. We found an age-dependent effect: chemogenetic reactivation increased freezing in adults, but not in infants, and had an intermediate effect in P25 mice. Together these data reveal specific changes in the activity of brain regions that coincide with the developmental transition from amnesic to persistent memories. RSP, which has not been studied in the context of infantile amnesia, may be key for this switch. In ongoing work, we are tracing developing RSC connections to identify circuit changes that could underlie RSP's role in the maturation of memory persistence across development.

Johnston MP, Garcia-Castaneda BI, Vargas V, Alexandre KF, Patel SK, Wanat MJ (*UT San Antonio*) Estrous-dependent effects of single and repeated stress on Pavlovian conditioning and dopamine release ABSTRACT: Stress can produce changes in physiology and behavior which endure well after exposure to stress has ended. Human studies demonstrate that stress facilitates responding to reward-predictive cues, as well as producing deficits in flexible responding. Our lab has demonstrated that a single stressful experience can enhance Pavlovian conditioned responding to rewards in male rats. This stress-enhanced conditioned responding is dependent on an increase in reward-evoked dopamine release in the ventral lateral striatum (VLS), but not the ventral medial striatum (VMS). However, there are sex differences in the effect of stress on behavior. This project examines how stress impacts conditioned responding in females. To address this, female rats were exposed to either single or repeated stress prior to Pavlovian conditioning. Preliminary findings indicate that stress enhances conditioned responding when administered during metestrus/diestrus. Interestingly, stress does not have an effect on conditioned responding when administered during proestrus/estrus. We additionally found that female rats exposed to repeated stress exhibited deficits in updating behavioral responding when the cue-reward contingency was changed. Ongoing fiber photometry experiments are examining how stress impacts dopamine release in the VMS and VLS of females undergoing Pavlovian conditioning. Collectively, these results suggest that cycling sex steroids may be acting on the dopamine system to regulate the behavioral effects of stress. This work was supported by National Institutes of Health grants MH127466 and DA051014 and the NSF Graduate Research Fellowship Program (Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF).

Kaplan K, Toennies L, Hunsberger H (*Rosalind Franklin*) Alprazolam impairs fear memory and alters dorsoventral CA1 neuronal ensembles in female mice ABSTRACT: Benzodiazepines (BZDs), are commonly prescribed anxiolytic drugs that act on GABAa receptors, and can result in anterograde amnesia, or the inability to form new memories. Although BZDs have been in use for over 60 years, the brain regions and neuronal mechanisms responsible for this detrimental side effect are largely unknown. Continued use of BZDs could potentially lead to memory impairment and cognitive decline later in life. To analyze the effects of BZDs on long term memory,

ArcCreERT2 x eYFP mice were injected with Alprazolam 30 minutes prior to a 3-shock contextual fear conditioning (CFC) procedure. This novel ArcCreERT2 x eYFP mouse model allows us to tag neuronal ensembles that are active during an experience or memory by using Arc as an immediate early gene (IEG) promotor. We then re-exposed mice to the same context 5 days later and euthanized mice 1 hr after exposure to examine retrieval cell activation using a second IEG, cFOS. This method allows us to determine which brain regions undergo changes after BZD injection and how these cellular changes relate to freezing behavior (a proxy for memory). Additionally, we address the question of whether BZDs induce state-dependent memory by altering the timelines of injection to include drug administration in both timepoints or a drug-vehicle administration to circumvent the sedative properties of BZDs. We also analyzed how BZDs affect the initial consolidation of a fear memory by injecting mice immediately after the CFC training. We found that 1) BZD-treated female mice exhibit a decrease in memory retention, 2) BZD-treated female and male mice show a decrease in memory retention with saline injection prior to re-exposure, and 3) BZD injection immediately after CFC training enhances memory in male mice. Cell counts revealed that BZD-treated female mice exhibit increased EYFP+ (encoding) and cFos+ (retrieval) activation in the dCA1 compared to controls. However, the opposite pattern was observed in the vCA1, where BZD-treated females showed less EYFP+ and cFos+ activation. These results suggest that ventral hippocampal activity is dampened with BZD injection, but the dorsal hippocampus is still actively encoding contextual information. We are further analyzing cell counts in other hippocampal and amygdala regions. Completion of this project will help us to better understand the long-term memory deficits associated with BZDs. We also have the potential to discover novel brain regions involved in these side effects and therefore offer alternative treatment options. Our future studies will examine the impact of chronic use of BZDs on aging and Alzheimer's disease.

Kim J, Bade I, Brenneman E, Erdley B, Hougham A, Keiss P, Leder J, Micek L, Miler J, Powell K, Vicknair E, Wang C, Pickens C (*Kansas State U*) Extremely high adolescent consumption of a sucrose solution, but not high consumption of an alcohol-sucrose cocktail, impairs devaluation in rats ABSTRACT: Many previous studies have shown that exposure to psychostimulants can lead to impaired devaluation in laboratory animals, but there is far less evidence for impairment after alcohol exposure. Previous research from our laboratory suggested that orally consumed alcohol did not impair devaluation (tested after an abstinence period) in a task where responses on levers in spatially separate locations with distinct cuelights above each lever earned different food reinforcers. However, consumption in this task was low and it seemed possible that higher levels of alcohol might lead to impaired devaluation. In addition, we subsequently found evidence that rats in this task might compensate for dysfunction of one brain area needed for devaluation by switching between a cuelight-based and lever-location-based strategy Specifically, we found that prelimbic cortex lesions did not impair devaluation if the lever-location and cuelight information was congruent between training and testing, but did impair devaluation if the lever-location and cuelight information was incongruent. Here, we examined whether higher levels of alcohol consumption and/or testing in a test that limits compensation between strategies would reveal impairments after alcohol consumption. In order to determine the long-term effects of voluntary adolescent access to alcohol on devaluation (with higher levels of exposure due to including sugar in the alcohol solution), we gave male and female rats access to an alcohol-sugar mixture (20% alcohol, 10% sugar, and 70% water), a sugar solution (10% sugar and 90% water), or 100% water group for 24-h three days a week for six weeks during postnatal day 26-66 (±1 day). After six weeks of exposure to alcohol-sugar, sugar, or water, we trained the rats that responses to two different cuelight-lever compounds would earn two different foods. Rats were then sated with 30g of food of one of the food before a choice test where both levers were available but the cuelight locations were incongruent to those during training. We then retrained the rats on both lever-cuelight-food associations and then tested after satiation to the other food. We found very high consumption of the alcohol-sugar solution (compared with our previous research) but even higher consumption of the solution with sugar alone. We also found a weak devaluation effect in the water group, a significant devaluation effect in the alcohol+sugar group, and a weak reversal of the devaluation effect in the sugar alone group. When we compared difference scores of responses on the nondevalued lever minus the devalued lever, we found that the difference scores in the sugar group differed significantly from those in the water and alcohol+sugar groups. Our results provide no support for alcohol exposure impairing devaluation even at elevated consumption levels. During my poster, I will also discuss possible reasons for the effect of sugar consumption on devaluation, including the possibility that rats consumed sugar exclusively for their calories (and ate little or no rat chow) on sugar access days and experienced some nutritional deficits as a result. This work was supported by: a K-State Arts and Sciences Undergraduate Research Awards to Jihyeon Kim, and NIGMS Grant P20GM113109.

Kim J, Waren O, Leder J, Bade I, Pickens C (*Kansas State U*) Behavioral training in a nicotine-paired environment impairs devaluation in male rats ABSTRACT: Prior exposure to drugs of abuse is often associated with impairments in devaluation in human drug users and laboratory animals, but the nature and reliability of this relationship is unclear. In laboratory animals, there are numerous demonstrations that past exposure to drugs of

abuse can lead to devaluation impairments weeks after the final drug exposure, with the majority of these demonstrations examining effects of exposure to psychostimulants such as cocaine, amphetamine, and methamphetamine. However, there has been minimal investigation into whether exposure to nicotine can also impair devaluation. In addition, much of the previous research on effects of prior drug exposure has found impairments in devaluation in tasks that are predisposed to lead to habitual behavior and there is less evidence for impairments in multi-response/multi-reinforcer devaluation tasks (which are less likely to lead to habit formation). One exception to this pattern is that testing in a chamber previously paired with drug exposure can lead to devaluation impairments in operant tasks that do not predispose rats towards habit formation. We examined whether repeated exposure to a low dose of nicotine (0.21 mg/kg, s.c.) would impair later devaluation if rats were trained in the nicotine-paired context or in a context non-associated with nicotine. One group (Conditioning Group) received injections of 0.21 mg/kg nicotine before placement in the operant chambers for 20 minutes, and saline injections before placement in an alternative chamber (an igloo cooler or beddingless brown cage) for 20 minutes. A second group (Neurotoxicity Group) received saline injections before placement in the operant chambers and nicotine before placement in the alternative chamber. The third group (Control Group) received injections of saline before placement in both chambers. All rats received 8 injections (with two-day cycles in which exposure to each context and injections alternated) prior to the start of the cued-trial operant training. After the first training session, rats received a two-day cycle of exposure to each chamber (with injections) prior to each subsequent training session. Once training was complete, a devaluation test was conducted in which rats were given unlimited access to one of two food pellets for one hour. This was followed by a choice test in which both levers were available but the location of the two cuelights was switched in order to differentiate if decision-making was based on cuelight or lever-location strategy. The rats then received retraining on the two lever-light compounds (with the 2-day cycle of injections and context exposure prior to each retraining session) before receiving satiation on the other pellet followed by a choice test otherwise identical to the first. We found that the male rats who were trained in the nicotine-associated environment exhibited impaired devaluation early in the test but prior exposure to nicotine paired with an alternative chamber did not impair devaluation. Females in all 3 treatment groups did not exhibit a significant devaluation effect. During my poster presentation, I will discuss the significance of these findings and describe our future directions for this research program. This work was supported by: A Johnson Cancer Research Center Undergraduate Research Award to Olivia Waren, a K-State Arts and Sciences Undergraduate Research Awards to Jendaya Leder, and NIGMS Grant P20GM113109.

King C, Pahua A, Warnes E, Neyhard J, Plakke B (*Kansas State U*) Effects of exercise on cognition and BDNF levels in the cortex of a rodent model of autism ABSTRACT: People with ASD (autism spectrum disorder) have altered cognition, communication styles, and exhibit repetitive behaviors. This study used the valproic acid (VPA) model to induce ASD-like symptoms in rodents. Past work has demonstrated that exercise improves cognitive performance in VPA females and that brain-derived neurotrophic factor (BDNF) levels in the brain are mediated by exercise. BDNF signaling is also disrupted in humans with ASD and animal models. Here, we exercised VPA and control animals to measure differences in BDNF and muscle-derived cytokine levels in the prefrontal cortex. Pregnant dams were injected with 600 mg/kg VPA or saline on gestational day 12. To control for the litter effect, one male and one female pup per litter were assigned to an experimental condition (exercise or sedentary, N=85). Rats performed set-shifting and rotarod tasks to measure cognition and motor coordination, respectively. Data analysis compared male and female data separately based on past findings (McKinnell et al., 2020). Researchers that conducted behavior and protein analysis were blind-to-condition. The exercised VPA animals had the largest improvements in motor coordination as measured by rotarod performance. There was improvement in cognitive performance consistent with our prior findings. Analysis of prefrontal tissues via a Luminex® multiplex protein assay is ongoing, and it is expected that animals in the exercise condition will have upregulated BDNF and muscle-derived cytokine levels in the cortex. Mediation of these cross-organ signaling pathways through exercise could be used as a cost-effective and non-invasive intervention to bolster neuroplasticity processes in those with ASD. This work was supported by: K-INBRE Developmental Research Project Award to Dr. Plakke (P20 GM103413) and NIGMS GM113109 CNAP.

Kinsky NR, Haddad J, Diba K (*U Michigan Med*) Combined Electrophysiology and Imaging to Investigate Hippocampal-Cortical Interactions During Trace Fear Memory Consolidation ABSTRACT: Systems consolidation theory posits that hippocampal-neocortical interactions following learning mediate plasticity in cortical circuits and allow for the creation of long-term episodic memories. One candidate mechanism for inducing cortical plasticity is the hippocampal sharp-wave ripple (SWR): a transient 150-250Hz oscillation which originates in the hippocampus during quiet periods and sleep and reverberates activity throughout the neocortex. However, testing the impact of this mechanism on cortical circuits has remained difficult due to technical limitations in tracking neural activity of the same neurons across days. Here, we perform simultaneous

in vivo electrophysiology in the hippocampus and calcium imaging in the pre-limbic (PL) region of freely moving rats before, during, and after a trace fear conditioning task. This approach allows us to characterize how post-learning SWRs influence the development of memory-related neural activity in PL neurons (10-50 cells per session). We first performed pilot behavioral studies where rats learned that a 10 second tone (conditioned stimulus, CS) predicted a mild foot shock (unconditioned stimulus, US) following a 20 second trace interval. Rats were tested 1-7 days later to assess freezing responses to both the CS and the conditioning arena. These studies revealed variability in CS responses: some rats exhibited clear freezing to the tone while others exhibited generalized fear. Based on these studies, we next performed simultaneous neural recordings while male and female rats underwent a modified trace fear conditioning task with which we incorporated a control tone (CS-) that was never paired with a shock. These rats developed a specific fear memory as exhibited by clear freezing responses to the CS, but not the CS-, 1-2 days following learning. Preliminary analyses revealed that PL neurons were persistently active during training and developed CS responses during memory recall. Future analyses will characterize the development of CS responses in PL neurons, determine how this plasticity relates to PL neuron co-activity with SWRs, and examine contextual fear related neural responses to the conditioning arena. This work was supported by: NIH/NINDS 1F32NS117732, NIH/NINDS R01NS115233, and NIH/NIMH R01MH117964.

Knox D, David N, Hause S, Biddle M, Mohammadmirzaei N, Sudheimer K, Jeun C, Barth M (*U Delaware/ S Illinois U School of Med*) Emotional properties of city and suburban landscapes ABSTRACT: US cities were the centers of economic and population growth through much of the 19th and early 20th centuries, but many cities have seen a hollowing out of population since the 1950s. Planners prioritizing sustainability, efficiency, social justice, and climate change have long advocated for more growth and development in urban (as opposed to suburban and rural) areas. However, the continued growth and development of suburban areas has proven to be problematic. Neural circuits for storing memories that surround fearful events are long-lasting and influence schema and behavioral strategies employed by organisms. The negative portrayal of cities in films, fiction, social media, and news coverage is commonplace. Cities are portrayed as environments where threats are commonly encountered. This portrayal may result in an association between the built environment of a city and fear generated by anticipated threats within a city. If this is true, then cities and the surrounding suburban regions may be linked with different emotional responses in the brain. To examine this, we used functional magnetic resonance imaging (fMRI) to measure brain activation changes that occurred when 18 people viewed city images and suburban images. We also measured the qualitative emotional experiences people had when viewing the images. City and suburban images were presented (in a counterbalance fashion) to subjects in a 3T Siemens scanner (~ 8 minutes). At the end of image viewing in the fMRI scanner, participants were presented with a subset of city and suburban images and their emotional experiences were assessed using a survey. Contrast analysis of brain activation in response to suburban vs. city images revealed a significant increase in the primary visual cortex (V1). Analysis of survey responses revealed that generally participants rated city images more negatively and that only some participants had an emotional experience during picture viewing. In the subset of participants that reported no emotional experience, city images evoked stronger brain activation than the suburban images in the prefrontal cortex, anterior cingulate, and insula. This pattern was not observed in participants that did report an emotional experience during the viewing of the pictures. The results of this study raise the possibility that city images can elicit negative emotions and can activate emotion-related brain regions. However, salient variables, like if an emotional response is evoked or not, may account for differences in neural reactivity to images of city and suburban landscapes. This work was supported by: Interdisciplinary Frontier Graduate and Postdoctoral Fellows Program; NIGMS-1P20GM103653.

Kokan N, Steidl S, Yee S, Boldt A, Rankin C (*U British Columbia*) Behavioral and genetic evidence that habituation at different interstimulus intervals involves dissociable processes ABSTRACT: Habituation is a simple form of learning that occurs when an organism decreases its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. The frequency of stimulation, or the time between stimulus presentation greatly impacts both the rate, depth, and memory of habituation. The goals of this research are a deeper understanding of how the time between stimulus presentations, or interstimulus interval (ISI), influences habituation, and the identification of genes that have an ISI dependent effect on habituation. Animals habituate more slowly and to a lesser extent at long ISIs, but the response decrement persists for longer than when the decrement is induced by short ISIs. This means that at longer ISIs habituation learning is slower, but memory of this learning lasts longer than at shorter ISIs. To investigate whether this is caused by different habituation processes being activated by short and long ISIs, I used our Multi-Worm Tracker to simultaneously monitor the behavior of dozens of Caenorhabditis elegans, on plates that receive mechanical taps at different ISIs. The worm's naive response to a tap is to reverse, moving backwards briefly; our Multi-Worm Tracker software generates detailed morphological and behavioral data for each worm as this reversal response habituates to repeated stimuli. As expected, this reversal response habituated more slowly

with longer ISIs than shorter ISIs, and the memory of habituation after long ISIs persisted for at least 15 minutes. In contrast, when the ISI was altered mid-experiment from a short ISI to a longer one, the response recovered within a single interval (i.e., 60s) to the asymptotic level of habituation at the longer ISI, demonstrating the transience of habituation at shorter ISIs. To further investigate the possibility of ISI specific habituation mechanisms, I tested worms with mutations in different genes at short and long ISIs looking for ISI-specific habituation defects. I found several genes such as avr-14, a glutamate gated chloride channel alpha subunit, and ogt-1, homolog of O-GlcNAc transferase, that appear to play important roles in habituation at long ISIs but only have a small impact at short ISIs. I have also found one gene, *acy-1* – a homolog of adenyl cyclase – which appears to only function in habituation at short ISIs. These data provide both behavioral and genetic evidence for ISI-specific habituation processes. Next, I will determine whether these genes are all acting in the same long ISI habituation pathway and where in the neural circuit this ISI-specific habituation is occurring. This work was supported by NSERC.

Kramer C, Ruble S, West L, Payne K, Auletti I, Diehl M (*Kansas State U*) Active avoidance under social conditions enhances fear responses and recruits the anterior cingulate cortex in male and female rats ABSTRACT: Avoiding danger is imperative for survival. To study active avoidance, we utilize the platform-mediated avoidance (PMA) task, in which rats avoid a tone-signaled foot shock by stepping onto a safe platform at the cost of access to a sucrose reward (Bravo-Rivera, et al., 2014). Previous PMA studies have largely focused on male rats learning PMA alone (Diehl, et al., 2019). The current study seeks to understand how rats acquire avoidance with a social partner. Male and female rats were trained in PMA under solitary (n=59) or social (n=42) conditions, whereby partners undergo PMA training together with access to their own platform and reward. Rats trained under social conditions showed similar levels of avoidance, but greater freezing ($p<0.001$) compared to rats trained under solitary conditions. Females showed increased avoidance compared to males ($p<0.01$) under solitary, but not under social conditions ($p=0.191$). We were next interested in whether activity in anterior cingulate cortex (ACC) was necessary for avoidance under social conditions, given its role in social learning (Apps, et al., 2016). To investigate this, we applied an optogenetic approach to photo-silence ACC neurons during avoidance expression. Following surgery, male and female rats were trained in PMA under solitary (ArchT n = 20, eYFP n = 15) or social conditions (ArchT n = 9, eYFP n = 13), then underwent a test of avoidance expression. Photo-silencing ACC impaired avoidance expression under social conditions both in the presence ($p<0.001$) and absence of the partner ($p = 0.002$). Under solitary conditions, photo-silencing ACC delayed avoidance in males but blocked avoidance in females ($p<0.001$ Male ArchT n=12, Female ArchT n=8). These findings suggest that active avoidance is mediated by the ACC, which may be dysfunctional in individuals suffering from trauma-related disorders. This work was supported by the Cognitive and Neurobiological Approaches to Plasticity (CNAP) from the NIGMS (#P20-GM113109), the Kansas iDeA Network of Biological Research Excellence (K-INBRE) from the NIGMS (#P20-GM103418), and the Department of Psychological Sciences.

Lacagnina AF, Dong TN, Iyer R, Khan S, Mohammed M, Clem RL (*Mt. Sinai*) Hippocampal somatostatin interneurons govern switching between memories of threat and safety ABSTRACT: Fearful experiences create enduring negative associations with the surrounding context. Emotional responses to these contextual cues normally subside in the absence of threat, a process known as extinction. Fear and extinction memories appear to be represented by competing neural ensembles; however, very little is known about the mechanisms governing the switching between these conflicting memories. Understanding neural circuits responsible for the gating of emotional memory expression can provide crucial insights for developing novel therapeutics for treating disorders of pathological fear. We identified activity of somatostatin interneurons (SST-INs) in the ventral hippocampal CA1 area (vCA1) as uniquely correlated with extinction retrieval. An extinction-specific recruitment of vCA1 SST-INs was confirmed using an intersectional activity-dependent tagging strategy. Optogenetically silencing vCA1 SST-INs impaired extinction retrieval, while stimulating these cells, either broadly or in an activity-dependent manner, prevented fear relapse. In contrast, manipulations of vCA1 parvalbumin-expressing INs did not affect expression of contextual fear or extinction. We confirmed that fear and extinction retrieval reactivate orthogonal vCA1 excitatory ensembles and, additionally, silencing excitatory projections from vCA1 to the prefrontal cortex specifically impairs extinction retrieval. Finally, vCA1 SST IN activity was both correlated and necessary for retrieval of an extinguished contextual reward memory, suggesting this mechanism is valence-independent. Our results suggest that retrieval of conflicting memories is mediated by vCA1 SST-INs. We hypothesize the vCA1 SST-INs gate the activity of orthogonal excitatory ensembles to suppress the activity of the ensemble associated with the initial contextual memory and promote the retrieval of the extinction-related ensemble.

