Dopamine in the Dorsal Bed Nucleus of Stria Terminalis signals Pavlovian sign-tracking and reward violations

Utsav Gyawali[1,2,4], David A. Martin[2], Fangmiao Sun[3], Yulong Li[3], Donna J. Calu[1,2,4]

Affiliations:

[1]Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, USA

[2]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA

[3]State Key Laboratory of Membrane Biology, Peking University School of Life Sciences; PKU-IDG/McGovern Institute for Brain Research; Peking-Tsinghua Center for Life Sciences, Beijing 100871, China

[4]Corresponding authors

Abstract

Midbrain and striatal dopamine signals have been extremely well characterized over the past several decades, yet novel dopamine signals and functions in reward learning and motivation continue to emerge. A similar characterization of real-time sub-second dopamine signals in areas outside of the striatum has been limited. Recent advances in fluorescent sensor technology and fiber photometry permit measurement of dopamine binding correlates, which can divulge basic functions of dopamine signaling in non-striatal dopamine terminal regions, like the dorsal bed nucleus of the stria terminalis (dBNST). Here, we record $GRAB_{DA}$ signals in the dBNST during a Pavlovian lever autoshaping task. We observe greater Pavlovian cue-evoked dBNST $GRAB_{DA}$ signals in sign-tracking (ST) compared to goal-tracking/intermediate (GT/INT) rats and the magnitude of cue-evoked dBNST $GRAB_{DA}$ signals decrease immediately following reinforcer-specific satiety. When we deliver unexpected reward or omit expected reward, we find that dBNST dopamine signals encode bidirectional reward prediction errors in GT/INT rats, but only positive prediction errors in ST rats. Since sign- and goal-tracking approach strategies are associated with distinct drug relapse vulnerabilities, we examined the effects of experimenter-administered fentanyl on dBNST dopamine associative encoding. Systemic fentanyl injections do not disrupt dBNST cue discrimination but generally potentiate dBNST dopamine signals. These results reveal multiple dBNST dopamine correlates of learning and motivation that depend on the Pavlovian approach strategy employed.

Introduction

Survival depends on learning to associate environmental cues with food or other natural rewards. Individual differences in learning and motivational processes support the acquisition, expression and updating of cue-reward associations. Recent evidence suggests that distinct learning strategies are predictive of dysregulated motivation for drug associated cues/conditioned stimuli (CS) (Chang et al., 2022; Martin et al., 2022; Pitchers et al., 2017; Saunders et al., 2013). Midbrain and striatal dopamine signals are broadly implicated in a diverse array of learning and motivational processes, including CS-reward associations underscoring the importance of dopamine (DA) in adaptive behavior that promotes survival (Langdon et al., 2018; Lee et al., 2022; Nasser et al., 2017). Yet considerably less is known about the role of DA signals in areas outside of the striatum during adaptive and maladaptive cue-reward learning. Recent advances in fluorescent sensor technology and fiber photometry permit measurement of DA binding correlates (Labouesse et al., 2020). These new techniques can reveal understudied functions of DA signaling in non-striatal DA terminal regions like the dorsal bed nucleus of the stria terminalis (dBNST), an extended amygdala nucleus, that is critical for dysregulated CS-triggered opioid relapse (Gyawali et al., 2020). Here, we characterize basic dBNST DA correlates by recording fluorescent $GRAB_{DA}$ signals during a Pavlovian task that distinguishes two distinct relapse vulnerability phenotypes.

Recent studies identify unique learning strategies that predict heightened CS-triggered relapse vulnerability (Chang et al., 2022; Martin et al., 2022; Pitchers et al., 2017; Saunders et al., 2013). In particular, a simple Pavlovian Lever Autoshaping task distinguishes two extreme tracking phenotypes: 1) sign-tracking (ST) rats that approach and vigorously engage with the reward predictive lever cue, even though cue interaction is not necessary to obtain food reward and 2) goal-tracking rats that interact with the foodcup during cue presentation where food reward is delivered after lever retraction (Boakes R.A, 1977; Flagel et al., 2007; Hearst &

82 Jenkins, 1974; Meyer et al., 2012). Sign-tracking rats show heightened CS-triggered drug
83 relapse vulnerability compared to goal-trackers. A third group called intermediates approach
84 both the food cup and lever at similar levels, and their relapse vulnerability is like that of goal-
85 tracking rats (Saunders and Robinson 2010). Fast scan cyclic voltammetry recording of real-
86 time dopamine indicated that sign-, but not goal-tracking, evokes increases in phasic
87 fluctuations in DA in the nucleus accumbens (NAc) during CS presentation (Flagel et al., 2011).
88 NAc DA is necessary for both the expression of sign-tracking and for sign-trackers heightened
89 CS-triggered drug relapse, but not for goal-tracking or their relapse behavior (Saunders et al.,
90 2013). Given the critical role of dBNST in CS-triggered relapse, we aimed to determine whether
91 there are similar individual differences in dBNST DA signaling in sign- and goal-tracking rats
92 using the Pavlovian Lever Autoshaping task (Buffalari & See, 2011; Gyawali et al., 2020;
93 Silberman & Winder, 2013).

94 Midbrain dopamine neuron activity strengthens cue-outcome associations by serving as
95 a bidirectional prediction error signal where, unexpected reward delivery increases and omitted
96 reward decreases dopamine neuron firing relative to expected reward (Montague et al., 1996;
97 Schultz, 2015; Schultz et al., 1997). Over the course of learning, the phasic dopamine activity
98 transfers from the unconditioned stimulus (US) to the CS (Montague et al., 1996; Schultz, 2015;
99 Schultz et al., 1997). In the NAc, transfer of dopamine signals from US to the CS occurs more
100 robustly in ST compared to GT rats (Flagel et al., 2011; Lee et al., 2018; Saddoris et al., 2016)
101 and NAc DA antagonism reduces sign-tracking but not goal-tracking behaviors (Saunders &
102 Robinson, 2012). Together, these studies support the Pavlovian lever autoshaping task (and
103 sign-tracking) as a reliable framework for studying dopamine's role in regions of the brain
104 critically involved in cue-motivated natural and drug reward seeking behaviors.

105 The dBNST receives dense dopaminergic input from several midbrain regions including
106 the ventral tegmental area, ventral periacqueductal gray, and to a much lesser extent, the

107 substantia nigra (Hasue and Shammah-Lagnado, 2002; Meloni et al., 2006). dBNST dopamine
108 is associated with a variety of reward-motivated behaviors. dBNST dopamine release is
109 increased during intra-oral sucrose infusion and in response to cues that predict intracranial
110 self-stimulation of the medial forebrain bundle (Lin et al., 2020; Park et al., 2012, 2013). Further,
111 dopamine antagonist injections in the dBNST reduce responding for sucrose in a binge eating
112 paradigm (Maracle et al., 2019). All major drugs of abuse, including opioids, increase
113 extracellular dopamine in the BNST and dBNST dopamine antagonism reduces cocaine self-
114 administration and ethanol seeking (Carboni et al., 2000; Eiler et al., 2003; Epping-Jordan et al.,
115 1998). Despite these studies implicating dBNST dopamine in motivated behaviors, a
116 comprehensive characterization of endogenous dBNST dopamine dynamics in cue-induced
117 behaviors is lacking. To address this, we used a dopamine sensor $GRAB_{DA}$ in combination with
118 fiber photometry to examine basic properties of the dBNST dopamine signals; their role during
119 lever autoshaping, reward violations, outcome specific-satiety and during systemic fentanyl
120 administration (Sun et al., 2018).