Laing PAF, Dunsmoor JE (*UT Austin*) Pattern separation of fear extinction memory in humans ABSTRACT: The relapse of fear following extinction is often attributed to the dominance of highly-generalizable fear memories over extinction memory, which is stimulus/context-specific. Human studies typically assess extinction memory via indirect measures of physiological fear, rather than explicit memory of fear and safety-conditioned stimuli. We report

results from separate behavioral and fMRI studies, examining how fear and extinction are differentially represented in episodic memory. Using hybrid conditioning/episodic memory paradigms, subjects encoded non-repeating category exemplars during fear conditioning and extinction, followed. 24-hours later by a surprise memory test, which included old, similar, and novel category exemplars. Behavioral results showed strong dissociation between pattern completion (generalization) versus pattern separation (discrimination) in episodic memory for items encoded during fear conditioning versus extinction, respectively. These data suggest that directly threat-conditioned stimuli are better recognized at the expense of mnemonic precision, whereas discrimination is enhanced for extinguished stimuli. Overly precise extinction memory may be a contributing factor to fear relapse. Emerging findings from an fMRI extension of the paradigm will also be presented. This work was supported by a National Institutes of Health grant to J.E.D (R01 MH122387).

Lamparelli AC, Vilchez GE, Patel K, Wassum KM (*UCLA*) Projections from the basolateral amygdala to the anterior cingulate cortex enable stimulus-generated reward value expectations ABSTRACT: Every day we use information in the environment to infer the availability and value of rewards to meet our needs. Reward-paired stimuli guide decision making by facilitating reward predictions that allow us to prospectively consider the value of obtaining a specific rewarding outcome. This process enables adaptive control over reward-directed behaviors, and may become disrupted in psychiatric disorders marked by decision-making deficits (e.g. substance use disorder, depression, schizophrenia etc.). A deeper understanding of how the brain fundamentally uses stimuli to guide behavior will help determine the neural bases of behavioral deficits observed in such disorders. The basolateral amygdala (BLA) coordinates with multiple regions in the medial prefrontal cortex to enable stimuli to generate reward expectations, but little is known of how direct projections between the anterior cingulate cortex (ACC) and BLA enable reward value expectations to inform reward pursuit behavior. Using chemogenetic inactivation of direct projections from BLA to ACC in a sensory-specific satiety devaluation paradigm, we found that BLA principal neurons projecting to the ACC enable rats to use Pavlovian stimuli to generate reward value expectations and adjust behavior based on current reward value. Interestingly, the BLA to ACC pathway is not needed for retrieving reward representations broadly, as both instrumental devaluation and Pavlovian-to-instrumental transfer were intact under BLA to ACC inactivation. Rather, the activity in BLA inputs to ACC is specific to the ability of stimuli to trigger reward value expectations to modulate reward pursuit. These results add further insights into the nuances of how the BLA coordinates with specific subregions of the medial prefrontal cortex. This work was supported by: NIH F31DA053104 (AL), NIH R01DA035443 (KW).

Le QE, Hereford D, Borkar CD, Aldaco Z, Klar J, Alam T, Resendez A, Fadok JP (*Tulane*) ABSTRACT: Traumatic experiences can have negative long-term effects on physical and emotional well-being, and recovery from trauma-related fear disorders is impeded by persistent fear responses that resist extinction. The central amygdala (CEA) contains somatostatin-positive (SOM+) and corticotropin-releasing-hormone-positive (CRH+) cells that are involved in fear response modulation and post-extinction fear renewal. Thus, it is critical to understand how these CEA populations contribute to maladaptive defensive responses and impaired fear extinction. To investigate their role in directing defensive behavior, we utilized chemogenetics to transiently activate SOM+ and CRH+ CEA cells in male and female adult SOM-Cre and CRH-Cre mice in a Pavlovian conditioning paradigm that paired footshock with a serial compound stimulus (SCS) consisting of distinct tone and white noise (WN) periods. Previously, we found that conditioned animals in this SCS paradigm would transition between freezing during tone to explosive jumping and darting behaviors during WN, with tone-evoked freezing diminishing and WN-evoked jumping behaviors being replaced with freezing and darting over extinction sessions. However, chemogenetic excitation of SOM+ cells in the CEA during extinction did not significantly alter freezing or flight responses to either portion of SCS, but it did seem to reduce jumping behaviors in favor of darting during WN. Though fewer CRH-Cre mice were tested, behavioral trends indicated that chemogenetic CRH+ excitation also did not affect defensive responses. Given the relative lack of changes brought about by chemogenetic manipulation, we are currently using optogenetics to activate/inhibit SOM+ and CRH+ CEA populations during WN presentation during extinction to observe how they affect the expression and magnitude of defensive behaviors within the SCS paradigm. We are also investigating the role of connections between the ventral hippocampus and the CEA in extinction-dependent defensive ethograms.

Lefner MJ, Moghaddam B (*OHSU*) Valence-specific gating of behavioral flexibility by medial prefrontal cortex projections to the ventral tegmental area ABSTRACT: In order to efficiently traverse an environment, one must learn to distinguish between cues that predict outcomes of different modalities. In a dynamic environment where cues predicting rewarding or aversive outcomes unexpectedly change, it is adaptive to flexibly update behavioral responding while retaining the ability to recall previous associations. Impairments in the ability to appropriately respond to cues according to the outcome are evident in a number of psychiatric disorders. Both dopamine and γ-aminobutyric acid (GABA) neurons in the ventral

tegmental area (VTA) are involved in appetitive and aversive learning. Furthermore, the medial prefrontal cortex (mPFC) is necessary for behavioral flexibility. We hypothesize that flexible updating of behavior in response to changes in learned associations is valence-specific, and is modulated by mPFC projections to the VTA. To quantify initial learning as well as updating cue-outcome associations, adult male and female Long-Evans rats were trained on a flexible contingency learning (FCL) task developed in our laboratory. During initial FCL training sessions, three distinct auditory cues are paired with either an appetitive outcome (sugar pellet reward), an aversive outcome (mild foot shock), or no outcome. After learning, the appetitive and aversive outcomes reverse such that the cue previously paired with a shock will instead precede reward delivery and vice versa. There were no sex differences in behavioral responding during the FCL task. Fiber photometry recordings were performed in transgenic rats in order to measure changes in calcium activity and characterize VTA dopamine and GABA neuron responses throughout all stages of FCL. Our findings indicate a dissociation between responses of VTA cell groups: the dopamine cell population increases calcium activity in response to initial learning and reversal of reward associations, whereas the GABA population encodes initial learning and reversal of both appetitive and aversive associations. We next probed whether projections from the mPFC (including both prelimbic and infralimbic subregions) to the VTA modulated flexible responding to changes in outcome contingencies using pathway-specific chemogenetic inhibition during the reversal of associations in the FCL task. Inhibiting the mPFC-VTA pathway enhanced conditioned responding selectively to the appetitive-paired cue that was previously associated with the aversive outcome. Taken together, our data highlights a role for top-down regulation of neural dynamics and behavioral responding during flexible learning.

Leptich EJ, Arey RA (*Baylor College of Med*) Uncovering novel neuropeptide regulators of associative behaviors ABSTRACT: Cognitive decline is a prominent feature of aging across organisms that significantly compromises quality of life in humans. Therefore, it is critical to identify mechanisms that boost learning and memory function. *C. elegans* is a fantastic model for studying this problem, given their short lifespan, invariant cell lineage, and wealth of genetic tools available. Most importantly, *C. elegans*' associative memory behavior is molecularly conserved and declines with age. Our previous research in *C. elegans* shows that gain-of-function mutants in Gαq signaling (egl-30(gf)) have enhanced long-term associative memory (LTAM) behavior as young adults and slowed cognitive aging phenotypes. The enhanced memory ability of young adults requires neuropeptide signaling from a single sensory neuron, the AWC. Growing evidence indicates neuropeptides regulate learning and memory, but their roles in these behaviors are less well-studied than classic neurotransmitters in the context of learning, memory, and cognitive aging. Thus, the identities of memory-promoting neuropeptides and their roles in cognitive aging are unknown. Here, we sought to identify the neuropeptides and their target receptors that boost learning and memory behavior. Using an RNAi-based approach, we screened candidate AWC-expressed neuropeptides for their role in learning and memory behavior in egl-30(gf) animals. We found multiple neuropeptides from different families (neuropeptide-like proteins, FMRFamide-like proteins, and insulin-like peptides) are necessary for enhanced learning and memory behavior. One of the insulin-like peptides we find to be necessary for learning ability, INS-17, is a known antagonist of the *C. elegans* insulin receptor homolog DAF-2. Since daf-2 mutants are known to have enhanced learning ability with age, our findings suggest that INS-17 may play a role in daf-2-dependent learning. Moreover, we have identified evolutionarily conserved target receptors of peptide hits from our screen as novel regulators of learning and memory behavior. In ongoing work, we aim to identify the neuronal sites governing these behaviors. We are also examining if peptide administration using a novel feeding-based approach can slow cognitive aging in the worm. Because many known pathways that slow cognitive aging are shared between species, this research has the potential to uncover novel therapeutic targets for cognitive impairment in higher organisms.

LeVasseur GW, Timothy SC, Perrine SA, Norrholm SD (*Wayne State U School of Med*) Development of robust fear potentiated startle protocol optimized for observation of within-session extinction learning in rats ABSTRACT: Fear conditioning in rodents, and its neurobehavioral sequelae, are considered to be models of the fear-related symptoms of posttraumatic stress disorder (PTSD). In other words, Pavlovian fear conditioning is thought to underlie the strong emotional memories and reactions commonly observed in PTSD. The gold standard treatment for PTSD currently is exposure therapy, which can also be modeled in the laboratory using fear extinction training (repeated non-reinforced presentations of a cue previously paired with an aversive outcome). When fear conditioning is conducted in rodents, the percent of time spent freezing is commonly used as an outcome measure related to fear. A potentially more translational measure of fear in the laboratory is fear potentiated startle (FPS). FPS makes use of the acoustic startle reflex; a response that is modulated by fear/anxiety states and can be measured across species. Several groups make use of FPS in their studies, yet there is no widely accepted standard protocol for conducting the learning tests involved in this procedure. In this study we conducted a series of experiments with 48 male Sprague Dawley and 16 male and female Wistar rats to better determine effective FPS parameters for fear acquisition learning (ACQ) and extinction learning (EXT). Our goal was to develop a model

that could be used to study manipulations that facilitate or protract EXT. Therefore, emphasis was placed on ACQ and EXT parameters that resulted in a robust, persistent startle signal evident during fear expression and EXT training while also allowing for effective assessment of retained EXT learning (EXT RET). Several parameters were altered in these experiments, including the number of CS-US ACQ pairings, number of EXT trials, number of days for both ACQ and EXT training, the presence or absence of startle probes during EXT training, and the presence or absence of pre-test startle probe habituation sessions. We provide evidence that 30 ACQ pairs over 2 days, results in effective fear expression and 120 EXT trials over 2 days results in effective extinction learning. Presenting the startle probe during only half of the EXT trials is still sensitive enough to observe within session EXT learning, and that the presence of pre-test startle sessions reduces inherent variability of the startle response.

Lozano-Ortiz K, Felix-Ortiz AC, Ramos AR, Velazquez-Hernandez G, Rodriguez-Romaguera J, Burgos-Robles A (*UT San Antonio*) Contribution of medial prefrontal cortical areas to the development of social phobia ABSTRACT: Social phobia is a maladaptive disorder in which individuals exhibit excessive fear and anxiety during social interactions or situations. Despite its high prevalence, the neural mechanisms underlying the development of social phobia still remain to be elucidated. To examine this, we implemented a "social threat learning" paradigm in which mice received electric shock punishment (0.4mA for 1s) every time they engaged in social interactions with unfamiliar conspecifics (i.e., social stimuli) that were confined within smaller non-electrified devices. This paradigm resulted in the development of robust responses associated with fear and anxiety, including freezing, avoidance, retreat, and darting. During a subsequent test session in a different context and in the presence of new unfamiliar social stimuli, mice that received shock punishment the previous day continued to exhibit significantly greater fear and anxiety responses compared to no-shock controls. This is consistent with the idea of formation of lasting states of social phobia. We next implemented optogenetic approaches to examine the contribution of distinct subregions of the medial prefrontal cortex, which have been shown to regulate multiple forms of fear and anxiety behaviors. While silencing principal neurons in the infralimbic subregion (IL-PFC) during social threat learning produced no significant effects, silencing principal neurons in the adjacent prelimbic subregion (PL-PFC) abolished the formation of lasting social phobia. Therefore, our results suggest that neural processing in the PL-PFC is necessary for social threat learning and the development of lasting social phobia. New experiments are on the way to examine whether NMDA receptor activity contributes to local plasticity in the PL-PFC to promote social threat learning.

Ly A, Root DH (*UC Boulder*) Bed nucleus of the stria terminalis (BNST) GABA signaling in backward conditioned suppression ABSTRACT: Conditioned suppression is a classic psychology-based task used to understand how learned cues interrupt ongoing reward-seeking behavior. Traditionally, conditioned suppression studies use forward conditioning pairings where a cue precedes an unconditioned aversive stimulus. However, backward conditioning where an unconditioned aversive stimulus precedes a cue, also has utility as an ambiguous cue. Specifically, with limited training, backward cues promote conditioned responding, whereas with extensive training, backward cues promote conditioned inhibition. Here, we compared conditioned suppression between a few and many pairings of forward and backward shock-predictive cues. Mice were trained to lick from a sucrose bottle in context A until there were no session differences in total number of licks between the last 3 sessions. Mice underwent shock conditioning in context B to either: 12 pairings of forward CS+, 96 pairings of CS+, 12 pairings of backward CS+, or 96 pairings of backward CS+. The following day, mice were placed back into context A with 15 CS+ presentations. We administered 0.5 mg/kg diazepam, a positive allosteric modulator of the GABA-A receptor, after conditioning to determine the sufficiency of increasing GABA signaling in decreasing conditioned suppression. We also examined BNST GABA neuron calcium signaling during and after conditioning because the BNST is a dense GABAergic region with evidence to suggest its role in stress regulation and motivation. Forward CS+ and few pairings of backward CS+ produced conditioned suppression; mice consumed sucrose less frequently during the forward CS+ and few pairings of backward CS+. Diazepam prevented conditioned suppression to few pairings of backward CS+. Freezing behavior was observed with forward CS+ but not backward CS+, and diazepam had no effect on freezing or locomotion. During conditioning, BNST GABA neurons signaled shock but not the backward CS+. After conditioning, BNST GABA GCaMP signaling increased in response to sucrose reward but not to the backward CS+. While GABA signaling is necessary for backward conditioned suppression, BNST GABA neurons do not signal backward CS+. Rather, BNST GABA neurons appear to signal both rewarding and aversive unconditioned stimuli. This work was funded by a NIMH F31 NRSA.

Lyvers DP, Mangrum JS, Sheehan T, Privratsky MA, Sangha S (*Indiana U School of Med*) Influence of prior stress and alcohol on safety behaviors, shock sensitivity, and subsequent alcohol consumption ABSTRACT: Stressful events can have lasting and impactful effects on behavior, especially in terms of appropriate regulation of fear and reward processing. Increasing evidence suggests stress disorders and chronic alcohol use may alter the ability to

discriminate between different stimuli leading to behavioral dysregulation. We have previously shown that an acute stressor increases home cage alcohol consumption in both male and female Long Evans rats, with females showing particularly pronounced increases in ethanol consumption. We have also shown that an acute stressor results in high fear generalization to a sucrose-associated cue, and this generalized fear was positively correlated with subsequent home cage alcohol consumption. Here, we used the same acute stress paradigm, along with home cage access to both water and alcohol to assess the influence of prior stress and alcohol on conditioned inhibition of fear and reward, shock sensitivity and subsequent alcohol consumption. Male and female Long Evans rats had home cage intermittent 24h access to water and 15% alcohol for 5 weeks prior to the acute stressor, which consisted of 15 unsignaled 1.0 mA footshocks in Context A (ETOH, Stress). One week after the stressor, rats had continuous access to water and 15% alcohol while receiving discrimination training in Context B to a reward cue paired with 10% sucrose, a fear cue paired with 0.5 mA footshock, and an inhibitor cue without sucrose or footshock. One day after the 4th discrimination training session, a summation test for conditioned inhibition was delivered in Context B by presenting the same cues as during discrimination training, along with summation trials of the fear+inhibitor cues without footshock, and reward+inhibitor cues without sucrose. Separate control groups (ETOH, No Stress) of males and females received access to water and alcohol but instead of receiving the acute stressor, were simply exposed to Context A without footshocks. Additional control groups of males and females only had access to water and either received the acute stressor (Water, Stress) or just Context A exposure (Water, No Stress). Separate groups of 1) ETOH, Stress, 2) ETOH, No Stress, 3) Water, Stress, and 4) Water, No Stress did not receive any behavioral conditioning but were assessed for shock sensitivity by assessing freezing, jumping and darting behaviors in response to increasing shock intensities from 0.3 mA to 1.0 mA. This was to determine if prior alcohol consumption and/or stress exposure altered the sensory perception of the footshock.

Macdonald EE, Ma J, Authement ME, Alvarez VA, Penzo MA (*NIMH*) A thalamostriatal circuit that shapes dopamine-mediated safety signaling during avoidance learning ABSTRACT: Active avoidance (AA) is an adaptive defensive strategy employed to minimize threat encounters. However, excessive avoidance is a core feature of anxiety disorders in humans, thus highlighting the necessity of understanding the neural mechanisms that underlie avoidance behaviors. A key brain region involved in the expression of avoidance is the nucleus accumbens (NAc), located within the ventral striatum. Inactivation of the NAc diminishes avoidance, and dopamine (DA) release within this region is classically associated with both the learning and execution of avoidance behavior. For instance, DA release immediately after successful avoidance responses (termed 'safety period') is thought to mediate avoidance learning via positive reinforcement. Still, the mechanisms that shape this process remain unknown. Here, we discovered that NAc-projecting neurons of the paraventricular nucleus of the thalamus (PVT) – a structure that is critical for the expression and maintenance of AA behavior – are selectively engaged during the safety period of the AA task. Importantly, optogenetic inhibition of the PVT–NAc pathway during the safety period diminished avoidance learning. These data suggested that safety related DA signaling in NAc and modulation of PVT input during these episodes might be related. In agreement, we found that activation of PVT terminals can promote DA release in NAc and that silencing the PVT–NAc pathway impairs safety-related DA. Additional evidence collected from us point at local modulation of cholinergic interneurons as a plausible mechanism by which the PVT shapes DA release in NAc. Overall, our results provide novel evidence of a thalamostriatal pathway that is critical for the learning of active avoidance likely by promoting the reinforcing actions of DA release during the safety period.

Markowitz SM, Fanselow MS, Sharpe MJ (*UCLA/ U Sydney*) The Role of Lateral Periaqueductal Grey During Associative and Non-Associative Fear Learning ABSTRACT: Approximately 10% of people who experience a highly stressful event, or trauma, will go on to develop Post-Traumatic Stress Disorder (PTSD). As such, it is critical that we understand the neural substrates involved in PTSD. The stress-enhanced fear learning (SEFL) phenomenon (SEFL) parallels some of the characteristics seen in people with PTSD. Rats receive a stressful event in one context (i.e., context A: 15 x 1mA shock). Then, rats receive a mild fear conditioning protocol in another context that differs in scent, texture, light, and shape (i.e., context B: 1 x 1mA shock). Rats given the stressful experience show greater fear to context B after this protocol, indicating stress-enhanced fear learning (SEFL). The SEFL effect is believed to be non-associative because the SEFL effect is seen even when the stressor is of different sensory modality to that experienced during the fear conditioning protocol (e.g., loud noise, tail shock). The periaqueductal gray (PAG) is a key brain region for defensive response behavior. It is well established that activity in the PAG is both necessary and sufficient for both fear and panic response toward external stressors. In the present experiments, we investigated c-Fos activity in PAG subregions during exposure to significant and nonsignificant stress. We found a significant difference in activity in the lateral PAG (lPAG) and ventral-lateral PAG (vlPAG) between shock stress, noise stress, and homecage animals. Of these two regions, lPAG has previously been found to be necessary for panic, which we reasoned may extend to the ability of stress to produce enhanced fear learning (i.e., SEFL). In order to investigate whether lPAG activity during shock stress is

necessary for SEFL, we inhibited this region using optogenetics. Rats were bilaterally injected with AAV8-CaMKII-NpHR-eGFP (NpHR) or AAV8-CaMKII-eGFP (eYFP) in the lPAG. This allowed us to inactivate pyramidal neurons in the lPAG during the shock exposures in context A. Surprisingly, we found that lPAG inhibition during the stressor did not attenuate the SEFL effect. In fact, we saw a trend suggesting the non-associative SEFL effect was enhanced in the NpHR group. In contrast, we saw evidence for a reduction in the fear seen to the stressful Context A in our NpHR group, which may indicate a reduction in the associative fear learning. Now, we plan to optogenetically inhibit lPAG during Pavlovian fear conditioning to further investigate how this region contributes differentially to associative and non-associative fear learning.