121 **Results**

122 **BNST $GRAB_{DA}$ signals respond to cues and reward in a Pavlovian learning task**

123 We sought to determine if BNST $GRAB_{DA}$ signals correlate with individual differences in
124 approach to Pavlovian cues (Experiment timeline in Fig. 1A). First, we trained rats in PLA for
125 five days (Fig. 1B) to examine the acquisition of lever- and food cup-directed behaviors across
126 training in sign and goal tracking/intermediate rats. Representative and histological inventory of
127 $GRAB_{DA}$ expression from these rats is shown in Fig. 1C. We analyzed the behavioral PCA score
128 using a mixed ANOVA with between subject factors of Tracking (ST, GT/INT) and within subject
129 factors of Session (Day 1, Day 5; Fig. 1D). ST rats show greater PCA score on Day 5 compared
130 to GT/INTs (Fig. 1D, PCA score: Session: $F_{(1,14)} = 67.3$, $p<0.001$, Session x Tracking: $F_{(1,14)} =$
131 15.04, $p=0.002$, Tracking: $F_{(1,14)} = 11.59$, $p=0.004$; post-hoc, Day 5 ST vs. GT/INT presses: $t_{14} =$

4.92, p<0.001). Next, to confirm rats could discriminate the reinforced and non-reinforced lever cues, we examined the difference between CS+ and CS- presses (Δ presses) and pokes (Δ pokes) using a mixed ANOVA with between subject factors of Tracking (ST, GT/INT) and within subject factors of Session (Day 1, Day 5, Fig. 1E). ST rats show better discrimination for lever directed behavior (Δ presses) on Day 5 compared to GT/INTs (Session: $F_{(1,14)} = 35.75$, $p<0.001$, Session x Tracking: $F_{(1,14)} = 11.66$, $p<0.001$, Tracking: $F_{(1,14)} = 17.81$, $p=0.001$; post-hoc, Day 5 ST vs. GT/INT presses: $t_{14} = 3.93$, $p=0.002$). In contrast, GT/INTs show better discrimination for food cup directed behavior (Δ pokes) on Day 5 compared to STs during the CS (Fig. 1F: Session x Tracking: $F_{(1,14)} = 4.90$, $p=0.044$, Tracking: $F_{(1,14)} = 15.17$, $p=0.002$; post-hoc, Day 5 ST vs. GT/INT pokes: $t_{14} = -3.92$, $p=0.002$).

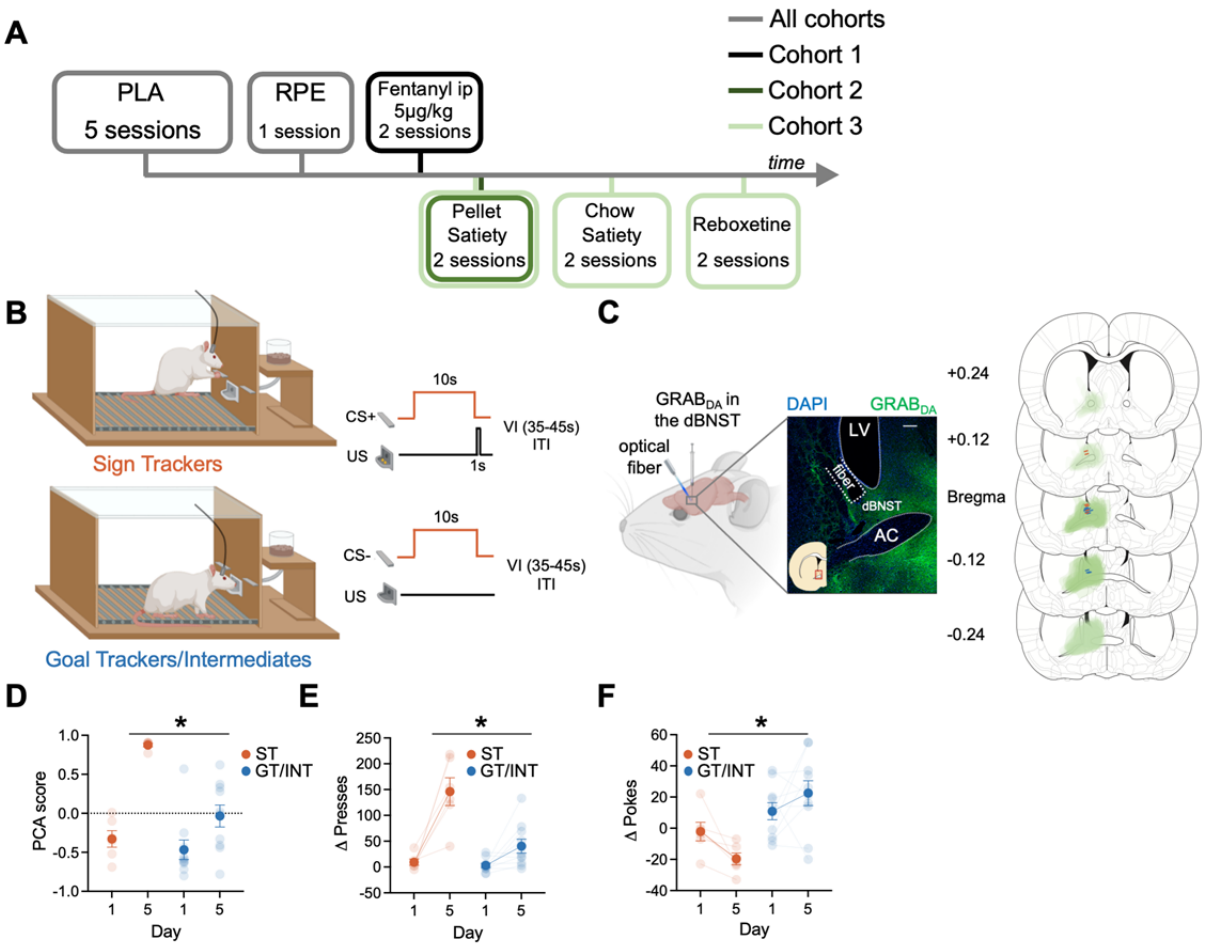

Figure 1: Individual differences emerge during Pavlovian Lever Autoshaping (PLA). (A) Experimental timeline. We trained all rats for five daily reinforced PLA sessions to determine their tracking groups followed by a single reward prediction error (RPE) session. We injected the first cohort of rats with i.p fentanyl in PLA and tested the second and the third cohort of rats on 2 counterbalanced PLA pellet satiety sessions. We tested the third cohort of rats on 2 counterbalanced PLA chow satiety and with reboxetine i.p. injection sessions. **(B)** PLA sessions consisted of presentation of 10s of cue (either conditioned stimulus, CS+ or CS- lever, pseudorandom order with an intertrial interval (ITI) varying (variable interval (VI)) between 35 and 45s) followed by lever retraction and delivery of two food pellets in the foodcup. Some rats (Sign Trackers, STs) engage with the cue while others (Goal trackers, GTs) wait for the food pellets in the food cup during cue period. Others display both lever and food cup behaviors (Intermediates, INTs) **(C)** Left: representative expression of $GRAB_{DA}$ construct and fiber placement in dorsal Bed Nucleus of Stria Terminalis (dBNST). White scale bar: 250µm. Right: The extent of $GRAB_{DA}$ expression and fiber placement across five coronal planes with anterior distance from bregma (millimeters) in the dBNST in STs (orange) and GT/INTs (blue). Drawings were adapted from Figures 31, 32, 33, 34 and 35 from Paxinos and Watson (2007). **(D)** Average PCA scores for STs and GT/INTs on Day 1 and Day 5 of PLA. **(E)** Average Δ Presses (CS+) – (CS-) on Day 1 and Day 5. **(F)** Average Δ Pokes (CS+) – (CS-) on Day 1 and Day 5. Data are mean±SEM. *p<0.05