Matsumura K, Nicot A, Choi IB, Asokan M, Le N, Natividad L, Dobbs LK (*UT Austin*) Endogenous opioid system modulates cocaine reward in a sex-dependent manner ABSTRACT: Cocaine-associated contexts exert powerful control over behavior and can incite drug seeking. This kind of context-dependent cocaine seeking is encoded within striatal circuits, and these circuits and behaviors, in part, are regulated by endogenous opioid peptides and receptors. Here, we investigated how enkephalin, an opioid peptide with high affinity for mu opioid receptors that is expressed in striatal medium spiny neurons (MSNs), regulates conditioned cocaine seeking. We previously showed that enhanced levels of enkephalin in the striatum facilitates acquisition of cocaine conditioned place preference (CPP) and opioid receptor antagonists attenuate cocaine CPP. However, whether striatal enkephalin is necessary for acquisition and maintenance of cocaine CPP remains unknown. We generated mice with a targeted deletion of enkephalin from dopamine D2-receptor expressing MSNs (D2-PenkKO) and tested them for cocaine CPP. Cell-selective deletion of striatal enkephalin had no effect on acquisition or maintenance of cocaine CPP; however, we found that acute naloxone attenuated expression of cocaine preference selectively in females. In addition, females, regardless of genotypes, extinguished cocaine preference faster than males, and extinction of cocaine CPP was rescued by pairing naloxone with cocaine-associated context during extinction training. These data suggest that striatal enkephalin is not necessary for acquisition, expression, or extinction of conditioned cocaine reward. We also conclude that naloxone has divergent effects on extinction of cocaine reward selectively in females, which implies opioid peptides other than striatal enkephalin as the substrate for modulating expression and extinction of conditioned cocaine reward. These data implicate sex as an important consideration for use of opioid antagonists in the treatment of cocaine use disorder. This research was supported by: NIDA R01DA054329 to LKD, UT Austin Rising STARs Award to LKD, NIAAA R00AA025393 to LN, and a Bruce Jones Predoctoral Fellowship to KM. We are grateful to Dr. Andreas Zimmer for generously providing the floxed Penk mice and to Dr. Ian Riddington and the Mass Spectrometry Facility at UT Austin for scientific assistance.

Mauk MD (*UT Austin*) On stimulus trace explanations for the Inter-Stimulus-Interval function ABSTRACT: The inter-stimulus-interval (ISI) function is a defining feature of Pavlovian conditioning. It refers to the amount or robustness of conditioning as a function of the interval between CS and US onsets during conditioning. The concept of stimulus trace, where the onset of a CS activates a process that is permissive for learning and that is the shape of the ISI function, is a surprisingly well-accepted mechanistic explanation for the ISI function. Using what's known about the cerebellar mechanisms of Pavlovian eyelid conditioning, and drawing upon insights from a large scale computer simulation of the cerebellum that produce all major behavioral features of eyelid conditioning, we will argue for the implausibility of stimulus trace explanations for the ISI function. I will show that in this simulation, which contains no parameter that comes anywhere close to the trace interval, the ISI function mostly arises from competition between the plasticity that supports learning (LTD) and the plasticity that supports extinction (LTP). We will further argue that the implausibility of simple stimulus trace explanations must apply to any form of Pavlovian conditioning where there is a degree of timing seen in the conditioned responses. We believe that analysis of all forms of Pavlovian conditioning would benefit from a reevaluation of the underlying assumptions regarding the basis for the ISI function.

Met Hoxha E, Robinson PK, Trask S (*Purdue U*) Generalization and discrimination of inhibitory avoidance differentially engage retrosplenial subregions ABSTRACT: In a variety of behavioral procedures animals will show selective fear responding in shock-associated contexts, but not in other contexts. However, several factors can lead to generalized fear behavior, where responding is no longer constrained to the conditioning context and will transfer to novel contexts. Here, we aimed to develop a paradigm in which generalization could be assessed in memories of the same age and strength and to assess if generalized avoidance behavior engages the retrosplenial cortex (RSC). Male and female Long Evans rats received inhibitory avoidance training or training and testing in the same context or a shifted context in two distinct rooms; one room that had fluorescent lighting (Light) and one that had red LED lighting (Dark). We found that animals tested in the light context maintained context-specificity; animals tested in the same context as training showed longer latencies to cross and animals tested in the shifted context showed shorter latencies to cross. However, animals tested in the dark generalized their avoidance behavior; animals tested in the

same context and animals tested in a shifted context showed similarly high latencies to cross. We next examined expression of the immediate early gene *zif268* in two regions important for contextual fear learning: the RSC and basolateral amygdala (BLA). We found that *zif268* increased in the anterior retrosplenial cortex (aRSC) of tested animals, but only increased in the posterior retrosplenial cortex (pRSC) of animals tested in the light. Furthermore, while activity in the BLA corresponded with overall levels of avoidance behaviors, aRSC activity corresponded with context-specific learning and pRSC corresponded with generalization. These findings suggest that there is differential engagement of retrosplenial subregions to these processes with aRSC activity present during discrimination and pRSC activity present during generalization. This work was supported by: Research Corporation for Science Advancement (Award: #29107) to S.T.

Mitchell JR, Ziane L, Bergeron E, Shansky RM (*Northeastern U*) Chemogenic manipulation of prefrontal-periaqueductal gray circuits in female and male rats during Pavlovian Fear Conditioning ABSTRACT: Darting, an escape-like conditioned response, occurs more frequently in females and is reliably accompanied by a distinct behavioral phenotype: Darters have heightened unconditioned responses, such as shock and post-shock response, which emerge before the first dart occurs, and also show enhanced extinction retention, as measured by decreased freezing. The dorsal periaqueductal gray (dPAG) is active during escape-like threat responses and receives direct input from the infralimbic cortex (IL). The IL plays a role in emotionally regulated behavior and is critical for extinction learning. The neural circuitry underlying darting is unknown, but the IL-dPAG circuit is a potential mediator of this and associated behaviors. In our first experiment, male and female Sprague Dawley rats underwent a 7 CS-US auditory, cued-fear conditioning paradigm. Animals were exposed to a 0.3mA, 1mA, or no shock. Ninety minutes after completion of fear conditioning, animals were perfused and tissue was immunostained for cFos+ cells. cFos+ cells were quantified in the medial prefrontal cortex and the dorsal, ventral, and lateral columns of the periaqueductal gray. We found that males and females did not differ in the level of cFos expression in the dPAG, but females showed higher levels of cFos expression in the IL than males. This effect was partially driven by higher cFos expression in female Darters when compared to female non-Darters. There was also a female-driven effect of shock intensity in the dPAG. There was an “inverted-U " effect of shock intensity in the IL in males, with 0.3mA males having the highest levels of cFos expression. These results suggest that activity in the IL and dPAG during fear conditioning is potentially a driver of the sex-dependent behavioral phenotype associated with darting as a conditioned response. For the second experiment, we used a cre-based intersectional DREADDs approach to activate or inhibit the IL-dPAG pathway before Pavlovian fear conditioning. Male and female Sprague Dawley rats underwent stereotaxic surgery to deliver a retro-cre virus into the dPAG and a Gq/Gi/mCherry control cre-dependent virus into the IL. After five weeks, animals went through fear conditioning, extinction learning, and extinction retention tests on three consecutive days. One hour before fear conditioning, animals were given an injection of clozapine-N-oxide to activate the DREADD. Using ScaredyRat, a custom Python tool designed in our lab to analyze raw Ethovision data files, we quantified conditioned (freezing, darting) and unconditioned responses (shock and post-shock response), as well as behavior during extinction learning and retention. A sex-dependent effect was seen when exciting or inhibiting the IL-dPAG circuit: Excitation of the circuit led to increased conditioned freezing in males and inhibition led to decreased shock response, while having no effect on conditioned responding during fear conditioning. The results from this experiment suggest that the role of the IL-dPAG circuit in fear conditioning is sex dependent. Taken together, these data suggest a novel, sex-dependent role of the IL during fear conditioning for both conditioned and unconditioned behaviors. This work was supported by: NIH grant 1R01MH123803-01

Moaddab M, Qian S, Boyce JB, Gordon NT, DuBois AM, Fitzpatrick AC, McDannald MA (*Boston College*) Ventral pallidum-defined pathways modulate fear-related behavior during threat discrimination ABSTRACT: The ventral pallidum (VP) is a critical node in the mesolimbic system, contributing to reward-related behavior. Previously, we reported the VP as a neural source of a dynamic, relative threat signal. The VP receives direct projections from the nucleus accumbens (NAc) and paraventricular nucleus of the thalamus (PVT), both important to organizing components of fear behavior. However, it is unclear if the VP, or its inputs from the NAc and PVT, modulate responding or expression of fear-related behavior. Here, we examined the role of the NAc and PVT input pathways to the VP in multi-cue fear discrimination. Male and female Long Evans rats received bilateral infusions of a retrogradely transported adeno-associated virus (AAV) vector encoding Cre recombinase (AAV/retro-eSYN-EGFP-T2A-iCre-WPRE, 0.3 µl per side) in the VP. Controls (n = 16) were injected with an AAV containing mCherry (rAAV5/Ef1a-DIO-mcherry, 0.3 µl per side). Cre-positive cells in the VP, NAc, or PVT were selectively deleted via cre-dependent viral caspase (rAAV5-Flex-taCasp3-TEVp). Cre-caspase was injected into the VP (n = 16, 1.0 µl per side), NAc (n = 16, 1.0 µl per side), or anterior/posterior PVT (n = 16, 0.75 µl per site). Following recovery, rats were food deprived to 85% of their body weight and trained to nose poke to receive food pellets. Rats underwent 16 sessions of Pavlovian fear discrimination before moving on to one extinction session. In fear discrimination sessions, rats were presented with

three 10-s auditory cues, each associated with a unique probability of foot shock; danger (p = 1.00), uncertainty (p = 0.25), and safety (p = 0.00). The schedule for rewarded nose poking was completely independent of auditory cue presentation and foot shock. Fear was measured using suppression of rewarded nose poking. Caspase-mediated deletion of local VP neurons and NAc or PVT inputs to the VP accelerated the recovery of nose poking after foot shock introduction. Caspase deletion of all three areas had no impact on direct responding to the three cues. Caspase and control rats showed high fear to danger, intermediate fear to uncertainty, and low fear to safety. However, cue-specific effects of caspase deletion emerged during the extinction. We will present the complete histological analysis of the caspase deletion and complete behavioral analyses of fear discrimination and extinction.

Mohammadmirzaei N, Biddle M, Hekmatyar K, Cai X, Kulkarni P, Knox D (*U Delaware*) Sex differences in the traumatic stress effects on functional connectivity, brain volume, and mu-opioid receptor levels within the reward circuit ABSTRACT: Post-traumatic stress disorder (PTSD) and opioid use disorder (OUD) frequently co-occur. Women are more likely than men to develop PTSD and OUD following trauma exposure. The neurobiological mechanisms through which trauma exposure increases the susceptibility to OUD in both males and females have not received sufficient attention. OUD is characterized by changes in the functional connectivity, brain volume and mu-opioid receptor (MOR) levels within reward circuits. Traumatic stress may cause distinct changes in the components of the reward circuit in males and females that increase susceptibility to opioid abuse. Furthermore, sex differences in traumatic stress effects could help account for sex differences in the prevalence of OUD within PTSD. Single prolonged stress (SPS) model was used to examine the effects of traumatic stress on resting state functional connectivity, brain volume, and mu-opioid receptor levels within the reward circuits including the prefrontal cortex (PFC), amygdala (AMG), dorsal and ventral hippocampus (DH and VH), nucleus accumbens (NAc), ventral tegmental area (VTA), bed nucleus of stria terminalis (BNST) and thalamic nuclei. Western blot (WB) and immunohistochemistry (IHC) were used to assay MOR levels and phosphorylation of MORs. For volumetric and connectivity studies, 3D T2-weighted MRI and resting-state functional connectivity imaging (R-fMRI) were performed using a 9.4T Brucker scanner. Our molecular data showed sex differences in the effect of SPS on MORs expression in the mPFC. In male rats, SPS exposure decreased MORs expression in the anterior cingulate cortex (ACC) and prelimbic cortex (PL) of the mPFC while it had no effects on the MORs of the mPFC in the female rats. Our fMRI data also revealed sex differences in the functional connectivity within the reward circuit following SPS exposure. SPS decreased functional connectivity between ACC-infralimbic cortex (IL), ACC-PL, BNST-dorsal dentate gyrus (dDG) and vCA3-medial dorsal thalamic nucleus (MDT) and increased functional connectivity between the anterior amygdala nucleus-entorhinal cortex, NAc(core)-IL, NAc(core)-PL, insular cortex-PL and vCA1-ACC in male rats. However, it had no effects on the functional connectivity of these regions in the female rats. In female rats, SPS exposure increased functional connectivity between CA1 subregion of the hippocampus and nuclei of the thalamus (e.g., central medial, medial dorsal and paraventricular thalamic nuclei) while it had no effects on the functional connectivity between these brain regions in the male rats. Analysis of the structural MRI data showed significant increase in the ACC volume in both male and female rats following SPS exposure, however the increase is larger in males in comparison to females. The results of this study suggest that the impact of traumatic stress on MORs expression, brain volume, and functional connectivity within nodes of the reward circuit are different between males and females and could contribute to sex differences in the prevalence of OUD within PTSD.

Moore E, Harris H, Slover W, Simpson J, Meyer W, Campolattaro MM (*Christopher Newport U*) Contextual Control of Classically Conditioned Eyeblink Responses in Rats ABSTRACT: The ability to generalize responding demonstrates that learned behavior is flexible, and it tends to occur most frequently when contexts are highly similar to one another. Previous work from our lab has shown that rats do not immediately generalize classically conditioned eyeblink responses between different tone-off CSs (i.e., absence of a 2-kHz tone vs 8-kHz tone). This lack of immediate generalization suggests that the background tone frequency used during initial training might be a critical part of the sensory experience that ultimately triggered a CR when it went quiet. Importantly, the tone-off CS was structurally the same sensory cue (i.e., a quiet period immediately leading up to the US) used during the initial and generalization tests. This finding led us to wonder if immediate generalization is also prevented to other CSs that are structurally the same during initial and generalizations tests. To test this possibility, we used delay eyeblink conditioning procedures to train rats to associate a 500-msec light CS with a 25-msec periorbital electrical stimulation US (2-3 mA) in the presence of a static 2-kHz background tone. We then tested for immediate generalization by presenting rats with light-alone trials while a different background tone (8-kHz) was played. We found that rats showed robust immediate generalization when the background tone frequency was changed. This outcome stands in contrast to the results obtained in our prior experiment. The difference in findings between the experiments can probably be attributed to the sensory demands imposed during initial training. When the background was a sound and the CS was a light, the visual neural pathway was engaged and the background sound was seemingly ignored. In the

circumstance when both the background sound and the tone-off CS were processed within the auditory system, it was the absence of a specific frequency that mattered, not merely the presence of a quiet period.

Moran KM, Enstrom AE, Jarrell L, Khashchuluun M, Tran A, Delville Y (*UT Austin*) Hamsters as an animal model for stress and obesity ABSTRACT: Hamsters present unique characteristics reminiscent of humans, such as weight gain under stress, similar metabolic and lipid profiles, and food hoarding behavior. This makes them well-suited for studies on obesity. In hamsters, a two-week exposure to chronic social stress in adolescence causes a consistent 10% increase in weight gain, food intake, and body fat. The present studies addressed effects of stress exposure on food-related motivation through observations of food hoarding and conditioned place preference (CPP). Male Golden Hamsters (n=7-8/group) were exposed to a resident-intruder or clean cage control condition for 20 minutes per day from postnatal day 28 to postnatal day 42 (early- to mid-adolescence). In the last five days of the stress period, subjects were allowed to habituate to a V-shaped arena with a blank start area and two distinctly textured wings for 10 minutes at least one hour after stress. After two habituation days, a bowl with banana-flavored pellets was placed in one wing of the arena with subjects allowed to collect as much as desired. On the fifth day (CPP), the food bowl was absent. On the food hoarding day, stressed and non-stressed subjects both spent more time in the food wing. Although animals from both groups spent equal time in the food wing, stressed subjects collected twice as much food as controls. However, on the CPP day, stressed hamsters spent significantly less time in the food-associated wing and more time in the opposite wing. Food hoarding differences likely exemplify that stressed individuals value food more or require more food to reach similar feelings of reward compared to controls. However, CPP differences are likely associated with a form of frustration having to do with lack of expected rewarding stimuli. Indeed, adult hamsters exposed to unexpected long delays in previously rewarding behaviors rapidly cease those behaviors and spend less time in areas previously associated with rewards. Together, altered motivation for food and frustration in response to delayed rewards are contributing factors to later-life increased body weight.

Moriarty SK, Schoenberg HL, Winterbauer NE, Hammack SE, Toufexis DJ, Todd TP *(U Vermont*) ABA and AAB fear renewal in male and female rats ABSTRACT: Pavlovian extinction reduces the performance of conditioned responses and occurs when the conditioned stimulus (CS) is presented repeatedly in the absence of the unconditioned stimulus (US). However, when the CS is experienced in a different context from the extinction context, there is a recovery of the conditioned response, a phenomenon known as renewal. Context includes both external components (e.g., sensory features such as olfactory, tactile and visual factors) as well as internal states such as stress and hunger. There is evidence that male and female rats behave differently during a test of appetitive renewal, such that female rats fail to exhibit renewed responding. In addition to sex differences in appetitive ABA renewal, there is recent research that suggests there may be a sex difference in AAB fear renewal as well. In both fear and appetitive preparations, the lack of renewal in females has been postulated to be related to cycling ovarian hormones. Thus, in the present set of experiments we directly compared males and females in ABA (experiment 1) and AAB (experiment 2) fear renewal. We found that males and females exhibited fear renewal in both ABA and AAB designs. Additionally, in experiment 3, we tested the idea that hormonal state may play a role in ABA renewal by including an ovariectomized (OVX) female group in addition to males and intact females. Replicating experiment 1, both males and females exhibited fear renewal. Furthermore, we observed renewal in the OVX female group as well. Together, these results suggest that there may not be a sex difference in fear renewal as previously thought.

Mousset M, Trombetti K, Ruben E, Ruben M, David J, Chu P, Huynh TN (*Midwestern U*) Chronic mild stress leads to anxiety-like behavior and decreased p70 S6K1 activity in the hippocampus of male mice ABSTRACT: Major affective disorders are highly prevalent in the United States, but current treatments are limited in their effectiveness due to a lack of understanding in underlying molecular mechanisms. Recent studies have shown that reduced activity of p70 S6 kinase 1 (S6K1), a downstream target of the mechanistic target of rapamycin complex 1 (mTORC1), is linked to anxiety-like and depression-like behavior in both humans and rodents. The purpose of this study was to investigate the relationship between S6K1 and anxiety-like behavior following chronic mild stress (CMS) and drug induced inhibition of S6K1. Following CMS, anxiety-like behavior was evaluated using the open field (OF) and elevated plus maze (EPM) in adult male C57/Bl6 mice. After behavior analysis, samples of the hippocampus were harvested for quantification of S6K1, S6 ribosomal protein, GSK3β and beta tubulin via western blot. Our results demonstrate that CMS mice exhibit anxiety-like behavior in the OF and EPM and reduced activity of S6K1 in the HPC. We measured phosphorylation levels of GSK3β and found that GSK3β phosphorylation was also reduced following CMS compared to control mice. Furthermore, pharmacological inhibition of S6K1 with PF-4708671 in male mice was sufficient to produce anxiety-like behavior in the OF and EPM. These results further support the significant role of S6K1 in the pathogenesis of anxiety and affective disorders.

Msengi HD, Felix-Ortiz AC, Magalhães G, Diehl MM, Burgos-Robles A (*UT San Antonio*) New insights on the mechanisms of active avoidance behavior during signaled threat: Prefrontal contributions and behavioral differences across sex and age groups ABSTRACT: Flexible adaptation of behavior during imminent threat is critical for safety and survival. A type of behavioral adaptation that is typically implemented during environmental threat is active avoidance in which specific actions or behavioral sequences are dynamically engaged to prevent harm from threat. While previous studies suggest that the medial prefrontal cortex (mPFC) is implicated in the learning of active avoidance responses, the role of discrete subregions of the mPFC – known in rodents as the prelimbic (PL-PFC) and infralimbic (IL-PFC) cortices – still remain unclear. In this project, we are implementing a novel behavioral paradigm in which mice are capable of developing flexible avoidance responses during a tone cue that predicts electric shock punishment, by stepping onto a small non-electrified platform that is dynamically presented into the behavioral chamber during tone-shock trials. Our results have so far revealed that over the course of five daily training sessions (20 trials/day), a large proportion of young-adult mice can develop robust active avoidance responses to avoid incoming shocks. However, optogenetic-mediated silencing of principal glutamatergic neurons in the IL-PFC subregion led to a significant impairment in the development of active avoidance responses, whereas neuronal silencing in the PL-PFC subregion produced no significant effects. In addition, our results suggest that female mice can learn active avoidance in more optimal manners than male mice, even in significantly aged groups (16 months old). Although potential differences in IL-PFC signaling across sexes and ages still remain to be elucidated, we hypothesize that IL-PFC signaling could be an important factor that promotes better avoidance learning in certain groups and/or individuals.

Murray JA, Ke-Lind PL, Schuh KM, Alltop KW, Tronson NC (*U Michigan*) Partial reinforcement prevents blocking: considering a role for affect in Pavlovian conditioning ABSTRACT: Pavlovian fear conditioning has been extensively used to study the neurobiology and psychology of fear- and anxiety-related disorders including Post-Traumatic Stress Disorder (PTSD) and phobias. And yet, whereas we typically describe learned associations in fear conditioning as being predictive (animals learn that a cue predicts the shock occurrence), PTSD and other anxiety disorders are characterized by an excessive fear in situations that do not predict danger. Moreover, there are many experimental paradigms that result in strong conditioned fear responses but poor prediction (e.g., partial reinforcement). In appetitive Pavlovian conditioning, it is widely accepted that animals learn both prediction and motivation/affective information including valence, value, and incentive motivation. In this project, we aim to determine whether context fear conditioning results in multiple types of associations including motivation/affective information in addition to predictive associations. We used context fear conditioning and varied predictability with high- vs low-prediction training (continuous vs partial reinforcement) and varied US valence with high vs moderate shock intensity (0.8mA vs 0.4mA). We assessed Blocking - the ability of the context paired with shock to block new CS (tone)-shock conditioning - as an index of prediction. We observed significant blocking of the new tone-shock association in continuous reinforcement-trained mice (CRft) at both 0.8mA and 0.4mA shock levels. Notably, this blocking effect was driven by male animals. We also observed strong context generalization in partial reinforcement-trained mice (PRft), an effect that was stronger at 0.8mA compared with 0.4mA shock levels. This generalization of freezing from training to test context complicated the interpretation of blocking in PRft animals. Nevertheless, differences in context generalization between CRft and PRft and between 0.8mA and 0.4mA shock levels suggests that additional non-specific/non-predictive processes are more evident after PRft-training and at higher shock intensities. Together, these findings support the idea that prediction is not sufficient to explain all fear conditioning, and that fear conditioning memories are likely constructed from multiple associations incorporating prediction as well as factors including value, valence, and other affective/motivational information. These non-predictive associative elements and their underlying neural circuits in learned fear may be critical factors for understanding the etiology and neurobiology of PTSD and other anxiety disorders. This work was funded by UMOR APSF to NCT.

Nerz JH, Bond SR, Miranda A, Gillespie C, Leising KJ (*Texas Christian U*) Reinforcer Value Affects the Emergence of the Differential Outcomes Effect in Rats ABSTRACT: Learning to make different responses in the presence of different stimuli is facilitated by the delivery of different outcomes (e.g., food vs. water) for each response. The current research aimed to extend this differential outcomes effect (DOE) to rats performing a visual discrimination and to compare the effects of using higher valued reinforcers (i.e., chocolate pellets) to lower valued reinforcers (i.e., chow pellets) on learning. During training, a left lever (LL) press was reinforced in the presence of one discriminative stimulus (SD, e.g., flashing light), whereas a right lever (RL) press was reinforced during the other visual stimulus (e.g., steady light). Rats in the differential outcomes (DO) groups received a different outcome for each correct response. For half of the DO group, correct responses were reinforced with chow pellets and an 18% (w/w) sucrose solution, and the other half received chocolate pellets and 30% (w/w) sucrose as reinforcement. Mixed-outcome (MO) groups received both outcomes (either chow pellets and 18% sucrose or chocolate pellets

and 30% sucrose) for both responses. As expected, the DO conditions acquired the discrimination faster than their respective MO control groups. When compared to chance (.50), performance in the MO chow pellet group rose above chance one block sooner than the MO chocolate group, which could be due to the unpredictability of the high-value reinforcers.

Nishimura KJ, Paredes D, Nocera NA, Drew MR (*UT Austin)* Role of the paraventricular thalamus in stress-induced fear sensitization ABSTRACT: 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. Next, c-fos analysis was performed to identify candidate brain regions that mediate fear sensitization. We identified several brain regions that were hyperactive in stressed animals including the paraventricular thalamus, dorsolateral periaqueductal gray, and the lateral parabrachial nucleus. Using fiber photometry, we confirm *in vivo* that stress leads to potentiated activity in the paraventricular thalamus in response to aversive or threatening stimuli. Finally, we used chemogenetic manipulations to show that the paraventricular thalamus is both necessary and sufficient for fear sensitization to novel tone. 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.

Oak SS, Lauraine E, Nguyen C, Rincón-Cortés M (*UT Dallas*) Effects of early life scarcity-adversity on developmental milestones in male and female rats ABSTRACT: Developing rats go through many fundamental maturational milestones that can be divided into categories: some are physical, whereas others involve developmental reflexes. There is evidence that early life adversity interferes with normal growth and development in rodents, and that effects may be sex-dependent even at an early age (Demaestri et al. 2020; Eck et. al, 2020; Demaestri). Moreover, although many studies have shown a long-term negative impact of early life adversity in rodents, literature regarding early life scarcity-adversity effects on maturational milestones in rats is scarce. We hypothesized that early life scarcity-adversity would delay physical and reflex development in male and female rats. To this end, we assessed the impact of scarcity-adversity, implemented via reduced bedding during postnatal days (PND) 2-9, on physical landmarks by measuring weight gain, incisor presence, fur development, eye opening, and pinnae detachment (n=7 litters per group). We also assessed the effect of early life adversity on developmental reflexes by measuring surface righting reflex (PND 6), grasp reflex (PND 8), negative geotaxis (PND 10), cliff avoidance (PND 12), bar holding (PND 14), and auditory startle (PND 16). Our findings suggest that scarcity-adversity negatively impacts a subset of physical landmarks and developmental reflexes in preweaning rodents in a sex-dependent manner. Our results extend previous research examining the effects of early life adversity, and specifically scarcity-adversity, on developing male and female rodents. In the broader context of the published work, our findings suggest that early life scarcity-adversity results in more pronounced detrimental effects during later development (adolescence, adulthood).

Oleksiak CR, Plas SL, Carriaga D, Vasudevan K, Maren S, Moscarello JM (*TAMU*) Ventral hippocampus regulates inter-trial responding in a two-way signaled active avoidance task in male and female rats ABSTRACT: The hippocampus has a central role in regulating contextual processes in memory. We have recently shown that pharmacological inactivation of ventral hippocampus (VH) attenuates the context-dependence of signaled active avoidance (SAA) in rats. Here we explored whether the VH and its efferents to the bed nucleus of the stria terminalis (BNST) mediate intertrial responses (ITRs), which are presumed to be context-dependent avoidance responses that occur in the conditioning context. In Experiment 1, male rats underwent SAA training and subsequently received intra-VH infusions of saline or muscimol immediately before a retrieval test in the conditioning context. While muscimol had no effect on avoidance responses, rats that received muscimol performed significantly fewer ITRs compared to rats that received vehicle. In Experiment 2, we asked whether chemogenetic activation of the VH would increase the vigor of ITRs. In male and female rats expressing excitatory (hM3Dq) DREADDs, systemic CNO administration produced a robust increase in ITRs that were not due to nonspecific increases in locomotor responding. In Experiment 3, we examined whether these effects are mediated by VH projections to the BNST, a brain area that has been implicated in both defensive responses to aversive contexts and SAA performance. To selectively activate VH terminals in BNST, we expressed hM3Dq in the VH and

made intra-BNST CNO infusions prior to retrieval testing. Chemogenetic activation of hM3Dq-expressing VH terminals in the BNST did not affect ITRs during retrieval testing. However, systemic administration of CNO once again increased ITRs when animals underwent retrieval testing in a novel context. There were no sex differences in any of these effects. Finally in Experiment 4, to determine if ITRs are mediated by context-US associations, we exposed rats to the conditioning context for three days after training to extinguish the context. Rats submitted to context extinction did not show a reliable decrease in ITRs during a retrieval test, suggesting that context-US associations are not responsible for these responses. Collectively, these results reveal an important role for the VH in intertrial responding during SAA. Further work is required to explore the neural circuits and associative basis for these responses, which may underlie persistent and pathological avoidance that occurs in humans after a threat has passed.

Olvera ME, Raskin M, Cofresi RU, Gonzales RA, Monfils MH, Lee HJ (*UT Austin/ U Missouri*) Extinction after memory retrieval reduces conditioned response to alcohol cues in male and female rats with a history of alcohol dependence ABSTRACT: Monfils et al. (2009) initially showed that retrieval+extinction, a paradigm that conducts an extinction session following memory retrieval, is more effective than standard extinction in preventing return of conditioned fear. We recently extended the finding to alcohol use and showed that retrieval+extinction is also more effective at reducing alcohol seeking behavior than extinction alone, using male rats with a history of moderate drinking (Cofresi et al., 2017). In this study, we tested the effectiveness of retrieval+ extinction on both male (Experiment 1) and female (Experiment 2) rats with a history of heavy alcohol exposure and dependence. Long-Evans rats were induced into alcohol consumption using 2 bottle choice procedure (15% unsweetened ethanol, 15E) on an intermittent 24hr schedule lasting 5 weeks. Then, they were randomized to 10 days of chronic intermittent exposure (CIE) to ethanol vapor or air, forming the alcohol dependent group and control (non-dependent) group, respectively. Over the next 12 daily sessions, rats received a light cue paired with 15E via sipper. To compare retrieval+extinction and standard extinction paradigms, rats were assigned to either RET+EXT group or EXT group, respectively, and received 14 daily extinction sessions. 48hr after the final extinction session, we tested for return of alcohol seeking behavior by measuring sipper site approach in the presence of light alone and sipper licks in the presence of light and an empty sipper. When tested for return of alcohol seeking, neither male nor female rats assigned to the RET+EXT group showed a significant return of sipper site approach regardless of their CIE grouping. In contrast, male rats assigned to the EXT group showed a significant return of sipper site approach behavior, regardless of their CIE grouping. Female rats from the EXT group showed a significant return of sipper site approach, but their air counterparts did not. Nonetheless, both male and female rats assigned to the RET+EXT group did not show a significant return of sipper licks regardless of their CIE grouping. In the EXT group, regardless of their CIE grouping, male but not female rats showed a significant return of sipper licks. Our data replicates the results from our previous study done in males with a history of moderate drinking and further extends it to females. We also show the sustained efficacy of retrieval+extinction in both male and female rat models of alcohol dependence. Finally, our data suggest that there exists variability in alcohol-seeking behavior amongst the sexes as a result of extinction. This work was supported by R01 R01AA029386 (HJL) and T32 AA007471 (MEO).

O'Malley JJ, Ma J, Kreiker M, Penzo MA (*NIMH*) Non-canonical cortico-thalamic dynamics gate avoidance decisions ABSTRACT: The cortex exerts top-down control of the thalamus by either enhancing or suppressing incoming sensory signals to coordinate a behavioral response. The prelimbic area (PL) of the prefrontal cortex is critical for selection of avoidance behaviors. Yet it is unclear how the PL exerts top-down control over the thalamus to shape the selection of avoidance behaviors. Recent work has shown that the paraventricular nucleus of the thalamus (PVT), a part of the limbic system and has been shown to receive PL input, is integral for driving avoidance behavior. To assess if the PVT projecting PL neurons (PL-PVT) shapes the selection of avoidance behaviors, we used a 2-way active avoidance (2AA) paradigm. Briefly, mice were placed in a 2 chambered box and learned to avoid a footshock by shuttling to the opposite chamber in response to a warning signal (WS). Using fiber photometry, we recorded the activity of PL neurons during 2AA and found that PL-PVT showed bidirectional responses with increased activity to the WS and reduced activity when initiating shuttling to avoid or escape the footshock. The reduction in activity in PL-PVT is at odds with findings that PVT activity increases during avoidance, suggesting that PL might couple to PVT via disinhibitory mechanisms. Given that the PVT lacks interneurons, inhibition must come from elsewhere. One candidate for mediating this effect is the thalamic reticular nucleus (TRN), a GABAergic nucleus that solely targets the thalamus and receives cortical input that results in strong thalamic inhibition. To determine if the PL exerts a largely inhibitory effect over the PVT via the TRN, we used ex vivo slice electrophysiology with optogenetics and found that the PL sends weak excitatory inputs to the PVT whereas the TRN receives strong excitation from the PL and robustly inhibits the PVT. Next, using fiber photometry, we found that PL-TRN and TRN-PVT had bidirectional responses mirroring PL-PVT. Importantly, responses to the WS only occurred during avoidance trials, suggesting that the brief activity during the WS and subsequent reduction shapes the expression of avoidance.

Indeed, optogenetic excitation mimicking the brief increase in activity increased avoidance. A key feature of thalamic cells is rebound activity following inhibition. The brief activation and reduction of TRN may lead to increased activity in PVT as observed in a fiber photometry experiment, suggesting a timing mechanism through coordinated disinhibition and subsequent excitation via the PL. Taken together, our findings identify a novel circuit (PL-TRN-PVT) that is critical for the expression of avoidance. This work was supported by: NIGMS PRAT.

Ortega ME, Hoang IB, Sharpe MJ (*UCLA*) The motivational properties of drug-paired cues on model-free and model-based associations ABSTRACT: Our previous experience with rewards- such as food, drugs, or social interaction- guides our future decision-making toward those rewards. Decision-making is governed by instrumental processes, in which an individual can control the environment to cause outcomes. Pavlovian processes also influence decision-making and allow us to adjust our behavior in response to environmental cues (e.g. context, people, etc.) that predict outcomes. Decision-making is typically characterized as being either model-based or model-free in nature. Model-based systems favor a goal-directed approach in which a cognitive representation of the reward is used to guide responding, allowing for flexible behavior that can be updated on-the-fly. Model-free systems, however, rely on a habitual strategy, in which responding is based only on previous experience and does not involve a representation of the reward being pursued. Addiction is generally thought to result in part from maladaptive model-free (habitual) responding directed towards drug rewards. However, there is less understanding of how drug-paired Pavlovian cues in the environment influence decision-making toward drug rewards. In contrast to the habit theory of addiction, our lab has previously found that food-paired cues exert enhanced model-based control over instrumental responding following methamphetamine experience (Hoang et al., 2023). In the present study, we investigated how drug-paired Pavlovian cues might influence decision-making directed toward drug rewards using a Pavlovian-to-Instrumental transfer paradigm. This allowed us to examine whether drug-paired cues also promote model-based control over decision-making directed to drug rewards. These findings provide insights into the complexity of addiction and highlight the importance of drug-paired cues in influencing decision-making in addiction. Future research will examine the relationship between a history of drug use and susceptibility to drug-paired cues over decision-making.

Paredes D, Drew MR (*UT Austin*) Effects of stress on formation and retrieval of hippocampal ensemble representations of contextual fear memory ABSTRACT: The stress-enhanced fear learning (SEFL) model recapitulates important and understudied components of PTSD, such as stress-induced sensitization of fear learning. The SEFL procedure utilizes components of Pavlovian fear conditioning but generates a marked stress-sensitized fear response. The SEFL procedure entails exposing mice to footshock stress in context A (10 shocks, 2 s, 1 mA). On a separate day, mice receive a single shock at a lower intensity (0.75mA), in a separate context. 24 hrs later, mice are placed in context B and tested for fear recall. Stressed mice exhibit higher levels of freezing in context B compared to controls. We used the SEFL model to investigate the effects of stress on fear memory encoding and retrieval in the dentate gyrus and CA1. Fear memory representations in the hippocampus were investigated using activity-dependent neuronal tagging with FosTRAP/Ai6 mice, whereby cells activated during a discrete event are indelibly labeled using the immediate early gene (IEG) promoter elements to initiate the expression of a tag (zsGreen) following injection of tamoxifen. Prior research shows that tagged fear engram cells are reactivated (at a rate higher than chance) during fear memory recall, and the degree of reactivation of fear engram cells is correlated with the percentage of freezing during fear memory recall. We used the FosTRAP2/Ai6 system to investigate how prior stress exposure affects formation and reactivation of contextual fear ensembles. Male and female FosTRAP2/Ai6 mice received shock stress (or exposure to the context) on day 1. From days 2-5, mice were re-exposed to the stress context (context A) to extinguish fear of the stress context and reduce generalization to context B. On day 6, mice received 1-shock conditioning in context B and immediately received an injection of 4-OHT (55mg/kg). On day 7, mice were tested for fear recall and were perfused 90 minutes after testing. We examined 1) number of zsGreen+ fear engram cells), 2) number of recall-activated cells (Fos+cells), and 3) number/percentage of fear engram cells reactivated during recall (Fos+ and zsGreen+) in the dentate gyrus and CA1. Our preliminary results show that prior stress increases the number of fear engram cells in the dorsal dentate gyrus, but does not change the number of activated cells (Fos+) during recall, or the total number of reactivated engram cells (Fos+ and zsGreen+ cells/mm2). Thus, stress enhances the encoding of contextual fear memory, but does not affect the reactivation of a contextual fear memory. Ongoing experiments will determine if stress affects the number of cells in the fear engram, fear recall, or the number of co-activated cells in CA1 and will examine differentiation along the dorsal-ventral axis. These studies are expected to reveal novel mechanisms for the stress-induced sensitization of fear learning.

Parekh PK (*Weill Cornell*) Frontocortical circuits encode reward- and effort-related information and are sensitive to chronic stress ABSTRACT: The ability to integrate information to drive high utility behavior is critical for survival. Individuals must continually update and weigh the value of rewards available in the environment against real

and perceived effort costs and select appropriate actions. A deficit in this process, known as effort valuation (EV), is characteristic of anhedonia, a core symptom of several neurological and neuropsychiatric conditions including depression. Anticipation of reward availability and effort expenditure may be particularly relevant in depression, and involves a complex interaction of limbic and cognitive functions. Moreover, epidemiological studies provide ample evidence that chronic stress is a significant risk factor for depression in patients and elicits anhedonia in animal models. Understanding the circuit and molecular mechanisms that contribute to a depressive brain state and how they differ from the healthy condition is integral to the development of more efficacious therapeutic strategies. We employed two parallel approaches to examine the contribution of prefrontal circuits in supporting effort valuation. Using a barrier T-maze task integrated with fiber photometry recording and optogenetic inhibition, we found that corticostriatal signaling is necessary for maintaining future effortful reward seeking and is diminished with exposure to chronic stress. In order to determine encoding properties of individual prefrontal projection neurons, we developed a head-fixed EV task in which mice were conditioned to respond to reward- and effort-predictive stimuli by licking a spout. Concurrent 2-photon calcium imaging revealed that distinct prefrontal projection neurons exhibit heterogeneous responses to reward- and effort-predictive cues and varied feature selectivity at baseline, with coding of reward anticipation and consumption represented within each recorded population. Interestingly, exposure to chronic stress alters the selectivity of individual corticostriatal neurons and the accuracy of decoding trial features from population activity. Together, these results suggest that stress may bias animals toward low-effort responding in part by diminishing the coding efficacy of reward- and effort-related stimuli in relevant pathways.

Parker RK, Padival M, Selby D, Ferrara NC (*Rosalind Franklin*) Social instability interferes with fear elevation ABSTRACT: Disruptions to fear learning and memory processes are characteristic of several neuropsychiatric disorders, like post-traumatic stress disorder (PTSD). These fear processes are impacted by social circumstances, where the social environment can have protective effects when positive or negative effects when stressful. This highlights a need to understand interactions between the social environment and fear learning and memory. Here, we manipulated the social environment using social instability, where partners are rotated 4 times every 48-hours with novel age-matched conspecifics. The following day, groups underwent contextual fear conditioning consisting of 5 footshocks (0.5mA) followed by retrieval and testing sessions each separated by 24-hours. During the retrieval session, groups received two different treatments: 1) a standard retrieval, in which rats were exposed to the chamber in the absence of footshock, or 2) an elevation condition, in which rats received two additional mild footshocks (0.2mA). Consistent with prior work, we found that control housed rats showed more freezing in the elevation group relative to the standard retrieval group. However, groups exposed to social instability fail to show this elevation in fear compared to their retrieval counterparts, suggesting that social instability interferes with fear elevation. The basolateral region of the amygdala (BLA) is critical for the formation and storage of context fear memories and is sensitive to the social environment. The BLA may therefore show differential patterns of activity in response to fear retrieval day manipulations as well as social instability. To test this, we quantified neuronal activity in the BLA using extracellular in vivo recordings in anesthetized rats. We found greater spontaneous firing in BLA principal neurons in the control housed elevation group when compared to the control housed retrieval group, whereas this effect was absent in social instability groups. This work collectively suggests that fear memory retention can be impacted by the social environment, and BLA neuronal activity mirrors these environmentally-driven changes in fear.