To investigate the endogenous dBNST dopamine activity across PLA training, we used fiber photometry to monitor the fluorescent activity of the genetically encoded dopamine sensor, $GRAB_{DA}$ (Sun et al., 2018). We see evidence of associative encoding during PLA (Fig. 2). Both lever insertion and retraction/reward delivery increased dBNST $GRAB_{DA}$ signals in ST and GT/INT rats (representative heat map and population average traces on Day 1 and Day 5 for STs in Fig. 2A and GT/INTs in Fig. 2B). To determine whether ST and GT/INT rats show differences in cue-evoked dopamine signals across acquisition of PLA, we compared the strength of CS+ onset (Δ lever extension area under curve (AUC) = (CS+) – (CS-) AUC; 2s after CS onset) signals between Day 1 and Day 5 using a mixed ANOVA with between subject factors of Tracking (ST, GT/INT) and within subject factor of Session (Day 1, Day 5). While CS+ onset-evoked $GRAB_{DA}$ signals increased across conditioning for both ST and GT/INT (Fig. 2C, Session: $F_{(1,14)} = 19.69$, $p=0.001$) the magnitude of the CS+ signal increase differed between tracking groups (Session x Tracking: $F_{(1,14)} = 5.99$, $p=0.028$, Tracking: $F_{(1,14)} = 10.35$, $p=0.006$). Post hoc analyses revealed greater cue-evoked dBNST $GRAB_{DA}$ signal in ST compared to GT/INT on Day 5, which was not evident on Day 1 (Day 1: $t_{14} = 0.17$, $p=0.87$; Day 5: $t_{14} = 2.93$, $p=0.011$). Next, we asked whether $GRAB_{DA}$ signals correlated with the tracking phenotype. We observed a positive correlation between Day 5 CS onset $GRAB_{DA}$ signals and Day 5 PLA score

179 (Fig. 2D; R^2 = 0.41, p=0.009) but not Day 1 CS onset $GRAB_{DA}$ signals and Day 1 PLA score
180 (Figure 2 – figure supplement 1A; R^2 = 0.21, p=0.09).

181 Next, we examined tracking differences in the sustained $GRAB_{DA}$ signal between STs and
182 GT/INTs throughout the duration of the CS, during which STs and GT/INTs show differences in
183 lever and foodcup directed behaviors. We compared Day 1 vs. Day 5 CS+ maintained (Δ cue-
184 period AUC = (CS+) – (CS-) AUC during the full 10s CS lever insertion period) $GRAB_{DA}$
185 signaling. CS+ maintained $GRAB_{DA}$ signals increased across conditioning for both ST and
186 GT/INT (Fig. 2E, Session: $F_{(1,13)}$ = 11.45, p=0.005, Session x Tracking: $F_{(1,13)}$ = 3.07, p=0.1,
187 Tracking: $F_{(1,13)}$ = 16.5, p=0.001). Like cue onset, we saw a strong positive correlation between
188 Day 5 $GRAB_{DA}$ signals during CS interaction and Day 5 PLA score (Fig. 2F, R^2 = 0.49, p=0.004)
189 but not Day 1 $GRAB_{DA}$ signals and Day 1 PLA score (Figure 2 – figure supplement 1B, R^2 =
190 0.08, p=0.3) suggesting that as rats display ST behavior, there's an increase in sustained
191 $GRAB_{DA}$ signal.

192 Prior work shows that NAc dopamine shifts from US to CS after conditioning to a greater degree
193 in STs compared to GTs (Flagel et al., 2011; Lee et al., 2018; Saddoris et al., 2016). Since we
194 observed differences in CS evoked BNST $GRAB_{DA}$ signals between STs and GT/INTs, we
195 wanted to determine if there was similar tracking specificity in the US to CS shift for BNST
196 $GRAB_{DA}$ signals. We quantified the relative CS/US dynamics across conditioning using a
197 difference score (Δ cue-reward AUC = (CS+) – (US) AUC for the 2s after CS+ onset and reward
198 delivery) and compared between Day 1 and Day 5. We used a mixed ANOVA with between
199 subject factors of Tracking (ST, GT/INT) and within subject factor of Session (Day 1, Day 5).
200 The relative CS/US dynamics across PLA differed by tracking group (Fig. 2G, Session: $F_{(1,14)}$ =
201 4.79, p=0.046, Session x Tracking: $F_{(1,14)}$ = 8.9, p=0.01). We found no tracking group
202 differences in the (CS+) – (US) difference score on Day 1 but by Day 5, the CS/US difference
203 score was greater in STs compared to GT/INTs (ST vs. GT/INT, Day 1: t_{14} = -1.6, p=0.13; ST vs

GT/INT, Day 5: t_{14} = 2.43, p=0.029). While the correlation between (CS+) – (US) $GRAB_{DA}$ signal and Day 5 PCA scores was marginal (Fig. 2H, R^2 = 0.22, p=0.06) there was no relationship between these measures on Day 1 (Figure 2 - figure supplement 1C, R^2 = 0.025, p=0.56). Overall, these data indicate sign-tracking specific dBNST $GRAB_{DA}$ signals increase to Pavlovian cue onset and during cue-maintained sign-tracking behaviors, and back propagate from the reward to cue onset across conditioning.