Penna SR, Ng Q, Watts ME, Zhao A, Cazares VA (*Williams*) Severe stress produces maladaptive cognitive and emotional function via non-associative sensitization ABSTRACT: Psychiatric comorbidities and an increased risk of subsequent traumatic experience are common in post-traumatic stress disorder (PTSD). Stress-enhanced fear learning (SEFL) is an effective paradigm for modeling PTSD and trauma-induced maladaptive affective behavior. Following a severely stressful event, rodents exhibit exaggerated conditioned fear responses to mild unconditioned stimuli (US). This SEFL effect is a result of fear sensitization and has been found to be non-associative, potentially explaining deficits in other behavioral domains. We assessed the effects of a severe stressor, specifically repeated mild footshocks, on phenotypes unrelated to the initial SEFL procedure, such as depressive-like, anxiety-like, and reward-seeking behaviors. Furthermore, we compared neural activation between sensitized and non-sensitized fear memory traces or engrams. SEFL was induced using 10 unsignaled footshocks prior to a 1-US contextual fear conditioning protocol. As expected, fear sensitization was observed in an unreinforced context test among stress-exposed mice. Following the SEFL paradigm, open-field (OFT), forced swim (FST), and progressive ratio (PR) nosepoking tasks were performed. We found that the stress-exposed mice exhibited maladaptive anxiety-like behaviors as characterized by the OFT, and may have expressed diminished reward-seeking behaviors, as characterized by the PR task. Using dual labeling for targeted recombination in active populations (TRAP) and Fos immunostaining, we are currently investigating how neural representations for both sensitized and non-sensitized

fear memories differ. Upon examining the behavioral domains and neural populations that are affected by severe stressors, we can contribute to the understanding of the mechanisms driving increased susceptibility to trauma-induced behavioral dysregulation. This work was supported by: NIH R15-MH129947.

Piantadosi P, Perry S, Coden K, Sandon Veliz R, Coden K, Choi H, Spitz N, Schwab N, Authement M, Alvarez V, Schaffer J, Da Silva D, Goff J, Halfeld M, Holmes A (*NIAAA*) Amygdalar regulation of punished decision-making ABSTRACT: Cost/benefit analyses are disturbed in substance use disorders and have been suggested to be mediated by interactions between cortico-limbic-striatal circuits. Within these circuits, the basolateral amygdala (BLA) has been shown to be critical for aversive costs like punishment to produce flexible changes in actions. Yet, it remains unclear how the BLA facilitates such flexibility. Here, we used in vivo 1-photon imaging, fiber photometry, and optogenetic manipulation in male mice to detail BLA function during cost/benefit decision making, identifying a BLA to nucleus accumbens shell (NAcSh) projection that contributes to action flexibility. Mice were first trained to discriminate between one reward port that delivered a large amount of milkshake reinforcement, and another that delivered a small amount. Mice then received risky decision-making task (RDT) sessions whereby selection of the large reward was associated with a minor footshock that ascended in likelihood across three trial blocks (0, 50, and 75% probability). Selection of the small reward option was never punished. Throughout training and RDT sessions, GCaMP6-based fluorescence was imaged using microendoscopic 1-photon imaging. During reward magnitude training, subsets of BLA neurons displayed consistent responses to rewarded events during the pre-choice and post-choice period, while others developed or lost responsivity over multiple days of training. The introduction of punishment risk during the RDT dramatically affected behavior: mice deliberated longer prior to making a choice, displayed behaviors characteristic of anxiety-related indecision, and gradually shifted their choice towards the small, safe reward. This shift was associated with changes in BLA neuron activity: for example, many neurons encoded footshock punishment, with most being recruited from a pool of cells that were not reward-excited. We next assessed whether signaling in a discrete BLA-NAcSh pathway was sensitive to risk and causally related to flexibility. Using projection-specific fiber photometry and 1-photon imaging, we observed that the BLA-NAcSh population was less sensitive to footshock punishment than the overall BLA population, but pathway activity preceding actions was significantly inhibited by punishment risk. To test whether this inhibition causally contributed to actions, we optogenetically excited BLA terminals in the NAcSh, which produced a robust increase in risk-seeking. These data provide novel insight into the manner in which BLA processes punishing information, with ongoing experiments exploring further differences in projection-specificity and transcriptional profile.

Pifer GC, Bellfy L, Kwapis JL (*Penn State*) Examining sex differences in memory performance across the day-night cycle ABSTRACT: The brain's "master clock" is the suprachiasmatic nucleus (SCN) of the hypothalamus, but there are additional satellite clocks within other regions of the brain and body. For instance, the dorsal hippocampus (DH) has diurnal oscillations in activity that may affect a range of functions, including long-term memory consolidation. Long-term memory is defined as memory that persists over a long period of time, which is operationally defined as lasting 24 hours or more following acquisition. Our lab has previously shown that male mice demonstrate better long-term memory performance during the day than at night using the hippocampus-dependent Object Location Memory (OLM) task. Additionally, we have shown that the circadian gene *Per1* oscillates in tandem with this memory performance, with the peak of expression occurring during the day. Knocking down *Per1* locally in the hippocampus impairs memory formation but has no effect on either circadian rhythm or sleep behavior. This demonstrates the possibility of a non-canonical role for *Per1* in modulating memory directly within memory-relevant brain regions like the DH. However, as these studies exclusively used male subjects, it is unknown whether the same mechanisms exert similar diurnal control over memory in females. Here, we tested OLM in a female cohort at various diurnal timepoints, to determine whether memory oscillates in the same manner in male mice. We also measured *Per1* levels to determine whether this gene might also regulate diurnal oscillations in memory in females. Finally, we are also testing whether *Per1* contributes to age-related memory impairments in female mice, as age-related repression of hippocampal *Per1* is known to contribute to memory impairments in old male mice. Together, our work indicates that *Per1* is a critical mechanism that operates within satellite clocks, most notably the hippocampus, to regulate memory across the diurnal cycle.

Plas SL, Oleksiak CR, Moscarello JM, Maren S (*TAMU*) Acute Stress Facilitates Two-Way Signaled Active Avoidance in Male and Female Rats ABSTRACT: Post-traumatic stress disorder (PTSD) is a debilitating disorder characterized by excessive fear and dread, hypervigilance, and deficits in extinction learning. Stress enhanced fear learning (SEFL) models several features of PTSD, including the long-term sensitization of associative learning induced by acute stressors. Previous work has explored the consequences of the SEFL procedure on the acquisition of Pavlovian fear conditioning, but it is not known whether acute stress also impacts aversive

instrumental conditioning, such as avoidance learning. Here, we explored the effects of the SEFL procedure on two-way signaled active avoidance (SAA), a form of instrumental learning in which rats learn to prevent an aversive outcome (shock) by performing a shuttling response when exposed to a warning signal (tone). Male and female rats were submitted to a SEFL procedure in which rats were placed in a distinct context (Context S) and delivered 15-randomly timed shocks across a 90-minute period; control animals were placed in the SEFL context for 90 minutes in absence of shock. One week after the stress procedure, the rats were submitted to four days of SAA training (Context A) and 3 days of SAA extinction in the training context (Context A) or a novel context (Context B). SEFL did not alter the likelihood of animals acquiring the SAA contingency; the number of poor avoiders (animals with average avoidance responses of 6 or less across D2-D4 of training) was similar in the two groups. However, the SEFL procedure significantly enhanced SAA acquisition rate in females, but not males. In particular, female rats exhibited significantly greater avoidance responding on the first day of training relative to controls, reaching similar levels of performance by the second day. Males that underwent the SEFL procedure showed similar rates of acquisition to controls but exhibited resistance to extinction. This was manifested as both elevated avoidance and intertrial responding across extinction days relative to non-stressed controls, an effect that was not observed in females. Together, these results reveal that acute stress facilitates SAA performance in both male and female rats, though the nature of this performance facilitation takes different forms in the two sexes. Future work will elucidate the neurobiological mechanisms underlying the differential effect of stress on instrumental avoidance in male and female rats.

Porras A, Rodney-Hernández P, Rincón-Cortés M (*UT Dallas*) That's stimulating! Assessing the effects of early life sensory overstimulation on later life behavioral function in rodents ABSTRACT: Children today are immersed in electronic technology shortly after birth. For example, children now begin regularly watching television earlier than they did in the past, with many new programs being geared towards young infants and containing lots of lights, color and sounds. This excessive media exposure may constitute a form of sensory overstimulation during early life, which is thought to contribute to cognitive and behavioral changes in children. Here, we sought to model excessive media exposure in developing male and female rodents by adapting an audio and visual sensory overstimulation paradigm (Christakis, D et al., 2012), which started at postnatal day (PND) 10 and lasted 6 hours a day for 30 days. Following a 9-day rest period, animals underwent a behavioral test battery to assess anxiety-like, spatial/working memory, social motivation, and compulsive behavior. Sensory overstimulated animals (n=7-8 per group) spent significantly more time in the open arms ($p < 0.05$) and reduced time in the closed arms ($p < 0.05$) of the elevated zero maze compared to controls, suggesting reduced anxiety-like behavior in overstimulated rats. These results are in line with the original findings reported in newborn male mice (Christakis, D et al., 2012). However, no significant differences were found for novel object recognition, social approach, marble-burying, or T-maze. This study extends prior work conducted in mice into rats and includes both sexes. Our findings suggest limited effects of sensory overstimulation on later life behavior, although future work will be aimed at determining if these effects vary by sex.

Privratsky MA, Lyvers DP, Gabriel DBK, Lapish CC, Sangha S (*Indiana U School of Med*) Neural Correlates of Safety Signaling and Conditioned Inhibition of Fear in the Infralimbic Prefrontal Cortex ABSTRACT: The ability to accurately discern between safe and threatening situations is challenged in Post-Traumatic Stress Disorder (PTSD), often leading to maladaptive fear. Many PTSD individuals fail to show conditioned inhibition of fear, the downregulation of fear during the simultaneous presentation of a learned fear and safety cue (fear+safety cue). Our lab has a well-validated safety-fear-reward cue discrimination task, which was adapted for this study to produce a greater range in fear suppression in male and female Long Evans rats, such that approximately half of males and females show "good" fear suppression, while the other half do not. During this behavior we collected longitudinal single cell calcium activity within the prelimbic (PL) and infralimbic (IL) regions of the ventromedial prefrontal cortex (vmPFC) in freely behaving male and female Long Evans rats using miniscopes and GCaMP6m expressed via AAV1.GCaMP6m.WPRE.SV40 from Inscopix. These vmPFC regions play key roles in modulating fear, anxiety, and stress behaviors. We have shown in our safety task that the PL is necessary for fear expression, while the IL is necessary for conditioned inhibition of fear. Our data in the IL indicate that there is an ensemble of neurons highly correlated in its activity to the fear+safety and fear cues that is separate from an ensemble of neurons highly correlated in its activity to the fear+safety and reward cues. Preliminary results show that we can also follow these same cells for at least 12 days. Current analyses are examining cue-evoked calcium transients against expressed safety, fear and reward behaviors across the entirety of the 15 day task.

Pyon WS, Blaes SL, Orsini CA, Faraji M, Viera OA, Singhal SM, Frazier CJ, Bizon JL, Setlow B (*U Florida*) Investigating the functional role of ventral tegmental area dopamine neurons in decision making under risk of punishment ABSTRACT: A prevailing theory regarding the role of dopamine in reward-motivated behaviors is that dopaminergic neurons of the ventral tegmental area (VTA) signal discrepancies between predicted and actual outcomes of a particular event. This theory of reward prediction error

signaling further implies that VTA dopamine neurons also play a role in cost-benefit decision-making in which outcomes can include reward alongside the potential for punishment. To elucidate the functional role of VTA dopamine neurons in decision making under risk of punishment, male and female tyrosine hydroxylase (TH)-Cre transgenic rats were injected with Cre-dependent GCaMP and trained on a risky decision making task in which they made discrete choices between a small, "safe" food reward and a larger food reward accompanied by ascending risk of mild footshock (0%, 25%, 75%). *In vivo* fiber photometric recording of VTA dopamine neurons revealed an increase in neuron activity during receipt of the large reward in the absence of probabilistic punishment, and this increase was greater in blocks of trials in which there was some chance of punishment (25% and 75%) vs. no chance of punishment (0%). In contrast, VTA dopamine neuron activity was suppressed during receipt of the large reward when it was accompanied by punishment. To confirm that this change in VTA dopamine neuron activity was causal to risky decision making, Cre-dependent halorhodopsin was expressed in dopamine neurons of TH-Cre rats. After stable behavior was established, these neurons were then selectively inhibited during delivery of the large reward. Relative to baseline sessions, inhibition of VTA dopamine neurons during receipt of the large reward when it was unpunished reduced preference for the large reward. In contrast, inhibition of VTA dopamine neurons during receipt of the large reward when it was punished had no significant effect on behavior. These findings support a causal link between VTA dopamine neuron activity and decision making under risk of punishment through signaling errors in outcome predictions. These results also provide evidence for a "negative prediction error floor", whereby further inhibition of VTA dopamine neurons during an already suppressive event does not result in further shifts in choice preference. This work was supported by: F31AA030936.

Qureshi OA, Gostolupce D, Iordanova MD (*Concordia U*) Sensory preconditioned fear requires the pathway between the orbitofrontal cortex and the perirhinal cortex ABSTRACT: Sensory preconditioned fear requires the pathway between the orbitofrontal cortex and the perirhinal cortex ABSTRACT:Rats exposed to pairings of two innocuous stimuli, S2 and S1 (light and tone) and then to pairings of S1 and foot-shock display defensive responses (freezing) when tested with S2. Previous research using this sensory preconditioning protocol has shown the perirhinal cortex (PRh) is required for the retrieval and/or expression of fear to S2 at test. Here, we show that the orbitofrontal cortex (OFC) is also required for retrieval and/or expression of fear to S2 at test by inactivating the OFC immediately prior to S2 test (Experiment 1). We then show that contralateral inactivation of the PRh and OFC immediately prior to S2 test also disrupts fear to S2 (Experiment 2). This disruption requires the OFC → PRh pathway as chemogenetic inactivation of this pathway immediately prior to S2 test also disrupts fear to S2 (Experiment 3). Thus, both the OFC and PRh, and more specifically the pathway between these two regions, is required for the retrieval and/or expression of fear to S2 in a sensory preconditioning protocol.

Raskin M, Keller NE, Agee LA, Shumake J, Lee HJ, Monfils M-H (*UT Austin*) CO2 reactivity predicts fear expression after extinction and retrieval-extinction ABSTRACT: Maladaptive associations underlie persistent responding to previously neutral stimuli. For example, cues present during a traumatic event may result in fear responses. These responses can be attenuated through extinction learning, where cues are repeatedly presented without the previously learned outcome—a core component of exposure therapy. Exposure therapy is effective for some patients, but not all. We recently demonstrated that CO2 reactivity predicts fear extinction memory and orexin activation, and that orexin activation predicts fear extinction memory, suggesting that a CO2 challenge may enable identifying whether an individual is a good candidate for an extinction-based approach. Another method to attenuate conditioned responses, retrieval-extinction, modifies the original associative memory via distinct neural mechanisms (Monfils et al., 2009). The purpose of the present study was to determine whether the predictive power of CO2 reactivity for fear cues can be replicated and whether it is specific to extinction. Male rats were fear conditioned, received either extinction (n = 26) or retrieval-extinction (n = 28), and then underwent a long-term memory test and reinstatement, followed by a CO2 challenge, as outlined in Monfils et al. (2019). We used the best subset approach to linear regression to determine whether CO2 reactivity would predict extinction phenotype. In the extinction group, CO2 reactivity predicted fear extinction memory, explaining 42% of the variability in the total sample and 28% of the variability in the cross-validated (CV) holdout samples. In the retrieval-extinction group, CO2 reactivity predicted fear extinction memory, explaining 19% of the variability in the total sample and 9% of the variability in the CV holdout samples. To determine whether extinction or retrieval-extinction was better at preventing the return of fear, we conducted two 2x2 ANOVAs with group (extinction and retrieval-extinction) as the between subjects factor and session (extinction and LTM or LTM and reinstatement) as the within subjects factor. For the extinction-LTM ANOVA, there was a significant main effect of group, with reduced freezing in the retrieval-extinction group relative to the extinction group, which yielded a medium effect size ($\eta 2 = .072$). There was no significant main effect of session nor a significant interaction. For the LTM-reinstatement ANOVA, there was a statistically significant main effect of group with reduced freezing in the retrieval-extinction group relative to the extinction group,

which yielded a medium effect size (η2 = .067). There was also a statistically significant main effect of session with increased freezing during reinstatement relative to LTM with a medium-large effect size (η2 = .119). There was no significant interaction. To assess whether these group effects reflect meaningful differences in fear attenuation, we looked at the proportion of each group that met our data-driven criteria for remission (Shumake et al., 2018). We found that 23.1% of the extinction group and 42.9% of the retrieval-extinction group met criteria for remission at LTM, and 7.7% of the extinction group and 25% of the retrieval-extinction group met criteria for remission at reinstatement. Overall, we replicate previous findings that CO2 reactivity predicts fear extinction memory and that retrieval-extinction is overall more effective than extinction at preventing the return of fear. We find that the predictive power of CO2 reactivity is not specific to fear extinction, and generalizes to retrieval-extinction, albeit to a lesser degree. Given that CO2 challenge can safely be administered in humans (Smits et al., 2022), our results suggest that CO2 reactivity could be used to determine whether an individual is a good candidate for extinction-based therapeutic approaches.

Reichert A, Gogusoglu SA, Buck AL, Seipel KA, Foor, EB, Leslie TG, Quinn JJ (*Miami U/ Purdue U*) Role of the paraventricular nucleus of the thalamus in the expression of threat and safety ABSTRACT: A hallmark of posttraumatic stress disorder is the inability to register safety signals in a previously stressful or novel environment. The paraventricular nucleus of the thalamus (PVT) has recently been observed to integrate emotionally salient information and modulate anxiety. Given this information, it is possible this midline region is involved in the acquisition and/or expression of safety learning. We hypothesize that inhibition of PVT will reduce the expression of fear and safety learning. Rats were trained using a compound visual/auditory cue explicitly unpaired with footshock across two days; this establishes the context as a threatening stimulus and the visual/auditory cue as a safety signal predicting the absence of footshock. Rats received muscimol/baclofen or vehicle infusion into the PVT prior to test. Preliminary results show that vehicle control animals express robust threat and safety learning, whereas animals with PVT inhibition do not demonstrate reduced freezing in the presence of the safety cue. We are powering for analyses by sex throughout the experiment. With this information, future experiments will utilize a chemogenetic approach to more precisely inhibit neurons of the anterior or posterior PVT to determine whether the PVT plays a role in safety learning, independent of its involvement in threat expression.

Remmers BC, Nicot A, Choi IB, Matsumura K, Alvarez V, Dobbs LK (*UT Austin*) Mu Opioid Receptors have Divergent Effects on Cocaine and Opiate Behaviors ABSTRACT: Cocaine and opiate reward are mediated by increased dopamine transmission in the striatum. While it is not surprising that activation of Gi-coupled mu opioid receptors (MORs) is important for opiate reward, evidence also suggests MORs play an important role in cocaine reward. For instance, global deletion of MORs attenuates reinstatement of cocaine seeking. This effect appears to be localized to the striatum, as intra-striatal administration of MOR antagonists block the acquisition and expression of cocaine place preference. Further, this likely occurs through enhanced levels of the opioid peptide, and MOR agonist, enkephalin. Withdrawal from long-term cocaine increases striatal enkephalin levels and augmenting striatal enkephalin tone facilitates acquisition of cocaine place preference. However, within the striatum, MORs are expressed on the two populations of GABAergic output neurons: dopamine D1 and D2 medium spiny neurons (D1-MSN, D2-MSN). Thus, it is unclear which population of MORs is important for regulating drug reward. Since D1-MSNs and D2-MSNs have opposing effects on motivated behavior, with D1-MSNs driving drug reward and D2-MSNs restraining it, we hypothesized that MORs on D2-MSNs act to inhibit these neurons and support cocaine and opiate reward. To test this, we generated a knockout mouse with a targeted deletion of MORs from D2-MSNs (D2-MORKO) and tested them in cocaine, morphine, and fentanyl locomotor sensitization and conditioned place preference. D2-MORKO mice showed a functional loss of MORs, reflected by an inability of DAMGO, a MOR agonist, to suppress GABA transmission from D2-MSNs onto neighboring D1-MSNs. Lack of MORs from D2-MSNs slowed the acquisition of cocaine place preference and attenuated the expression of cocaine preference when tested in the presence of cocaine relative to littermate controls. In contrast, D2-MORKOs showed normal acquisition and expression of morphine and fentanyl place preference compared to controls. Deletion of MORs from D2-MSNs also had no effect on acute and sensitized locomotor responses following single-dose or repeated-administration of cocaine, morphine, or fentanyl. These findings suggest a divergent role for MORs expressed in D2-MSNs in mediating cocaine and opiate reward and further suggest these MORs facilitate conditioned cocaine reward. We suspect this occurs through cocaine-enhanced enkephalin, which acts on MORs to suppress D2-MSNs and facilitate cocaine reward. This work is supported by: NIDA Grant to Lauren K Dobbs (R01DA054329), Rising STARS Award from UT Austin to Lauren K Dobbs, Pre-Doctoral Training Grant in Interdisciplinary Neuroscience to Bailey Remmers (5T32DA018926).

Reyes VR, Sharpe MJ (*UCLA*) Role of VTA GABA neurons in encoding sensory specific expectations of rewards ABSTRACT: Decision making is generally dichotomized into model-free or model-based systems. Model-free learning is inflexible, lacks a representation of future outcome, and is dictated by long-term reinforcement

of behavior. In contrast, model-based learning involves chaining together related information to create a complex network of states that allow for flexible decision making based on future goals. Dopamine neurons in the ventral tegmental area (VTA) are critical for signaling prediction errors to produce learning, and we have shown that the learning supported by these neurons is model-based (Sharpe et al. 2017, Nature Neuroscience). GABAergic cells in VTA are thought to contain information about the prediction for reward that is relayed to dopamine neurons, but whether this population operates in a model-free or model-based fashion has yet to be investigated. Here, we test this question using Pavlovian conditioning and a devaluation procedure. We found that optogenetic inhibition of VTA GABA neurons produced an attenuated devaluation effect indicating that these rats were unable to flexibly shift their learning when the value of the outcome was changed, in line with the model-free strategy. This suggests that VTA GABA neurons may be responsible for retrieving the sensory specific expectations of reward in a model-based fashion. Now, we are employing a bi-conditional configural learning task to further examine the role VTA GABA neurons play in accessing model-based expectations of cues and rewards.

Robinson PK, Met Hoxha E, Williams D, Kinzig KP, Trask S (*Purdue U*) Extinction learning is impaired by normal aging ABSTRACT: Normal aging is accompanied by broad loss of cognitive function in humans and rodents, including declines in cognitive flexibility. In extinction, a conditional stimulus (CS) that was previously paired with a footshock is presented alone. This procedure reliably reduces conditional freezing behavior in young adult rats. Here, we investigated how normal aging affects extinction learning. Using young (3-month) and aged (20+ month) male and female Long Evans rats, we compared extinction (20 CS-alone presentations) to a no extinction control (equal exposure to the chamber without CS presentations) following delay fear conditioning. We found that young animals in the extinction group showed a decrease in freezing relative to their no extinction controls; aged animals did not. We next examined changes in neural activity using expression of the immediate early gene *zif268*. We found that in the BLA and aRSC, the young animals in the no extinction group had heightened *zif268* expression compared to the control group and the extinction group, suggesting extinction reduced activity in these regions. In aged animals, *zif268* expression in both the extinction and no extinction groups were elevated compared to controls. Further, *zif268* was elevated in the aged animals in every brain region examined, suggesting broad dysfunction of protein regulation in normal aging. Together, these results suggest that extinction is impaired in aged animals, demonstrating cognitive inflexibility in response to changing contingencies, and that this deficit is likely a result of aberrant neural activity. Ongoing work is assessing how changes in protein accumulation that precede memory impairment are caused by dysfunction of the ubiquitin proteasome system in aged brains. This work was supported by: Research Corporation for Science Advancement (Award: #29107) to ST.

Rodney-Hernández P, Porras A, Rincón-Cortés M (*UT Dallas*) Effects of sensory overstimulation in postpartum rodents ABSTRACT: Research in newborn rodents has shown that exposure to excessive sensory overstimulation leads to cognitive deficits, including impaired working memory, and increased risk-taking behaviors (Christakis, D et al., 2012). This paradigm is typically used in newborn mice and starts around postnatal day 10. Since this period of stimulation occurs prior to weaning, newborn rodents receive the excessive sensory stimulation in their home cage while the dam is present. However, the effects of excessive sensory stimulation during the postpartum period remain unexplored as potential effects on dams have not been evaluated. To this end, we adapted an excessive sensory overstimulation paradigm for use in rats and evaluated the behavioral effects of sensory overstimulation in dams. To assess behavioral changes in anxiety-like behavior, spatial learning/memory and social motivation, control and overstimulated dams (n=5-6 per group) underwent elevated zero maze, novel object recognition, and social approach tests. Additionally, we conducted maternal observations to determine whether the overstimulation exposure produced changes in the maternal behavior. We observed no significant changes in positive pup-directed caregiving behaviors such as nursing and licking or negative pup-directed behaviors such as stepping or dragging pups. However, nest-building was significantly reduced in sensory overstimulated dams compared to control dams. Furthermore, sensory overstimulated dams exhibited significant increases in pup shoving compared to control dams. With regards to the behaviors tested, there were no differences between control and overstimulated dams. These findings, being the first of their kind, lay the foundation in understanding how dams are being affected by postpartum sensory overstimulation.

Ruble S, Kramer C, Kettler C, West L, Auletti I, Diehl MM (*Kansas State U*) Active avoidance is enhanced when learned indirectly through observation ABSTRACT: Learning to avoid danger can be achieved through direct or indirect experience. Observational learning of active avoidance, achieved through indirect experience, has been demonstrated in rats using the two-way shuttle task (Del Russo, 1975) but has yet to be studied in platform-mediated avoidance (PMA). During PMA, rats learn to avoid a tone-signaled footshock by stepping onto a safe platform at the cost of access to a sucrose reward (Bravo-Rivera, et al., 2014). Here, we modified the PMA task to assess acquisition of PMA when learning through observation. During observational PMA, Observers were randomly assigned to watch a Demonstrator during early (Day 1; D1),

middle (Days 2-9; D2-9), or late PMA conditioning (Day 10; D10). After the observation phase, Observers were placed in the same location that the Demonstrator previously occupied and underwent a full PMA conditioning session alone. D2-9 Observers (n=7) exhibited the highest levels of avoidance compared to D1 (n=8) and D10 (n=8) Observers, suggesting that observing multiple PMA sessions is advantageous for learning avoidance. We next compared behaviors during observational PMA with behaviors during both solitary and social PMA. In social PMA, pairs of rats were conditioned together while separated by a perforated plexiglass barrier. D1 Observers showed no differences in avoidance or freezing compared to rats who underwent solitary (n=59) or social PMA (n=22). However, D2-9 and D10 Observers exhibited significantly increased avoidance compared to rats who underwent solitary or social PMA. Freezing was increased in D2-9 and D10 Observers compared to solitary but not social PMA rats. Our findings suggest that indirect observational learning enhances avoidance compared to direct learning with or without a partner. Ongoing studies are characterizing the neural circuitry underlying the observational learning of avoidance using cFos, optogenetics, and electrophysiology.

Russell EL, McDannald MA (*Boston College*) Ventral pallidum neurons are necessary to generalize and express fear-related responding in a minimal threat setting ABSTRACT: Fear-related behavior is beneficial when specific to events signaling harm (discrimination), but becomes problematic when displayed in response to neutral events (generalization). Here, we developed minimal threat learning procedures to distinguish discrimination from generalization. We then examined roles for the ventral pallidum - a region anatomically poised to modulate amygdalar threat function - in the generalization and expression of fear-related responding. Experiment 1 established behavioral procedures that would distinguish discrimination from generalization. Long Evans rats (n = 47, 23 females) were mildly food-deprived, trained to nose poke for food pellets, then assigned to one of three probability conditions. For each condition, a threat cue probabilistically predicted foot shock on 10% (n=15), 20% (n=16), or 30% (n=16) of trials. A neutral cue never predicted foot shock. All rats acquired discrimination over the 10 sessions. Rats in the 10% probability condition did not generalize responding to the neutral cue, whereas generalization was evident in the 20% and 30% conditions. During an extinction test, the 10% threat cue supported less responding than the 20% and 30% threat cues. Experiment 2 examined a role for the ventral pallidum in discrimination, generalization and expression of threat cue responding. A dual viral approach (AAV-eSYN-EGFP-T2A-iCre + AAV-flex-taCasp3- TEVp) was used to delete ventral pallidum neurons (Casp3VP, n = 11, 5 female), while neurons were left intact in the Control group (n = 12, 6 female). Control and Casp3VP rats were then assigned to either the 10% or 30% probability conditions. Control rats in the 30% condition, but not the 10% condition, generalized responding. The 30% threat cue supported greater fear related behavior during extinction than the 10% threat cue. Neither Casp3VP rats in the 10% nor the 30% generalized responding and both groups showed reduced fear-related behavior to the threat cue in extinction. Experiment 3 used the dual viral approach to delete nucleus accumbens neurons projecting directly to the ventral pallidum (Casp3NAc→VP). Control and Casp3NAc→VP rats were assigned to the 30% probability condition (n = 24). Pathway deletion had no impact on generalization or expression of fear-related responding to the 30% threat cue. The results reveal the ventral pallidum is necessary to generalize and express fear-related behavior in a minimal threat setting; however, this function does not depend on nucleus accumbens input.

Sattler KP, Hedges A, Miller R, Zelikowsky M (*U Utah*) The Ventral Hippocampus is necessary for trauma-altered social behavior ABSTRACT: A single, acute traumatic experience can result in a host of negative behavioral effects, such as increased aggression and exaggerated fear responses to mild stressors. Despite the large body of research on the neurobiology of trauma, we nevertheless have a poor understanding of how the brain encodes trauma-associated changes in behavior. The ventral hippocampus (VH) is well-suited for processing the multi-modal behavioral effects of trauma, as it receives inputs from sensory integration sites and sends output to regions involved in social and emotional behavior. Importantly, the VH projects to the basolateral amygdala (BLA), a central node for fear, the ventromedial hypothalamus (VMH) and bed nucleus of the stria terminalis (BNST), both implicated in aggression, and the prefrontal cortex (PFC), known to be involved in social processing. Thus, we hypothesized that the effects of trauma to alter fear, aggression, and social behaviors are controlled by projection neurons in the VH. To test this hypothesis, mice were injected with a virus encoding hM4D into the VH to allow for chemogenetic silencing of the VH during the Resident Intruder assay, as well as the 3-Chamber Sociability assay. We show that the VH is necessary for trauma-enhanced aggression and that trauma-altered social behavioral changes can be ameliorated by VH silencing. To further dissect the involvement of neuronal ensembles in the VH to encode the effects of trauma on enhanced aggression and altered social behavior, we tested whether ensembles activated by each phenotype have unique projection profiles using tract tracing and immunohistochemistry. Collectively, our findings suggest a role for the VH as a central hub underlying trauma-altered social behaviors and provide insight into how experiencing a traumatic event can lead to diverse behavioral changes.

Schamber G, LaViola M, Grisales K, Awad A, Herbst MR, Twining RC, Gilmartin MR (*Marquette U*) Prefrontal

cortical output to the mediodorsal thalamus encodes trace fear conditioning ABSTRACT: The ability to anticipate threat from available cues in trace fear conditioning requires activity within the prelimbic cortex (PL). Approximately 30% of neurons within PL show sustained firing to shock-predictive cues, which if disrupted impairs learning, suggesting a working memory or attentional role for PL in cued fear acquisition. It is unknown however, how this bridging signal is distributed to downstream emotional learning systems to support fear memory. Our recent work suggests that direct prelimbic output to the amygdala is important for learning, but fear acquisition can still occur in the absence of direct communication between the PL and amygdala (Kirry et al., 2020). Here we investigate the importance of the mediodorsal thalamus (MD) in trace fear conditioning. The MD is strongly interconnected with the PL and this connection is implicated in working memory. Moreover, the MD is connected with cognitive and emotional systems including the amygdala and brainstem arousal systems, which positions it as a potential node for integrating temporal and emotional information in memory. In this study, an intersectional viral approach was used to express GCaMP6f or ArchT in PL cells projecting to the MD. Fiber photometric imaging of PL-MD revealed 1) activation of this pathway during the CS and UCS in trace conditioned male (n=7) and female rats (n=8) and 2) potentiated CS-evoked activity at test compared with pre-training CS-alone trials. Additional experiments are underway to test the functional significance of PL-MD activity (ArchT silencing the PL-MD pathway during training (n=6-9/sex/group)) and to characterize the encoding profiles of MD neurons during trace fear conditioning. The outcome of this work will begin to reveal the role of this midline thalamic nucleus in the acquisition and expression of episodic fear memories. This work was supported by: Regular Research Grant, Marquette U (MRG), Neurosurgery Research & Education Foundation (AA).

Schuler H, Mandel Weinbaum A, Siemonsmeier G, Iyer E, Bagot RC (*McGill*) A novel protocol for simultaneous appetitive and aversive associative learning in male and female mice ABSTRACT: The affective valence of a stimulus ranges from appetitive (positive) to aversive (negative). Valence representations infuse stimuli with emotional significance that powerfully regulates motivation to approach and avoid. Appetitive and aversive stimuli are often considered as opposing ends on a valence spectrum and assumed to be represented in the brain by similarly distinct neural mechanisms. However, they are primarily studied in isolation preventing direct comparison that is necessary to determine unique and shared neurobiological underpinnings. Here, we developed a classical conditioning paradigm, in which mice are simultaneously trained to associate three distinct auditory cues to a positive (chocolate milk), negative (footshock) or non-reinforced outcome. Using machine learning approaches to profile behavioral repertoires, we find that both male and female mice acquire robust appetitive and aversive associations over 14 days of training and conditioned responses remain stable across three days of extinction. Finally, using in-situ hybridization we confirm that in the nucleus accumbens, a key region implicated in valence processing, both types of medium spiny neurons, dopamine receptor D1- and D2-expressing, are activated by both appetitive and aversive conditioned stimuli. Our novel dual-valence classical conditioning protocol provides a validated behavioural paradigm that can be used to probe mechanisms of valence processing in male and female mice in health and disease.

Sheppard VM, Mitchell JR, Shansky RM (*Northeastern U*) Sexual dimorphism in the anterior cingulate cortex-ventrolateral periaqueductal gray pathway ABSTRACT: Despite novel and innovative research progressing in neuroscience and mental health fields, we continue to lack an understanding of the underlying mechanisms that characterize maladaptive stress responses across sexes. My lab attempts to close this gap by improving the translational validity of pavlovian fear conditioning and garnering a clearer understanding of the relationship between stress, neuroanatomy, and behavior. During fear conditioning, rats display a plethora of behaviors including but not limited to freezing and darting. Females are more likely to engage in darting behavior which can be defined as an escape-like conditioned response that is associated with heightened shock reactivity. We aim to study this behavioral output at the circuit level as it may offer a clearer understanding of conditioned responses. Previous neuroanatomical inquiries indicated sparse connections existing between the ventrolateral periaqueductal gray (vlPAG) and anterior cingulate cortex (ACC); however, subject use was confined to males. Recently, pilot studies in our lab have discovered that this circuit predominates in females. The robust amount of labeling in females may be indicative of differing processes of fear acquisition and behavioral responses across sexes during fear conditioning. Our lab hypothesizes that darting emerges primarily in females due to sex-dependent ACC-mediated suppression of freezing via inhibition of vlPAG activity. To test this hypothesis, we injected a retrograde-transducing AAV-GFP into the vlPAG of Sprague Dawley rats. After five weeks, animals went through fear conditioning, extinction learning, and extinction retention tests on three consecutive days. We are currently analyzing behavior using ScaredyRat, a custom Python tool designed in our lab to analyze raw Ethovision data files, to quantify freezing, shock response, and darting. Though data collection remains ongoing our preliminary results indicate a relationship between darter status and number of cells quantified within the ACC. This may suggest that the ACC-vlPAG pathway during fear conditioning is a potential driver of the sex-dependent behavioral phenotypes associated with darting as a conditioned response.

Sheynin J, Baidya S, Shrestha G, Liberzon I (*TAMU/ U Michigan*) Learning and generalization of avoidance: A mismatch between expectancy and behavior and associations with anxiety vulnerability ABSTRACT: Excessive avoidance behavior is a key symptom in all anxiety disorders and posttraumatic stress disorder (PTSD). While two drivers are commonly thought to result in avoidance behavior (fear and cognitive expectancy), the exact mechanism that underlies individual differences in avoidance is still undefined. We used a computer-based task to specifically focus on the cognitive basis of avoidance behavior. Here, participants control a spaceship avatar and shoot an enemy spaceship to gain points, as well as learn to hide their spaceship in "safe areas" to avoid an aversive event (on-screen explosion and point loss). The avoidance behavior takes place during the presentation of signals that predict the upcoming aversive event with varying contingencies (0% - safety signal, and 60% and 100%, i.e., probabilistic and deterministic warning signals, respectively). Avoidance is quantified as the duration of hiding during these signals. Young adults (N=66) from University of Michigan and surrounding area were recruited. Avoidance behavior and expected risk associated with each of the signals, were recorded. Anxiety vulnerability was assessed by the behaviorally-inhibited temperament using the harm-avoidance subscale of the Tridimensional Personality Questionnaire. Overall, while participants successfully discriminated the deterministic and probabilistic signals on expectancy scores, they demonstrated similar avoidance behavior during these signals. Further, when participants were presented with novel "generalization" signals that share similarities with the previously learned signals, they showed more gradual generalization gradients (i.e., greater response to the novel signals) in expectancy scores than in avoidance behavior. Interestingly, the similar levels of avoidance during probabilistic and deterministic warning signals, and the lower avoidance during novel signals, were driven by participants with lower anxiety vulnerability. Taken together, these findings suggest that self-reported expectancy of negative outcome and operationalized avoidance behavior might follow different patterns, and hence, highlight the need to rely on objective measures of behavior. Further, they suggest that the greater avoidance behavior that has been reported in anxiety-vulnerable individuals is due to increased behavioral responding to cues that are novel or are associated with greater risk for aversive outcome. This work was supported by: Departmental funding (Department of Psychiatry) and Undergraduate Research Opportunity Program (UROP), University of Michigan.

Shilyansky C, Kochalka J, Raffiee M, Cordero A, Ramakrishnan C, Quirin S, Deisseroth K (*Stanford/ HHMI*) Prefrontal cortex controls large-scale neural dynamics coupled to fear during memory recall ABSTRACT: Cognitive processing engages activity across large-scale networks. However, recruitment of organized, network-wide activity during cognition is poorly understood. Using integrated optogenetics and multisite photometry in behaving mice, we causally examine control of network-wide neuronal activity across the default mode network (DMN) during aversive contextual memory recall. Optogenetically induced theta frequency activity in medial prefrontal cortex, a DMN node, drives sustained network-wide recruitment and behavioral transitions in fear expression during memory recall. In contrast, other network nodes, the retrosplenial cortex and ventral hippocampus, do not show behaviorally coupled network control despite participating in a highly correlated network structure driven by context recall. Therefore, dissociable mechanisms control the functional mode of this large-scale cognitive network, with the medial prefrontal cortex uniquely driving network engagement and fear coupling. This work was supported by a NARSAD YIA and NIMH grant K08MH11735005.

Siller-Perez C, Andrade EC, Smiley J, Cain CK, Sears RM (*EBI/ NKI/ NYU Langone*) Orexin promotes active avoidance via amygdalar and midbrain targets ABSTRACT: Identifying the neuromodulatory mechanisms orchestrating survival behaviors is critical to understanding anxiety- and stress-coping in health and disease. Perifornical (PFH) and lateral hypothalamus (LH) neurons expressing orexin (hypocretin) peptides project throughout the brain and mediate functions critical for survival behaviors, including vigilance, attention, and action selection. Here we delineate the role of two hypothalamic orexin system targets using a proactive threat-coping behavior model—signaled active avoidance (SigAA). In SigAA, subjects learn to escape a warning signal (WS) that predicts a painful unconditioned stimulus (US; e. g. shock) by emitting a specific avoidance response (AR; e. g. shuttling). Feedback (FB) stimuli encountered immediately after ARs are known to develop into safety signals, raising the possibility that "avoidance" is positively reinforced by safety. Based on appetitive instrumental studies and physiology findings, we hypothesized that orexin is necessary for active avoidance a) via projections to the central nucleus of the amygdala (CeA) to inhibit freezing reactions when safety is available and b) via projections to the ventral tegmental area (VTA) to invigorate avoidance/safety-seeking responses. To test this, Sprague Dawley rats received infusions of an orexin-specific viral vector containing an inhibitory opsin (AAV1-Ple112-Arch3.0-eYFP) into the PFH/LH, and optic fibers were implanted in the CeA or VTA. Following a 6–8-week incubation, rats were trained in the SigAA task. Animals received one Pavlovian trial [60s white noise WS paired with an inescapable foot-shock (1.0/0.7 mA males/females; 0.5 s)] to transform the WS into a conditioned threat. For all remaining trials, if animals shuttled during the WS (an AR), a FB tone was delivered (5

s, 80 dB), and the scheduled shock was omitted. If animals failed to avoid, the shock was delivered at the end of the 60s WS, and FB was omitted. Rats received 15 daily trials until reaching 80% successful avoidance for an entire session. During subsequent shock-free test sessions, orexin->CeA or orexin->VTA axon terminals were photoinhibited (green laser 532 nm, 10 mW) during the FB only. When inhibiting orexin->CeA terminals, AR expression was delayed; when inhibiting orexin->VTA axon terminals, AR expression was intact, but AR extinction was improved. These results suggest that the orexin system suppresses incompatible Pavlovian reactions (freezing) and promotes adaptive instrumental actions (active avoidance) via two separate neural pathways, highlighting a novel role for the orexin system as a 'master modulator' of proactive coping behavior.