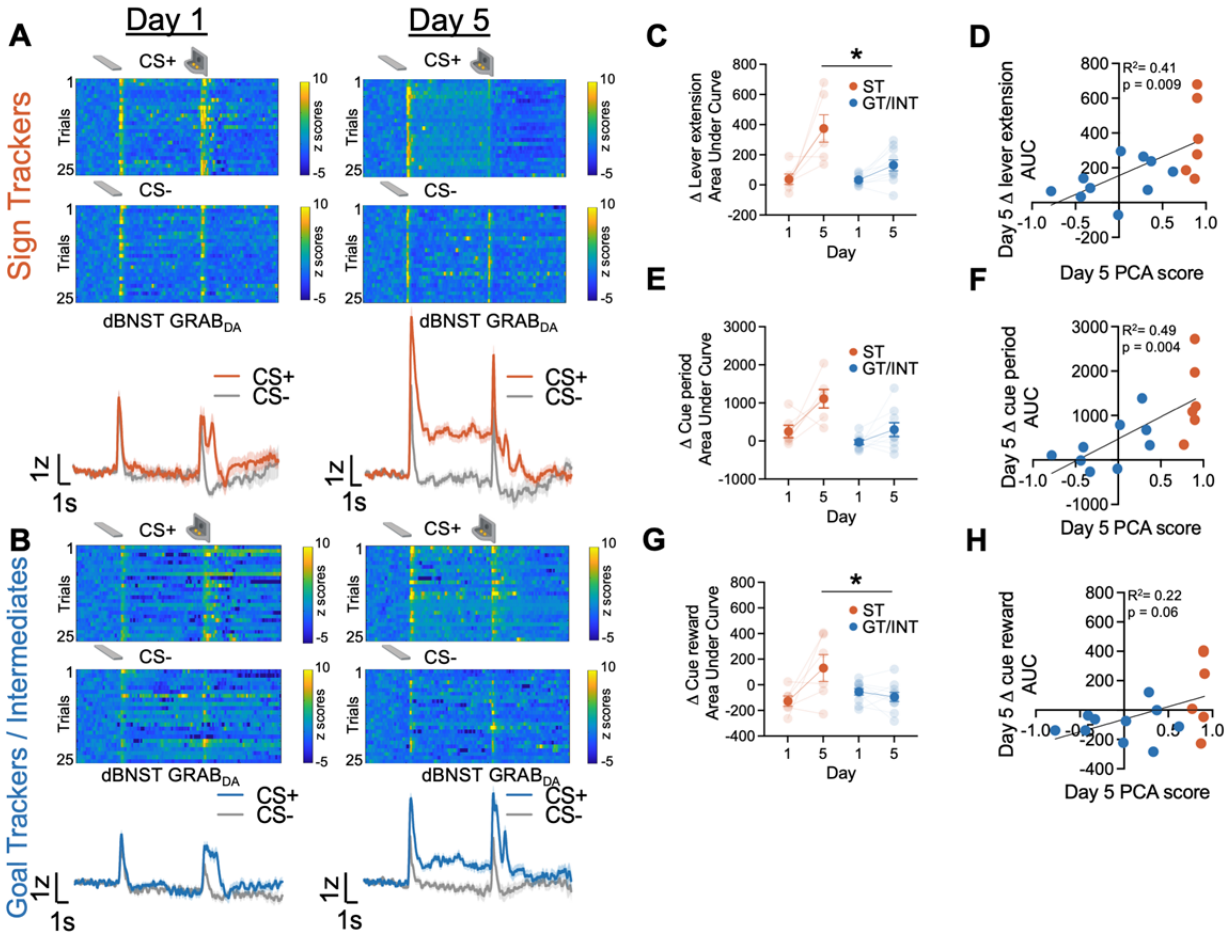

Figure 2: Dorsal bed-nucleus of stria terminalis (dBNST) $GRAB_{DA}$ signals during PLA between STs and GT/INTs. Representative heat maps illustrating $GRAB_{DA}$ signal changes (z-scores) during CS+ and CS- presentations on Day 1 (top left) and Day 5 (top right) and trial-averaged $GRAB_{DA}$ signal change (z-scored ΔF/F) during CS+ and CS- presentations on Day 1 (bottom left) and Day 5 (bottom right) in **A)** STs and **B)** GT/INTs. **C)** Trial averaged quantification Δ lever extension ((CS+) – (CS-); 2s) $GRAB_{DA}$ area under (AUC) between STs and GT/INTs. **D)** Correlation between Day 5 PCA scores and Day 5 Δ lever extension AUC. **E)** Trial averaged quantification of Δ cue period ((CS+) – (CS-); 10s) in AUC during cue period (10s) between STs and GT/INTs. **F)** Correlation between Day 5 PCA scores and Day 5 Δ cue

period AUC. **G)** Trial averaged quantification of Δ cue-reward (CS+) – (US), 2s) in AUC between STs and GT/INTs. **H)** Correlation between Day 5 PCA scores and Day 5 change in Δ cue-reward AUC. Data are mean±SEM. *p<0.05.

Figure supplement 1: No correlation between Day 1 PCA scores and Day 1 BNST $GRAB_{DA}$ signals.

Figure supplement 2: Norepinephrine reuptake blocker, Reboxetine doesn't alter PLA behavior or $GRAB_{DA}$ signals.

Figure supplement 3: Representative and population graph of signals from rats that had correct fiber placement and viral expression but under 2z peak.

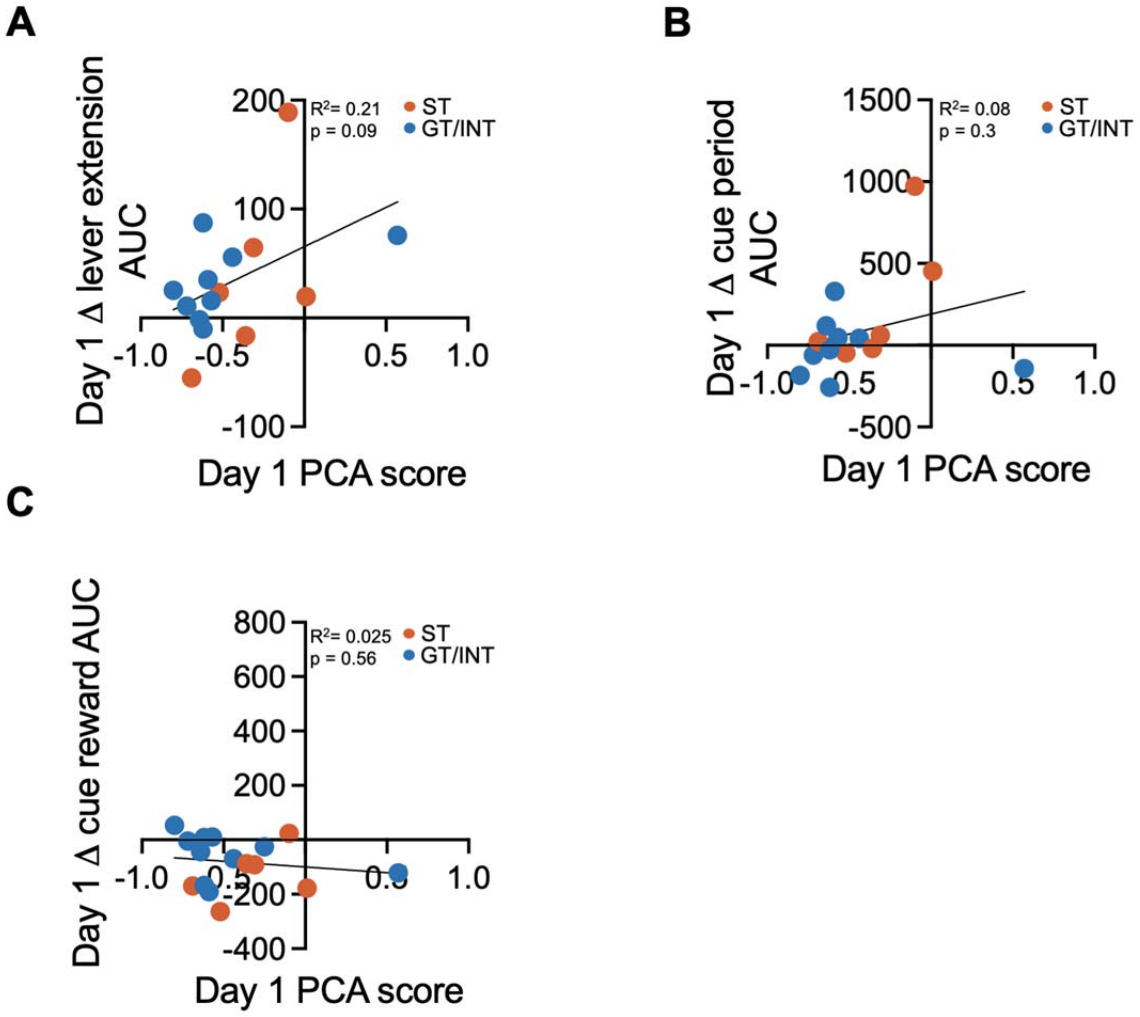

Figure 2 – Figure supplement 1: No correlation between Day 1 PCA scores and Day 1 BNST $GRAB_{DA}$ signals. Correlation between **A)** Day 1 PCA scores and Day 1 Δ lever extension AUC. **B)** Day 1 PCA scores and Day 1 Δ cue period AUC and **C)** Day 1 PCA scores and Day 1 Δ cue-reward AUC.

dBNST $GRAB_{DA}$ signals during PLA are specific to dopamine

238 Even though the $GRAB_{DA}$ construct we used is 15-fold more sensitive to dopamine than
239 norepinephrine **(NE)**; BNST **NE** plays an important role in motivated behaviors and dBNST
240 receives **dense** noradrenergic input **with relatively slow NE clearance measured *in vivo*** (Egli
241 et al., 2005; Flavin & Winder, 2013; **Park et al., 2009**; Sun et al., 2020). To validate that the
242 signals we recorded during PLA were dopaminergic and not noradrenergic, we injected a **NE**
243 reuptake inhibitor Reboxetine (1mg/kg) 30 minutes prior to PLA. **NE** levels in the brain remain
244 elevated at this dose for up to 3 hours peaking at ~20 minutes after injection (Page & Lucki,
245 2002). We found that Reboxetine injection did not alter behavior (Figure 2 – figure supplement
246 2B) or increase BNST $GRAB_{DA}$ signal to lever extension or reward consumption (Figure 2 –
247 figure supplement 2A,C,E), Epoch: $F_{(1,12)} = 3.82$, $p=0.074$, Epoch x Treatment: $F_{(1,12)} = 0.20$,
248 $p=0.66$, Treatment: $F_{(1,12)} = 0.21$, $p=0.66$) compared to saline injection. Further, there was no
249 difference in cue-interaction period between reboxetine and saline injected conditions (Figure 2
250 – figure supplement 2D; $t_6 = 1.14$, $p=0.3$). These data confirm that the signals we recorded
251 during PLA are not sensitive to noradrenergic reuptake inhibition and are most likely due to
252 fluctuations in DA signaling in the BNST.

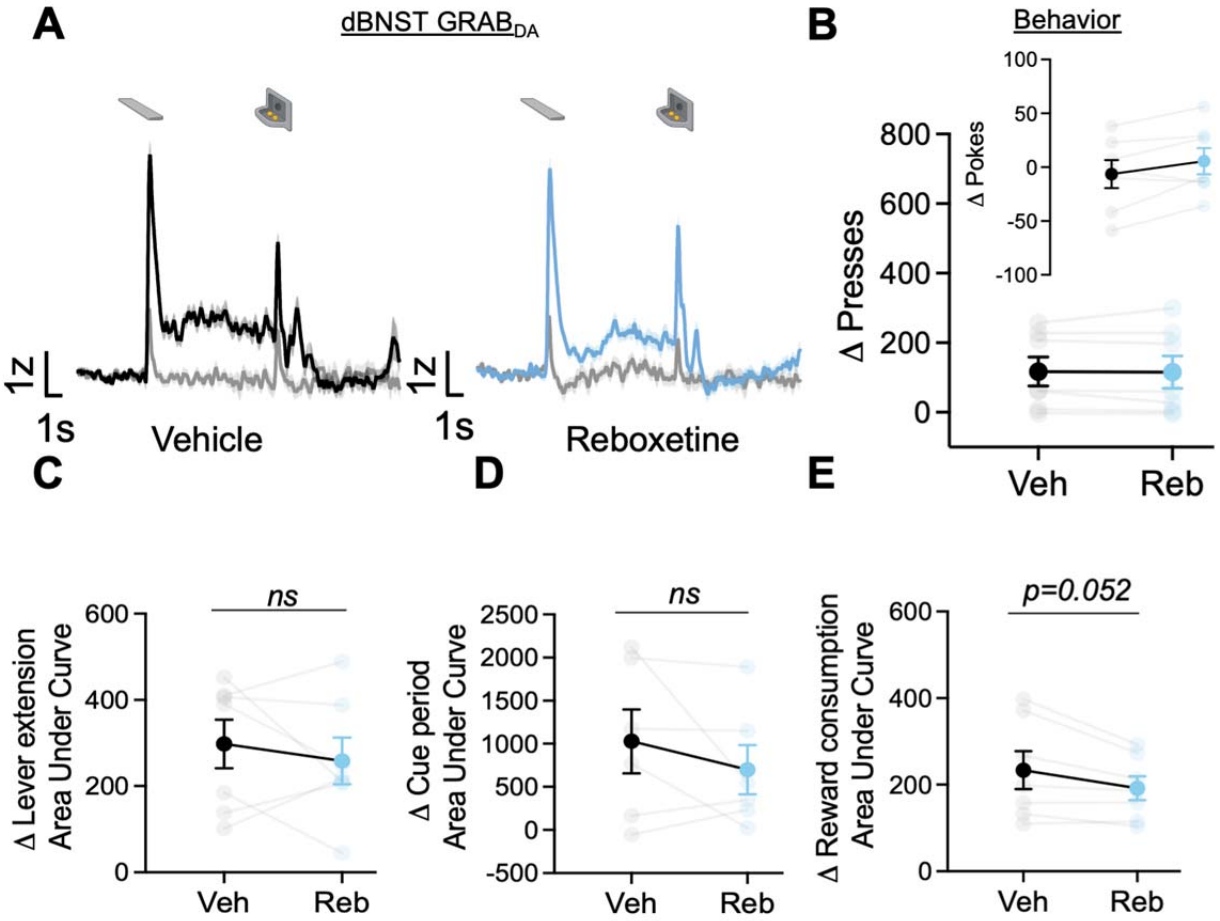

Figure 2 – Figure supplement 2: Norepinephrine reuptake blocker, Reboxetine doesn't alter PLA behavior or GRAB$_{DA}$ signals. A) Trial-averaged GRAB$_{DA}$ signal change (z-scored ΔF/F) and **B)** Average Δ Presses (CS+) – (CS-) and average Δ pokes (CS+) – (CS-) (inset) when rats were injected with vehicle or Reboxetine during PLA. Trial average quantification of change (CS+) – (CS-) in area under GRAB$_{DA}$ z-scored curve (AUC) during **C)** lever extension (2s), **D)** cue period (10s) and **E)** reward consumption between vehicle and reboxetine conditions. Data are mean±SEM. Veh = Vehicle, Reb = Reboxetine.