Simmons TA, Gonzales RA, Monfils MH, Lee HJ (*UT Austin*) Renewal of alcohol-seeking in males and female rats ABSTRACT: Environmental cues become conditioned stimuli when paired with alcohol consumption and facilitate alcohol-seeking behavior in users. Alcohol-seeking occurs even during abstinence and may ultimately lead to relapse. Extinction can reduce cue-conditioned responses by exposing discrete alcohol cues without alcohol consumption; however, extinguished cue-conditioned alcohol-seeking responses can return under certain conditions, such as renewal. Existing research suggests sex differences whereby males exhibit more renewal of both appetitive and fear responses (Anderson and Petrovich, 2015; Binette et al., 2022) than female rats; however, the whether there are sex differences in renewal of alcohol-seeking behavior has yet to be investigated. In this study, we investigated the impact of contextual shifts on renewal of alcohol-seeking behavior in rats. Male (n=7) and female (n=11) Long-Evans rats first underwent an induction phase: 15% unsweetened ethanol (15E) was provided MWF on a 24-hour schedule over a 5-week period. Then, Pavlovian conditioning took place in context A (standard conditioning chamber) where a 20-second light presentation was paired with a 10-second presentation of sipper containing 15E (8 trials/session, 14-17 daily sessions). Subsequently, extinction (12 trial/session, 12 daily sessions) and testing (4 trials) occurred in context B which consisted of smooth flooring, lemon scents, and black wall on the front and back of conditioning chambers. A 20-second light presentation was paired with a 10-second presentation of sipper without alcohol. Renewal testing (4 trials) occurred in context A. The results show successful extinction measured by low levels of sipper contact (reflecting reduced alcohol-seeking behavior) in context B. Male and female rats also exhibited an overall increase in sipper contact when placed back in the original alcohol associated context (context A), indicative of renewal. In conclusion, there was a main effect of renewal ($p<0.001$) but no sex effect or interaction effect of sex and renewal, suggesting no sex difference in renewal of alcohol-seeking behavior. This work was supported by: R01 1R01AA029386-01A1 (HJL).

Smies CW, Bellfy L, Wright DS, Bennetts SS, Urban MW, Brunswick CA, Kwapis JL (*Penn State*) The role of histone deacetylation in memory competition ABSTRACT: Memories are plastic records of experience that require active maintenance to maintain relevance, yet the mechanisms that allow existing memories to update are currently unclear. Transcription is one critical mechanism known to be required for both memory consolidation and reconsolidation-dependent memory updating. This process is regulated in part by epigenetic mechanisms, which modulate the transcriptional machinery's access to DNA to dynamically regulate gene expression. One major epigenetic mechanism critical for memory consolidation is histone deacetylation via HDAC3. HDAC3 represses a number of memory-relevant genes and inhibiting HDAC3 transforms a weak learning event into one that is encoded into long-term memory. The role of HDAC3 in reconsolidation-based memory updating, however, is less clear. Here, to better understand the mechanisms important for memory updating, we used the Objects in Updated Locations (OUL) paradigm. In OUL, mice learn the locations of two identical objects before an update session, in which one object is moved to a novel location. Memory strength is then tested by presenting four identical objects; two in the original locations, one in the updated location, and a fourth in a novel location. At test, mice demonstrate memory for the original and updated locations by preferentially investigating the object in the novel location. Here, we show that aged mice exhibit impaired memory updating even when the original memory is intact, demonstrating a unique aging-related deficit in memory updating. HDAC3 inhibition following the update session ameliorates this impairment, allowing old mice to successfully update spatial memories. In comparison, blocking HDAC3 after updating in young mice actually reduced the strength of the original memory, suggesting that the original and the updated information may compete for expression. Together, our work suggests HDAC3 contributes to age-related impairments in memory updating and may help regulate the strength of this information. Current experiments are systematically manipulating the strength of the original and update memories to determine if we can modulate which memory is expressed at test. We are also working to identify transcripts that uniquely support memory formation or updating to selectively target an initial or update memory in future studies and paradigms.

Sosa R, Saavedra P, Jiménez S, Lago G, Buenrostro-Jáuregui M (*UP/ UNAM/ ITESM/ UIA-México*) Dissociation between response restraint and response cancellation: Evidence from a Pavlovian protocol ABSTRACT: Response inhibition is an ecologically meaningful behavioral mechanism as it promotes a better fit to the environment by suppressing maladaptive (e.g.,

competing, costly, or risky) actions. Such actions can be mitigated through antagonizing behavioral tendencies either prior to or following their occurrence, thereby aligning with the definitions of response restraint and response cancelation, respectively. The dissociation of these constructs has been traditionally examined using protocols featuring instrumental contingencies, such as providing incentives for responding quickly to a cue or successfully suppressing the target action. We propose that this phenomenon could be explored using a Pavlovian feature-negative discrimination protocol, which does not impose explicit instrumental contingencies, thereby rendering a better model of how inhibitory tendencies unfold due to factors related to response cost and prediction-error adjustment. An additional advantage of this approach is that indices of response restraint and response cancelation can be derived simultaneously without imposing distinct task demands for each, as canonical protocols do. Specifically, response probability discrepancies between excitatory and inhibitory trials can serve as a measure for response restraint, while response duration discrepancies effectively quantify response cancellation. Using an heterogeneous sample of rats as subjects, we explore several paths for assessing the dissociation of these constructs through a range of complementary analytical tools. Our results collectively suggest that these components of response inhibition are indeed statistically independent at the level of inter-individual differences. We propose further procedural and analytical refinements to delineate this dissociation more accurately, which seems a promising strategy to investigate associative inhibitory phenomena. This work is supported by Universidad Panamericana (UP) and Universidad Iberoamericana (UIA).

Spring MG, Nautiyal KM (*Dartmouth*) Serotonin is released in the dorsal striatum in anticipation of a reward ABSTRACT: Serotonin signaling throughout the brain is involved in reward processing, threat detection, behavioral inhibition, and other facets of motivation and behavioral control. In the dorsal striatum (DS), pharmacological manipulation of the serotonin system disrupts behavioral control and the prospective encoding of rewards. In order to better understand the involvement of serotonin in striatal reward processing, we monitored serotonin levels using a G-Protein Activation Based (GRAB) fluorescent serotonin sensor (GRAB-5-HT) in the DS of adult mice during both consummatory and Pavlovian tasks. In the first set of experiments, we used GRAB-5-HT to measure serotonin levels during evaporated milk reward consumption in the Davis Lickometer. We saw a robust serotonin release in the DS that began rising one to two seconds prior to the onset of reward consumption, and peaked during the first lick. This serotonin signal also encoded the value of the reward, with higher reward concentrations associated with larger and more prolonged rises in serotonin. Mice were also given intermittent access to the highly palatable evaporated milk reward or water during varying homeostatic states including sated, food restricted, and water restricted conditions. Relative to the food restricted condition, water restriction increased the amount of water, but not milk, consumed during access periods, however serotonin release was only associated with the milk, but not water consumption. This suggests that the serotonin release in the dorsal striatum can be dissociated from the motoric aspects of liquid consumption, and potentially involved in aspects of reward anticipation, motivation, and/or reward valuation. In a second set of experiments, mice were trained in a Pavlovian delay conditioning paradigm in Bussey-Saksida touch screen chambers during which 8s long tones (CS+: white noise or 2 kHz tone counterbalanced) preceded delivery of evaporated milk, and an equal number of 8s long CS- tones (opposite of assigned CS+ cue) presentations that were not associated with reward delivery. After 10 training sessions, half of the animals displayed CS+/CS- discrimination (as measured by anticipatory approach during tone presentation), while the other half of mice did not. In animals that displayed accurate stimulus discrimination and approach, serotonin release during CS+ trials was significantly higher than during CS- trials. This was not the case for animals that did not display CS discrimination. Overall, these data suggest that striatal serotonin rises in anticipation of a reward, encodes the relative value of a reward, and seems to reflect the hedonic, rather than the consummatory aspects of a liquid reward. Additionally, the serotonin signal is associated with a cue that predicts a reward, rather than the sensory aspects of the cue. Our studies contribute to our understanding of how serotonin is involved in learning and representing reward, and ongoing studies are focused on establishing causality between serotonin release and behavioral processes through simultaneous GRAB-5HT monitoring and optogenetic inhibition of serotonin terminals in the DS.

Stevanovic KD, Wilson LR, Letsinger AC, Cushman JD (*NIEHS*) Systematic comparison of three *in vivo* fiber photometry recording methods ABSTRACT: The use of *in vivo* fiber photometry has rapidly expanded in recent years as technical advances in commercially available and custom-built recording systems have made it more accessible. Increasingly sophisticated fluorescent sensors combined with viral and transgenic targeting strategies have made photometry an indispensable tool for connecting behavior to the neural circuitry that underlies it. Multiple different photometry recording approaches have been developed, with each approach likely to have specific strengths, weaknesses, and caveats. It is also not clear whether these different approaches produce quantitatively or qualitatively similar results, which makes comparison across studies difficult. In order to systematically investigate this, we conducted a comparison of the three most used approaches: a spectrally-resolved fiber photometry system, a camera-based multi-wavelength photometry system and a

lock-in demodulation photometry system. We systematically rotated three groups of male C57Bl6/J mice through each photometry system in a counterbalanced manner and recorded a stimulus evoked GCaMP response in the locus coeruleus (LC). The presentation of white noise or LED light elicits a strong and consistent transient response in the LC, which allowed for a systematic comparison of each system. Here, we summarize the data processing pipeline for each system and systematically compare the results. These findings provide important quantitative comparisons that will aid in interpretation across studies utilizing these different methods and help future researchers determine the best in vivo fiber photometry system to use for their specific experimental needs.

Sweck SO, Binette AN, Maren S (*TAMU*) Chemogenetic activation of the locus coeruleus mimics stress-impaired fear extinction in male and female rats ABSTRACT: Extinction learning is an important mechanism for behavioral therapies of a variety of trauma- and stressor-related disorders. The infralimbic cortex (IL), a subdivision of the medial prefrontal cortex, has a critical role in fear extinction. Stress can induce impairments in fear extinction, including the "immediate extinction deficit" (IED) – an impairment that occurs when fear extinction is performed shortly after conditioning. Previous work in our lab suggests the IED is related to a stress-induced decrease in IL spike firing. Additionally, the IED can be induced or rescued by noradrenergic enhancement or blockade, respectively. Thus, we hypothesize that the locus coeruleus (LC), a major source of norepinephrine (NE) in the forebrain, mediates the stress-induced extinction deficit. To test this idea, we sought to describe the effects of chemogenetic activation of LC neurons on IL activity and behavior. Adult male and female (n=12) Long-Evans rats were bilaterally infused with AAV-PRSx8-hM3Dq-HA, an NE-specific excitatory designer receptor exclusively activated by designer drugs (DREADDs), in the LC and unilaterally infused with AAV-CaMKII-GCaMP6m into the IL. Additionally, a GRIN lens was implanted into the IL to allow for recording of calcium transients. To determine if LC-NE activation affects IL activity under basal conditions, animals underwent two days of recordings with injection of vehicle (VEH) or the DREADD ligand clozapine-N-oxide (CNO; 3 mg/kg, i.p.). We found that LC-NE activation drives increased freezing behavior and decreases IL activity by increasing the proportion of neurons suppressed after CNO injection relative to VEH. To ascertain whether LC-NE activation mimics footshock-induced changes in IL activity, animals were injected with VEH or CNO immediately prior to tone-only presentations or tone-footshock conditioning followed by 25 min of recording. Both footshock and LC-NE activation decreased IL principal activity with the former correlated to a weaker effect. Additionally, most recorded cells (67%) that were suppressed by LC-NE activation were also suppressed by footshock. Lastly, animals underwent standard delayed extinction and extinction retrieval protocols with either VEH or CNO onboard to determine if LC-NE activation prior to delayed extinction impairs retrieval and IL activity. CNO treatment prior to extinction resulted in higher levels of freezing during the first block of retrieval relative to VEH controls, suggesting that LC-NE activation is sufficient to impair fear extinction. Together, these results suggest that LC-NE signaling modulates IL activity and freezing behavior similarly to stress.

Torres JB, Williams E, Martin MJ, Bishop CA, Saddoris MP (*UC Boulder*) Cocaine self-administration experience alters phasic responses of prefrontal cortex in response to controllable stressors ABSTRACT: Stress can generate adaptive behaviors in acute settings, but prolonged and/or severe bouts of stress can conversely generate maladaptive behaviors including anxiety, post-traumatic stress disorder, and drug relapse. In addition to these broad effects of stress, it's also known that some individuals appear to be more susceptible to these stress-related effects, while others prove resilient. Significant work has been dedicated to understanding the behavioral and neural mechanisms that support resilience. In an animal model of resilience, rats that can perform an escapable response to shock have been shown to have greater resilience to future stressors than rats who cannot escape an identical shock, and this acquired resilience appears to depend on the integrity of the prefrontal cortex. However, our lab and others have shown that repeated experience with self-administered cocaine can persistently alter and impair the function of limbic circuits, particularly those that receive dense dopamine projections. We therefore hypothesized that cocaine experience could alter the functional response of prefrontal networks necessary to detect behavioral control, which in turn would impair the ability for drug-experienced animals to gain resilience from behavioral control. To test this, male and female rats were trained to self-administer daily either cocaine i.v. (0.5mg/ml/inf, 2h/d, 14d) or saline vehicle, followed by a period of enforced abstinence (30d). Rats were implanted with bilateral electrophysiological arrays in the prelimbic cortex, after which rats performed the stressor controllability task, followed by assays of Pavlovian reward and fear to assess the post-stress effects on these behaviors. Phasic responses recorded during controllability showed drug-related changes that were sex-dependent. In subsequent assessments of new fear conditioning, drug-experienced rats showed elevated responses to fear-predictive cues compared to saline Controls. Collectively, these data suggest that prior drug experience can prevent individuals from benefiting from acquired resilience, putting them at elevated risk for stress-related disorders, including relapse. This work was supported by: NIH (DA044980) and NARSAD Young Investigator Award.

Truckenbrod L, Garner M, Carlos N, Gore AC, Orsini CA (*UT Austin*) Contributions of estradiol and progesterone to female risk aversion ABSTRACT: Decision making is a complex cognitive process in which an individual must weigh options that differ in their expected rewards and their associated costs. Previous literature has established sex differences in decision making, particularly when decisions involve an explicit risk of punishment, with females displaying greater risk aversion than males. Recent evidence suggests that the ovarian hormone estradiol is a critical mediator of phenotypical female risk aversion. The role of progesterone in this form of decision making, however, is unknown. To address this gap in knowledge, female Long-Evans rats (n=19) were trained in a model of risk-based decision making (the Risky Decision-making Task, RDT) in which rats choose between a small, safe food reward and a large food reward that is accompanied by a variable probability of footshock punishment. After achieving stable behavioral performance, rats were ovariectomized (OVX) and re-tested in the RDT. Rats then received subcutaneous administration of estradiol benzoate (EB, 0.05mg/kg), EB and progesterone (EB+P; EB, 0.05mg/kg; P, 0.5mg/kg) or vehicle (sesame oil) daily for 7 days. Injections occurred following testing in the RDT each day using a randomized within-subjects design, such that each rat received 7 days of each treatment with a minimum of 8 days between treatments. During each treatment and the successive washout period, the rats' estrous cycles were assessed to confirm that the hormonal state of the rat was consistent with their treatment group. Consistent with previous work, OVX increased risk taking relative to rats' pre-surgical performance. Administration of either EB or EB+P attenuated this effect, causing a decrease in risk taking. To determine whether the decrease in risk taking in the EB+P condition was due to EB alone, rats underwent another regimen of hormone treatment in which P (or vehicle) was administered alone (identical duration and dosing as above). In contrast to the effects of EB alone, there were no effects of P on risk taking. These data expand our understanding of hormonal regulation of risk taking and indicate that estradiol is the critical ovarian hormone responsible for female risk aversion.

Tuna T, Totty MS, Peters S, Maren S (*TAMU*) Neuronal activity in the thalamic nucleus reuniens during the conditioning and extinction of fear in male and female rats ABSTRACT: The nucleus reuniens (RE) is a midline thalamic structure that interconnects the medial prefrontal cortex and the hippocampus via bidirectional connections. We have recently identified a critical role for RE in the extinction of fear memory. This work suggests that RE neurons may have an active role in suppressing conditional fear responses, and single-unit recording data provide some support for this hypothesis. However, the learning-related responses of RE neurons during fear conditioning and extinction have not been performed. To address this, we recorded, in separate experiments, calcium transients and electrophysiological responses of RE neurons during both auditory fear conditioning, extinction, and extinction retrieval. In the fiber photometry experiment, adult male and female Long-Evans rats (n=8) were injected with AAV8-CaMKII-GCaMP6f and implanted with an optic fiber in RE. Relative to habituation, RE neurons exhibited robust US-evoked responses during conditioning and acquired CS-evoked responses by the end of the conditioning session. Contrary to our expectations, CS-evoked responses were maximal at the outset of extinction training, decreased over the course of several extinction trials, and remained low during extinction retrieval testing. Interestingly, spontaneous fluctuations in RE calcium activity were highly correlated with freezing behavior in an extinguished context: increases in RE activity reliably preceded transitions from freezing to activity. To ascertain the dynamics of individual RE neurons, we made single-unit recordings in the RE from adult male and female rats (n=13) during fear conditioning (n=41 cells) and extinction (n=33 cells). Fear conditioning was associated with robust CS- (n=12 of 41) and US-elicited (n=22 of 41) activity in RE neurons. During extinction, reliable CS-evoked responding was observed in roughly half of the neurons recorded. Roughly half of the responsive cells (n=7 of 16; "fear" neurons) showed maximal CS-evoked firing in the early extinction trials that decreased over the session. In contrast, the other population of cells (n=7; "extinction" neurons) showed the inverse pattern, firing to the CS only in the latest extinction trials. These data reveal that there is considerable heterogeneity in RE neuronal activity during extinction. "Extinction" neurons in the RE play a particularly important role in suppressing conditioned fear responses.

Vasquez A, Kim G, Dominguez JM, Monfils M-H, Lee HJ (*UT Austin*) Oral hormonal contraceptives affect female rat gonadal function and lead to reduced amphetamine-preference during extinction ABSTRACT: Prevailing evidence suggests that increased levels of gonadal hormones (e.g., estrogens and progestins) lead to increased risk and maintenance of substance use disorders (SUD) in females. A common type of treatment for treating SUD's is exposure therapy, which is largely based on extinction. However, changes in gonadal hormone levels across the menstrual cycle, as well as those induced by hormonal contraceptives (HC) among women of reproductive age, affect extinction, but are rarely considered. HC's suppress hormonal fluctuations and levels of gonadal hormones in the body to prevent ovulation. Work by the Milad lab has demonstrated that extinction to a previously learned fear stimulus is specifically affected by high levels of gonadal hormones and by HC administration - where HCs impaired extinction training and led to decreased extinction recall, while high levels of estradiol enhanced extinction training. Additionally, work in our lab has shown that HC implants containing Levonorgestrel (LNG), a synthetic progestin

commonly used in HCs, led to a rapid reduction in preference for amphetamine (AMPH)-associated context. However, how HCs alter female reward-learning and extinction is yet to be determined. The current experiment expands on both studies by considering both the route and timing of LNG administration to assess LNG effects on AMPH-preference over extinction. Female rats underwent AMPH-conditioned place preference. They were then tested for their AMPH-preference either (1) over three sessions (ie., extinction learning sessions) while receiving oral LNG (250g/rat), or (2) during an estrous cycle stage associated with higher levels of gonadal hormones (i.e., proestrus/estrus). Both groups initially showed preference for the AMPH-associated context regardless of hormonal treatment. However, the LNG females showed no significant preference by the third extinction session, whereas naturally cycling females on proestrus/estrus stages still showed preference. Interestingly, estrous cycles of LNG females were not affected at the dose (250g/rat) that influenced AMPH extinction. A higher dose (500g/rat) of oral LNG still did not lead to persistent estrous stages associated with lower gonadal hormone levels (i.e., diestrus/metestrus), unlike what has been previously reported. Interestingly, uterine horn width, an index of prior exposure to high levels of estrogens, was significantly thinner in both high and low dose LNG rats as compared to proestrus/estrus rats, but not significantly different from diestrus/metestrus rats, suggesting that administration of the oral contraceptive was not without consequence. These findings are consistent with previous results from our lab and suggest a dose-dependent effect of LNG on gonadal function. Future work for this study will investigate the dose-dependent effects of LNG on AMPH-conditioning and extinction to assess conclusive effects of LNG on AMPH-extinction in females.