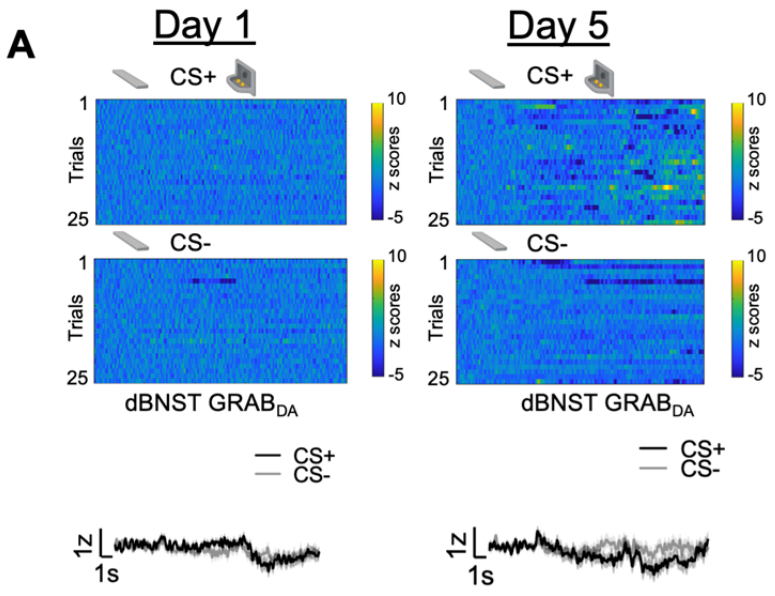

Figure 2 – Figure supplement 3: Representative and population graph of signals from rats that had correct fiber placement and viral expression but under 2z peak. A) Representative heat maps illustrating GRAB$_{DA}$ signal changes (z-scores) during CS+ and CS- presentations on Day 1 (top left) and Day 5 (top right) and trial-averaged GRAB$_{DA}$ signal change (z-scored ∆F/F) for N = 4 rats during CS+ and CS- presentations on Day 1 (bottom left) and Day 5 (bottom right).

BNST dopamine encodes reward prediction error

After five Pavlovian autoshaping sessions, we conducted a Reward Prediction Error (RPE) session in which we randomly intermixed expected food reward trials and unexpected food reward delivery with omission trials. Expected reward (Expected) trials are identical to those delivered during training, with a 10s CS+ lever insertion followed by retraction and food reward delivery. Unexpected reward (Positive) trials consist of randomly delivered food reward that is not signaled by a cue. Unexpected omission (Negative) trials consist of 10 s CS+ lever insertion and retraction, but no food reward is delivered. During these sessions we monitored BNST GRAB$_{DA}$ signals to examine whether dopamine signals track errors in reward prediction (representative heat map for each trial type in Fig. 3A-C; Schultz et al., 1997).

First, to determine whether BNST GRAB$_{DA}$ signals encode bidirectional reward prediction error **(RPE)**, we compare signals on expected, positive and negative trials. Notably, because lever

retraction occurs simultaneously with reward delivery, and sign- and goal-trackers may be in different locations at this time, we examine the signals during the six seconds **(three 2-s bins)** after reward delivery or omission, which captures the period corresponding to violations in reward expectations (Fig. 3D). We performed a repeated measures ANOVA **on z scores during the RPE session** including Trial Type (Expected, Positive, Negative) and Bin (three 2 s bins **(0-2 s, 2-4 s, 4-6 s**) as factors. We observed a difference **in dBNST $GRAB_{DA}$ signaling** between the three trial types in the bins following reward delivery/omission (Fig. 3D, Bin: $F_{(2,72)}$ = 13.65, p<0.001, Bin x Trial Type: $F_{(4,72)}$ = 13.99, p<0.001, Trial Type: $F_{(2,36)}$ = 3.49, p=0.041). Post-hocs confirm that in the second 2-second bin **(ie. 2-4 s)** after reward delivery/omission, BNST $GRAB_{DA}$ signals differed from one another for all three trial types, Expected vs. Positive (population traces in Fig. 3E; p=0.013), Expected vs. Negative (population traces in Fig. 3F; p=0.043) and Positive vs. Negative (p=0.0004). **Across all rats, we observe that dBNST $GRAB_{DA}$ signals reflect bidirectional reward prediction errors.**

Then to determine whether there are tracking differences in dBNST RPE signals, we separately analyzed **the z scores during RPE sessions in the two tracking groups. Again, we examine how $GRAB_{DA}$ signaling differs for the three trial types (expected, positive, negative) during the three 2-second bins after reward delivery** (population traces for STs and GT/INTs Fig. 3G-H). **In GT/INT rats we observed main effects of Trial ($F_{(2,12)}$ = 8.2, p=0.006) and Bin ($F_{(2,12)}$ = 4.9, p=0.027) and a Trial x Bin interaction ($F_{(4,24)}$ = 25.7, p<0.001). GT/INT rats showed evidence for both positive RPE (Trial (Expected, Positive) x Bin interaction $F_{(2,12)}$ = 14.5, p=0.001) and negative RPE Trial (Expected, Negative) x Bin interaction $F_{(2,12)}$ = 9.9, p=0.003; Fig. 3G inset). In GT/INT rats, we next examined the time course and found dBNST $GRAB_{DA}$ signaling on both positive and negative trials differs from expected trials during the second 2-second bin (ie 2-4 s) after lever retraction/pellet**

308 **delivery/omission (positive vs. expected p=0.04, negative vs. expected p=0.021). This**
309 **suggests GT/INT rats show evidence for dBNST $GRAB_{DA}$ bidirectional RPE signaling.**

310 **In a parallel analysis in ST rats considering all trial types (Expected, Positive, Negative)**
311 **we also observed main effect of Bin ($F_{(2,10)}$ = 10.4, p=0.004) and Trial x Bin interaction**
312 **($F_{(4,20)}$ = 5.4, p=0.004). ST rats showed evidence for positive RPE (Trial (expected,**
313 **positive) x Bin interaction $F_{(2,10)}$ = 6.8, p=0.014) but not negative RPE (Trial (expected,**
314 **negative) x Bin interaction $F_{(2,10)}$ = 2.8, p=0.153, Fig. 3H inset). Post-hoc analyses in ST**
315 **rats on the time-course failed to identify which bin GRABDA signals distinguished by**
316 **trial type, however a planned analysis on the relevant second 2-s bin (ie. 2-4 s after lever**
317 **retraction/pellet delivery/omission) indicates a main effect ($F_{(2,10)}$ = 5.3, p=0.027) is**
318 **marginally driven by Expected vs. Positive trial types ($F_{(1,5)}$=5.0, p=0.075 and not**
319 **Expected vs. Negative trial types ($F_{(1,5)}$= 2.0, p=0.216). This analysis suggests ST rats fail**
320 **to show evidence for dBNST $GRAB_{DA}$ bidirectional RPE signaling.**

321 We collected behavioral data during RPE sessions and examined pre-trial food cup checking
322 rate (response/10s prior to CS onset/reward delivery) on the trial after a reward violation, during
323 which prior studies establish invigoration of conditioned responses and orienting (Holland and
324 Gallagher 1993, Holland and Gallagher 1993, Calu, Roesch et al. 2010, Roesch, Calu et al.
325 2010). Rats increase their pre-trial foodcup checking on trials after a reward violation (Fig. 3I).
326 We performed repeated measures ANOVA including factors of Trial Type (Expected, Positive,
327 Negative) and Tracking (ST,GT/INT). While ST rats increase their pre-trial food cup checking
328 after both positive (p=0.042) and negative (p=0.016) trials, GT/INTs only increase their pre-trial
329 food cup checking following negative (p=0.013) trials (Fig. 3I, Trial Type: $F_{(2,22)}$ = 10.9, p=0.001,
330 Trial Type x Tracking: $F_{(2,22)}$ = 4.39, p=0.025, Tracking: $F_{(1,11)}$ = 1.77, p=0.21). These data
331 indicate that STs and GT/INTs use different reward seeking behavioral strategies following
332 violation of reward expectation.

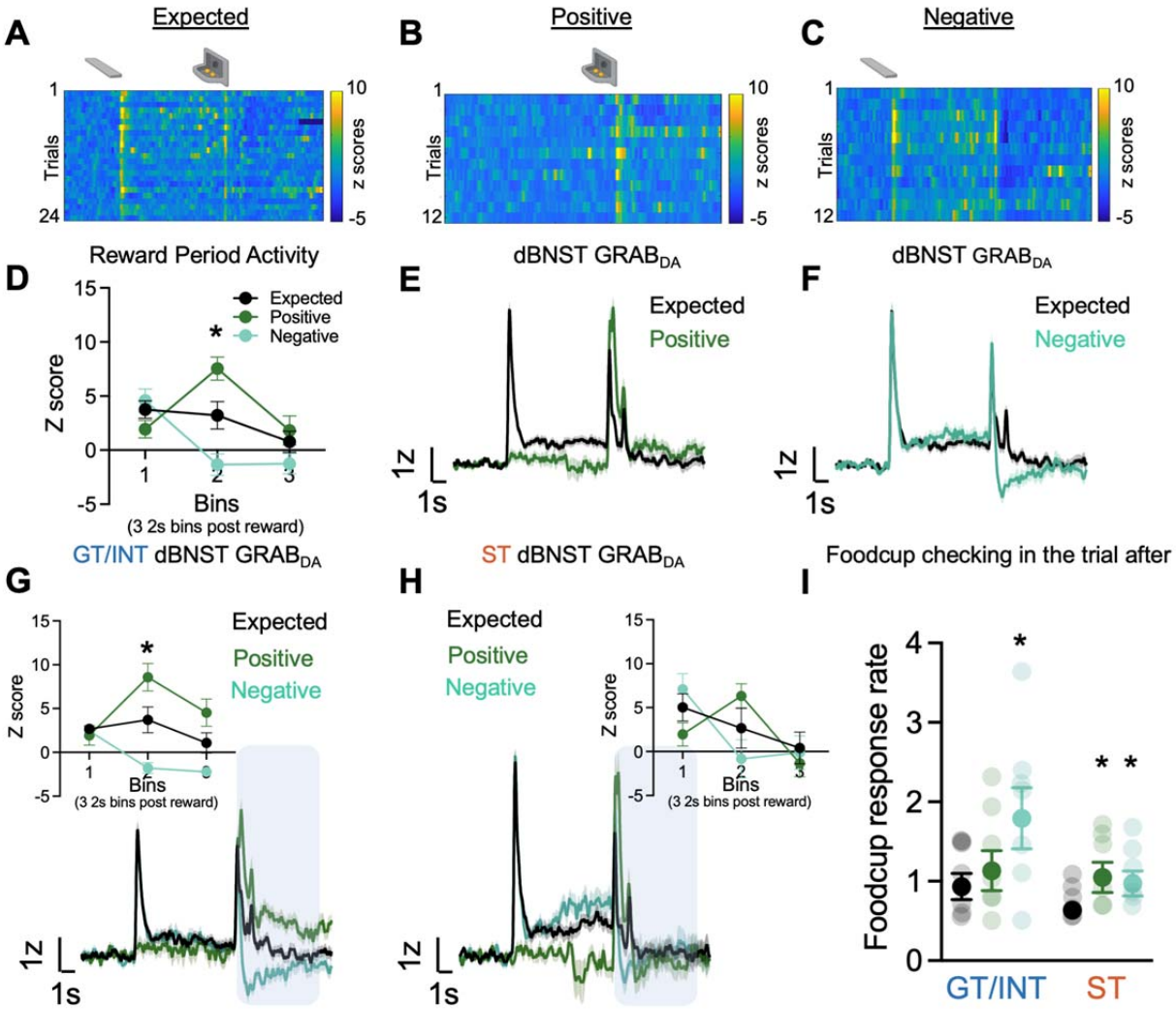

Figure 3: Individual differences in reward prediction error (RPE). A-C) Representative heat maps during Expected, Positive (unexpected reward) and Negative (unexpected omission) reward trials. **D)** Average binned z-scores (2s bins) during Expected, Positive and Negative trials 6s post reward delivery (bins 1-3). Trial-averaged GRAB$_{DA}$ signal change (z-scored ΔF/F) during **E)** Expected vs. Positive trials and **F)** Expected vs Negative trials **in all rats**. Trial-averaged GRAB$_{DA}$ signal change (z-scored ΔF/F) during all three trials and **average binned z-scores (2s bins) during Expected, Positive and Negative trials 6s post reward delivery (bins 1-3)** (inset) in **G)** GT/INTs and **H)** STs. **I)** Average foodcup checking response rate (responses/10s) during 10s pre-trial period on trial after expected, positive, and negative trials in GT/INTs vs STs. Data are mean±SEM, *p<0.05.

Reinforcer-specific but not general satiety attenuates cue-triggered GRAB$_{DA}$ signal

In the current and following sections, we **report the number of ST an GT/INT rats for each experimental phase but** do not report tracking differences due to **decreased** statistical power

348 **to detect group differences**. **Prior studies indicate that the midbrain and striatal**
349 **dopamine system tracks motivational state through satiety-dependent changes in the**
350 **magnitude of dopamine responses** (Cone et al., 2014; Hsu et al., 2018; Wilson et al., 1995)**.**
351 **Here we** determined whether motivational state **also decreases task-related BNST** $GRAB_{DA}$
352 signals during **lever autoshaping**. After rats completed 25 trials of PLA along with the $GRAB_{DA}$
353 recordings, we sated **rats (N = 11, ST = 4, GT/INT = 7)** on the training pellets presented in
354 ceramic ramekin in the homecage or presented a sham condition in which an empty ramekin
355 was placed in the homecage for 30 minutes. Immediately after, we recorded $GRAB_{DA}$ signals
356 during the remaining 25 trials of PLA sessions. First, we compared Δ presses and Δ pokes
357 ((CS+) – (CS-)) between hungry and sated or hungry and sham conditions using two-way
358 ANOVA with factors of State (Hungry, Sate) and Condition (Real, Sham). The number of
359 presses differed based on the satiety condition compared to hungry condition (State x
360 Condition: $F_{(1,20)} = 9.65$, p=0.006). Post hoc analysis revealed that rats sated on training pellets
361 decreased lever presses predictive of food pellet reward (Fig. 4A left, hungry vs sated presses:
362 $t_{10} = 3.02$, p=0.013; hungry vs sham presses: $t_{10} = -1.51$, p=0.16). In contrast, the number of
363 pokes generally but not differentially increased during sated and sham condition compared to
364 hungry condition (Fig. 4A right, State: $F_{(1,20)} = 6.73$, p=0.017, State x Condition: $F_{(1,20)} = 3.72$,
365 p=0.068). Similarly, we examined cue-evoked $GRAB_{DA}$ signal ((CS+) – (CS-); 2s after cue
366 onset) between hungry and sated or hungry and sham conditions using ANOVA with factors of
367 State (Hungry, Sate) and Condition (Real, Sham). The differential change in lever presses was
368 associated with difference in cue-evoked $GRAB_{DA}$ signal during sated and sham condition
369 compared to hungry condition (State x Condition: $F_{(1,20)} = 6.68$, p=0.018). Post hoc analysis
370 revealed that rats sated on pellets show a decrease in cue evoked $GRAB_{DA}$ signals but not in
371 sham conditions (Fig. 4E left, hungry vs sated: $t_{10} = 2.71$, p=0.022; hungry vs sham: $t_{10} = -0.95$,
372 p=0.35). While we observed a decrease in cue-triggered dopamine signals in sated conditions,
373 there was no change in reward consumption related dopamine signals in both sated and sham

374 conditions (Fig. 4E right, F's < 0.52, p's > 0.05). These results further bolster our finding that
375 BNST $GRAB_{DA}$ **signals encode** cue-outcome **associations, which similar to striatal**
376 **dopamine signaling,** is **blunted when the animal has** reduced motivational **drive** (Cone et al.,
377 2014; Wilson et al., 1995).