Vasquez LS, Stack SM, Taylor WW, Dias BG (*CHLA/ USC*) Learning, memory, and motivation in Prader-Willi Syndrome through the lens of *snord116* in catecholaminergic cells ABSTRACT: Prader-Willi Syndrome (PWS) is a rare genetic disorder that is primarily caused by a loss of genes within a critical region on the paternal allele of chromosome 15. Phenotypically, PWS presents with perturbations of learning, memory, and motivation (Copet et al., 2010; Cassidy et al., 2012; Li et al., 2019; Dykens et al., 2007). Of the paternal alleles deleted on chromosome 15, whole brain knock-outs of the paternal allele of the *snord116* gene cluster in mice results in PWS phenotypes similar to humans (Adhikari et al., 2019; Ding et al., 2008; Bieth et al., 2015). Missing from such analyses are the effects of deleting the paternal allele of *snord116* in specific neuronal populations. With catecholamines implicated in learning, memory, and motivation, we used a CRE-loxP approach in mice to determine how these processes are affected after knocking-out the paternal allele of *snord116* in catecholaminergic cells. Experimental TH-CRE+ animals had the paternal allele of *snord116* knocked-out in catecholaminergic cells, while control animals retained a functional copy of the paternal allele. Auditory fear conditioning and extinction training was performed to investigate learning and memory. Operant progressive ratio schedule of reinforcement (PR) was used to assay motivation. Changes in learning and memory were observed in CRE+ animals during recall of extinction training. Motivation was not impacted in CRE+ animals as measured by breakpoint ratio in the PR test. Our data suggest that knocking-out the paternal allele of *snord116* in catecholaminergic cells results in altered learning and memory. This approach has the potential to shed light on the neurobiology underlying deficits in learning and memory in the context of PWS.

Vazquez KE, Parsons RG (*SUNY Stony Brook*) Contextual fear expression activates frontal cortex projections to the ventral periaqueductal gray ABSTRACT: Studies of fear expression have largely focused on neural circuit interactions involving the amygdala. However, there are projections originating from multiple regions of the medial prefrontal frontal cortex (mPFC) that terminate across the different columns of the periaqueductal gray (PAG). Despite the fact that both mPFC and PAG have been implicated in regulating fear behavior, knowledge about the function of the connections between the mPFC and PAG is scant. As a first step, we infused a viral retrograde tracer into the ventral PAG in male rats and trained half of them in a contextual fear conditioning task, while the other half was exposed to the same context without receiving shock. The following day, all rats underwent a 10 minute context test in the conditioning chamber. 1 hour after testing, rats were perfused and the brains were harvested. Immunohistochemistry was performed and data were analyzed by counting the number of cells that were labeled by the viral tracer and cells that were positive for EGR1 in the anterior cingulate cortex (ACC), prelimbic cortex (PL), and infralimbic cortex (IL). Rats that underwent training and testing showed an increase in the proportion of viral infected cells that express EGR1 in the PL compared to rats that had only received context exposure. There were no differences between groups in the proportion of tracer labeled cells that were EGR1 positive in the ACC or IL. Experiments are ongoing to test if the pattern of findings is similar in females, and whether or not manipulating the function of PL-vPAG affects the expression of contextual fear.

Verma M, Toennies L, Kaplan K, Ferrara N, Hunsberger H (*Lake Forest/ Rosalind Franklin*) Sex differences in cognitive decline, affective behaviors, and PV interneuron activation in AD mice after isolation ABSTRACT: As innately social beings, humans are motivated by and dependent on social relationships throughout their lifetime. The importance of social interaction is emphasized by studies that demonstrate social

isolation as a risk factor for negative health outcomes such as cardiovascular disease, increased risk for mortality, depression and anxiety, and cognitive decline. Elderly people are particularly susceptible to becoming isolated and cognitive decline is higher among seniors who report loneliness, and this is greater among females. In contrast, intact social networks are highly protective against dementia in both humans and rodents. While there are familial AD mutations, most cases of AD are sporadic and the cause is unknown. However, one promising avenue to reduce AD prevalence by 4% is to eliminate social isolation as a risk factor. This would provide a greater reduction in prevalence than combatting physical activity (2%) and hypertension (2%). Moreover, the recent COVID-19 pandemic forced millions into repeated social isolation periods ranging from several weeks to months, a situation which could occur in the future. Therefore, it is essential to understand risk factors, such as isolation, for early interventions. The goal of this proposal is to understand the sex-specific effects of social isolation on memory and social interaction in aging and AD mice. Our central hypothesis is that social isolation alters both long-term memory and social behavior to a greater extent in AD phenotypes that will be differentially affected between sexes. To explore the impact of social isolation on AD, we ran a battery of social, anxiety, and cognitive behavioral tests. Although most isolation studies result in memory impairment, our AD female mice exhibited enhanced memory retention after acute isolation as measured by contextual fear conditioning. Isolated male control and AD mice showed a memory deficit compared to group housed mice. Social interaction was decreased after a stressor in all groups and this was exacerbated by isolation in female mice. We are currently examining brain tissue to determine excitatory/inhibitory cell activation using cFos, an immediate early gene, and Parvalbumin (PV), an inhibitory interneuron. We predict increased cFos and decreased PV activation in isolated AD females, a disturbance in the excitation/inhibition balance in the amygdala and hippocampal regions. The results from the proposed project would provide foundational evidence for the impact of the social environment on age-related cognitive decline. These results would mechanistically identify a novel pathway through which social circumstances impact degenerating neural circuits to drive age-related alterations in behavior. This work was supported by: LFC Summer Intern Program.

Vogt GS, Sheynin J, Nickelsen T, Lokshina Y, Abelson JL, Liberzon I (*TAMU*) Effects of context processing on physiological fear responses during learning and recall of fear and extinction memories ABSTRACT: Altered contextual processing is hypothesized to underlie memory abnormalities in PTSD. Specifically, altered pattern separation (PS) and/or pattern completion (PC), two processes that underlie contextual processing, might contribute to impaired consolidation and recall of fear and extinction memories. We examined the relationship between skin conductance response (SCR) during fear conditioning, extinction, extinction recall and fear renewal, and PS and PC performance. Ninety-two participants (46% female, mean age (SD) = 30 (11) years) underwent a fear conditioning and extinction paradigm. During fear conditioning in a danger context, one conditioned stimulus (CS+; colored light) was paired with an aversive unconditioned stimulus (US; loud noise) at 60% reinforcement, while the other (CS-) was not. During extinction in a safe context, both CS's were presented without the US. Extinction recall and fear renewal were assessed the following day and SCR was collected throughout. Participants also performed the Mnemonic Similarity Task (MST) to distinguish memorized items from novel items or lures, and a novel Context Separation and Completion (CSC) task where they identified the type of room presented ("office" vs. "living room") from incomplete available details. PS and PC scores were derived from MST and CSC, respectively. Scores were z-transformed, and Pearson correlations were run between PS, PC and SCR. We hypothesized that greater PS would be associated with higher SCR in response to CS+ and lower SCR in response to CS- during conditioning, that greater PC would be associated with lower SCR in response to CS+ during extinction, that greater PC would be associated with higher SCR in response to both cues during extinction recall, and that greater PS would be associated with higher SCR in response to CS+ while greater PC would be associated with higher SCR in response to CS- during fear renewal. During fear conditioning, PS positively correlated with SCR in response to CS+ ($r=.249$, $p=.019$) and CS- ($r=.211$, $p=.047$). Neither PS nor PC were correlated with SCR in response to either cue during extinction. During extinction recall, PC positively correlated with greater SCR in response to both CS+($r=.254$, $p=.028$) and CS- ($r=.263$, $p=.022$), while during fear renewal, PS positively correlated with SCR in response to CS+ ($r=.238$, $p=.025$) and PC positively correlated with SCR to CS- ($r=.278$, $p=.011$). Overall, our findings suggest that PS performance is associated with the identification of cues as dangerous and the renewal of fear responses, whereas PC performance is associated with altered safety learning. Data collection from both healthy controls and PTSD patients is ongoing, and future analyses will test the differences between these two groups.

Welch HF, Thorn CA (*UT Dallas*) Exploring right versus left VNS effects on motor performance and cortical plasticity ABSTRACT: Left vagus nerve stimulation (l-VNS) paired with motor rehabilitation is FDA approved for aiding in stroke recovery, with improved clinical outcomes correlated with increased neuroplasticity in the motor cortex (M1). This enhanced neuroplasticity is facilitated by l-VNS driven neuromodulatory signaling, including in the cholinergic, noradrenergic, and serotonergic

systems. Although the dopaminergic system is also known to promote plasticity, it is not required for l-VNS-induced M1 plasticity. However, right vagus nerve stimulation (r-VNS) activates midbrain dopaminergic "reward" circuits, a mechanism not shared by l-VNS. Prior research has shown that this distinct mechanism of r-VNS can lead to behavioral differences, including the ability to sustain self-administration behavior and induce appetitive responses. We hypothesize that r-VNS driven dopaminergic signaling may enhance cortical plasticity and appetitively reinforce learned behaviors, providing greater potential for post-stroke recovery compared to l-VNS. To test these hypotheses, female Long-Evans rats were trained on a VNS-paired skilled reaching lever-press task that required the rats to press a lever to receive a food pellet reward. After achieving stable performance, rats received a VNS cuff implanted around the left or right cervical vagus nerve. After recovery, rats underwent 5 days of training-paired VNS treatment, in which VNS pulses were triggered upon detection of a correct lever press. Sham treatment groups underwent the same procedures but did not receive stimulation in the final 5 days of training. Within 24 hours after the last training session, M1 was mapped using intracortical microstimulation to assess l-VNS versus r-VNS induced plasticity. To test whether l-VNS and r-VNS differentially reinforce skilled behavior, we compared behavioral performance across groups during the treatment period. This research aims to elucidate the extent to which r-VNS driven dopaminergic signaling may be targeted to enhance the therapeutic potential of VNS in stroke rehabilitation. This work was supported by: R21 DA055166, R01 NS126816, and UT Dallas.

West L, Davis I, Smith TR, Southern R, Panfil K, Kirkpatrick K (*Kansas State U*) Chemogenetic inactivation of the pre-limbic cortex increases impulsive decision-making in rats ABSTRACT: Impulsive decision making is associated with many maladaptive behaviors and mental health disorders. Impulsive choice can be defined as choosing a smaller-sooner (SS) reward over a larger-later (LL) reward when the LL reward is the more optimal choice. Our lab has established that time-based interventions are effective for decreasing impulsive decision-making. The improvements in timing behavior correlates with improvements in self-control, however the neural mechanisms underlying these improvements remain unclear. The prelimbic (PL) cortex has been implicated in processes underlying self-control, delay tracking, and interval timing. To elucidate the role of the PL during time-based interventions, we used designer receptors exclusively activated by designer drugs (DREADDs) to inactivate the PL during the intervention or choice assessment. The intervention was a forced choice exposure to both an SS and LL lever wherein the SS lever always resulted in 1 pellet following a 10-s delay and the LL lever always resulted in 2 pellets following a 30-s delay. The choice assessment was identical to the intervention but included free-choice trials and the SS delay increased from 10 to 30 s. Inhibiting the PL with DREADDs during either the intervention or choice phase of the experiment increased impulsive choices relative to control rats. Rats that had their PL inactivated during the choice phase of the experiment were also less sensitive to the SS delays. These results highlight the importance of the PL for learning to be self-controlled based upon experience with timing delays.

Wheeler AW, Truckenbrod LM, Garner M, Orsini CA (*UT Austin*) Relationships between fentanyl self-administration and risk-taking behavior in rats ABSTRACT: Individuals with opioid use disorder (OUD) display impaired decision-making behavior and elevated risk taking. To understand the relationship between opioid use and elevated risk taking, our lab employs a rodent model of decision making involving risk of explicit punishment (Risky Decision-making Task; RDT). In this task, rats choose between a small, safe food reward and a large food reward that is accompanied by increasing risk of mild footshock punishment. In Experiment 1, we examined whether individual differences in risk preference in the RDT predicted aspects of self-administration of the synthetic opioid fentanyl. Male Sprague-Dawley rats were characterized on the RDT and then underwent fentanyl self-administration (6 hours/day) for 21 days. During self-administration, rats escalated their fentanyl intake, but neither the rate of escalation nor overall fentanyl intake correlated with risk preference in the RDT. These data suggest that increased risk taking in individuals with OUD is likely a consequence of drug exposure. To explore this possibility, male and female rats were trained on the RDT until stable behavior emerged and then underwent fentanyl self-administration (6 hours/day) or sucrose self-administration for 14 days (Experiment 2). Following the cessation of self-administration, rats remained undisturbed for 3 weeks and were then re-tested on the RDT to assess fentanyl-induced changes in risk taking. Relative to performance before self-administration, rats that self-administered fentanyl displayed an increase in risk taking, an effect that was absent in sucrose control rats. Increased risk taking in rats that self-administered fentanyl could not be attributed to impaired behavioral flexibility, augmented motivation to work for food or diminished sensitivity to footshock. These findings support our hypothesis that the high rates of risk taking in individuals with OUD are a direct result of chronic opioid exposure. Future work will determine whether there is a similar lack of relationship between drug-naïve risk taking and aspects of fentanyl self-administration in females and will also focus on identifying the neurobiological mechanisms that contribute to fentanyl-induced increases in risk taking. Collectively, this work will provide important insight into the neurobehavioral mechanisms underlying the relationship between opioid use and altered risk taking. This work was

supported by: R00DA041493 (CAO), R21DA053462 (CAO), 5T32DA018926-18 (AW).

Williams BL, Demaestri C, Berry GC, Darling AM, Bath KG (*Columbia/ NYSPI*) Exploring the impact of early life adversity on stress-enhanced fear learning and fear expression in mice ABSTRACT: Early life adversity (ELA), including altered maternal care induced by resource scarcity, has been shown to alter the development of stress systems in the brain and increase the risk for pathology later in life. In humans, ELA has been associated with increased sensitivity to stressors and may contribute to elevated risk for the development of post-traumatic stress disorder (PTSD). However, the mechanisms by which multiple stressors across the lifespan contribute to increased vulnerability for pathology remain poorly understood. Stress-enhanced fear learning (SEFL) has been established as a robust model of PTSD-like behaviors, in which animals receive 4 unsignaled, 1mA foot shocks in a given context, resulting in sustained fear sensitization that is resistant to extinction. Here, we tested the impact of the limited bedding and nesting (LBN) model of ELA combined with SEFL in adulthood, and measured fear expression in a novel context in response to an unconditioned tone. Using male and female C57BL/6N mice, we found that ELA reared males exhibit reduced freezing to the unconditioned tone and enhanced extinction to the shock context. This reduction in freezing persisted four weeks later upon reexposure to the tone. Females in all groups demonstrated increased freezing in response to a novel tone, which was further amplified after a four-week incubation period. Interestingly, ELA females exhibited reduced augmentation of freezing at this later time point, compared to their control reared counterparts. These changes in SEFL-associated freezing, and the sex differences we observed, are potentially linked to altered fear expression dynamics and sex differences in the behavioral response to threat. Ongoing work aims to address this by examining sex and SEFL effects on the startle response and the expression of active fear. We will also explore the potential sex-specific effects of ELA on the activity of corticotropin-releasing hormone and somatostatin positive neurons in the central amygdala, deciphering their role in susceptibility or resistance to non-associative fear sensitization. Together, this work sheds light on the intricate interplay between ELA, sex, stress-enhanced fear learning, and fear expression dynamics, which points to potential mechanisms underlying vulnerability to stress-related disorders. Understanding these mechanisms may inform novel therapeutic strategies targeting PTSD and related conditions. This work is supported by: NSF GRFP.

Wilson LR, Plummer NW, Evsyukova IY, Patino D, Stewart CL, Smith KG, Konrad KS, Fry SA, Deal AL, Kilonzo VW, Panda S, Sciolino NR, Cushman JD, Jensen P (*NIEHS/ NIH/ Social and Scientific Systems, Inc./ UConn*) Partial or complete loss of norepinephrine differentially alters contextual fear and catecholamine release dynamics in hippocampal CA1 ABSTRACT: Contextual fear learning is heavily dependent on the hippocampus. Despite evidence that catecholamines contribute to contextual encoding and memory retrieval, the precise temporal dynamics of their release in the hippocampus during behavior is unknown. In addition, new animal models are required to probe the effects of altered catecholamine synthesis on release dynamics and contextual learning. In this study, we generated two new mouse models of altered locus coeruleus norepinephrine (LC-NE) synthesis and utilized them together with GRABNE and GRABDA sensors and *in vivo* fiber photometry to investigate norepinephrine (NE) and dopamine (DA) release dynamics in dorsal hippocampal CA1 during contextual fear conditioning. We report that aversive foot-shock increases both NE and DA release in dorsal CA1, while freezing behavior associated with recall of fear memory is accompanied by decreased release. Moreover, we find that recall of recent fear memory is sensitive to both partial and complete loss of LC-NE synthesis throughout prenatal and postnatal development, similar to prior observations of mice with global loss of NE synthesis beginning postnatally. In contrast, remote recall is compromised only by complete loss of LC-NE synthesis beginning prenatally. Overall, these findings provide novel insights into the role of NE in contextual fear and the precise temporal dynamics of both NE and DA during freezing behavior, and highlight a complex relationship between genotype, sex, and NE signaling.

Zeidler ZE, Moonkyu P, DeNardo LW (*UCLA*) Reorganization of cortical fear memories across time ABSTRACT: Emotionally salient memories are persistent and can endure for a lifetime, yet the neural representation of a memory can change dramatically. The medial prefrontal cortex (mPFC) is interconnected with hundreds of brain regions, including key areas involved in learning and memory, making it a prime candidate to orchestrate memory transformation and retrieval. While the PFC is involved in all stages of memory, a remarkably different subset of PFC neurons is active at different memory timepoints. Here we reveal novel functions for a projection-defined mPFC cell class innervating the temporal association area (TeA), a region known to associate auditory stimuli with emotional salience. Optogenetic inhibition of these projections impairs memory exclusively at remote but not recent memory timepoints. Using miniscopes to record, align, and track the activity of both PFC and TeA-projecting PFC neurons across time, we show that while the overall PFC population maintains a stable proportion of stimulus-encoding neurons, the identity of those neurons shifts across time to TeA-projecting neurons. Overall, these data support a model of temporally evolving prefrontal fear memory ensembles that recruit long-range projection neurons over time, forming a remote memory network that preferentially utilizes cortical processing of emotionally salient memory

cues. More broadly, this may suggest that the dynamic reorganization of memory serves to enhance modality-specific cortical processing before executing subcortical fear routines.

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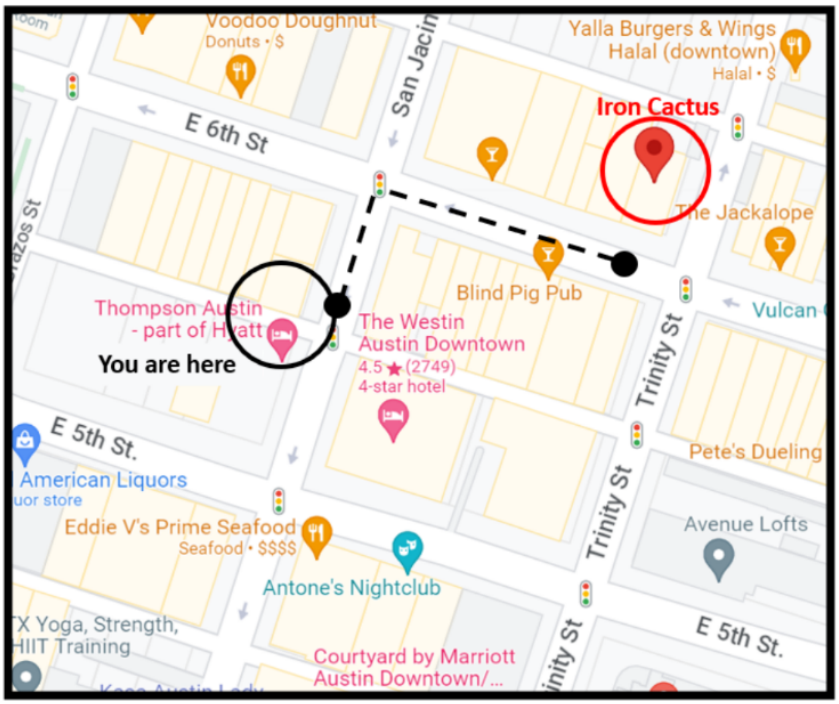

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