378 Next, we examined whether the reduction in cue evoked $GRAB_{DA}$ signal is specific to the
379 training pellet or whether it is sensitive to a general satiety state by sating rats on homecage
380 chow **(N = 7, ST = 3, GT/INT = 4)**. We conducted a similar analysis in pellet satiety experiment.
381 When we sated rats on chow, the number of presses differed based on the satiety condition
382 compared to hungry condition (State x Condition: $F_{(1,12)} = 5.86$, p=0.032), however, there was no
383 change in cue-evoked $GRAB_{DA}$ signals (Fig. 4B,D,F, F's < 1.8, p's > 0.05). Similarly, the number
384 of pokes also differed based on the satiety condition compared to hungry condition (State x
385 Condition: $F_{(1,12)} = 9.61$, p=0.009). Post hoc analysis revealed that rats decreased their poking
386 when sham compared to hungry ($t_6 = 2.87$, p=0.03). This is presumably due to a concurrent
387 non-significant increase in lever presses (sham sate presses: $t_6 = -1.92$, p=0.1). But this
388 decrease in foodcup pokes was not accompanied with a change in reward consumption evoked
389 $GRAB_{DA}$ signal (F's < 1.3, p's > 0.05). These results suggest that when rats are sated on the
390 outcome associated with the Pavlovian cue, there is an attenuation in $GRAB_{DA}$ signals while a
391 general satiety doesn't attenuate cue responding or $GRAB_{DA}$ signals.

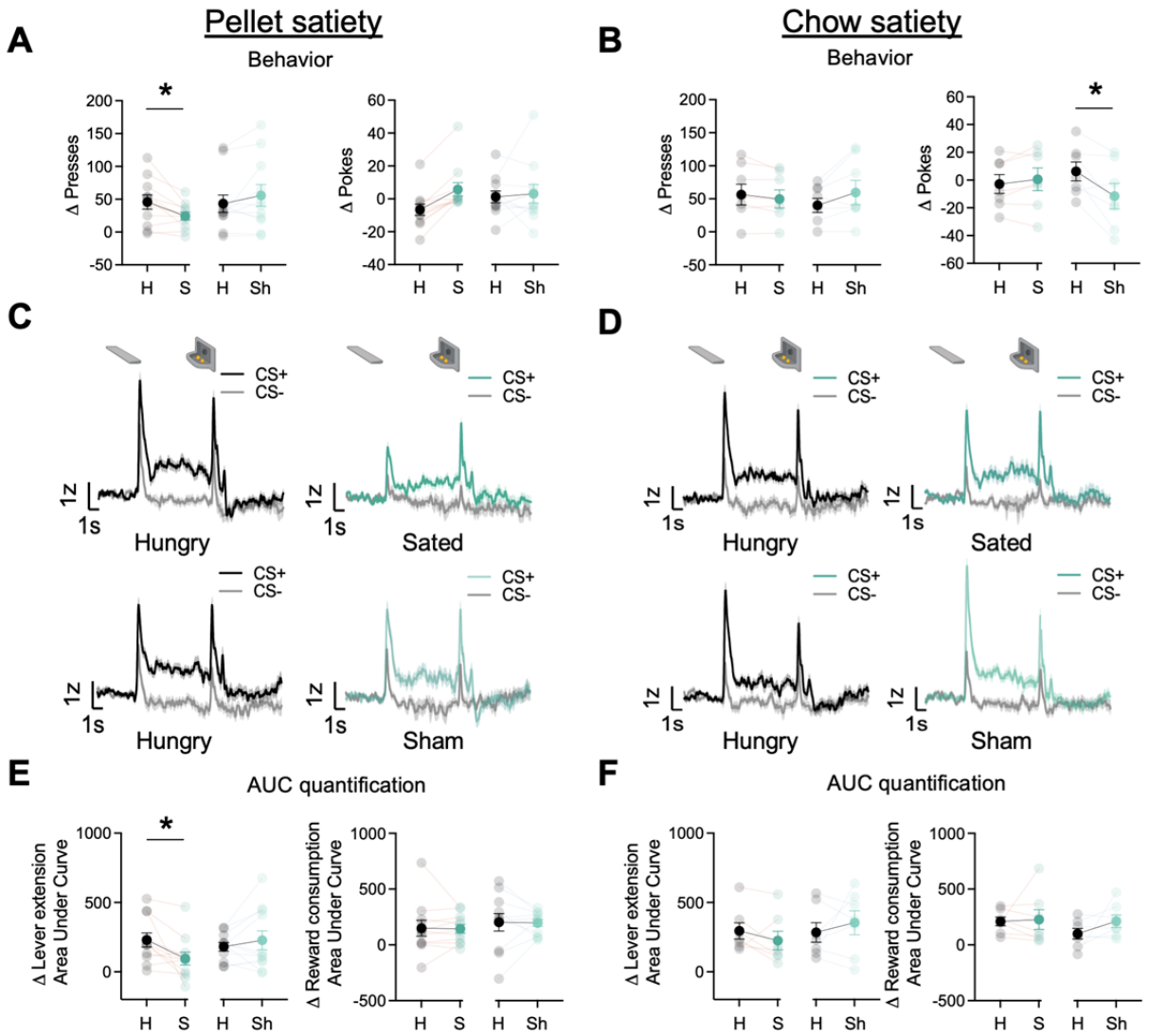

Figure 4: dBNST $GRAB_{DA}$ signals attenuate after reinforcer-specific but not general satiety. **A)** Average Δ Presses (CS+) – (CS-) (left) and average Δ pokes (CS+) – (CS-) (right) when rats were either sated on training food pellets in ramekin or sham-sated (ramekin only). **B)** Average Δ Presses (CS+) – (CS-) (left) and average Δ pokes (CS+) – (CS-) (right) when rats were either sated or sham-sated on homecage chow. **C)** Trial-averaged $GRAB_{DA}$ signal change (z-scored ΔF/F) during CS+ and CS- presentations when rats were hungry versus sated (top) and when rats were hungry versus sham-sated (bottom) on food pellets and **D)** on homecage chow. **E)** Trial average quantification of change (CS+) – (CS-) in area under $GRAB_{DA}$ z-scored curve (AUC) during lever extension (2s) (left) and reward consumption (right) between food pellet sated and sham and **F)** between homecage chow sated and sham conditions. Data are mean±SEM, *p<0.05. H = Hungry, S = Sated, Sh = Sham conditions.

Systemic fentanyl administration boosts $GRAB_{DA}$ signals to reward related cues

Opioids potentiate NAc activity and NAc DA responses to natural rewards and natural reward-associated cues (Bassareo et al., 2013; Castro and Berridge 2014; Mahler et al., 2007; Pecina and Berridge 2005). Here, we sought to determine whether opioids also potentiate task-related BNST $GRAB_{DA}$ signals during natural reward seeking during lever autoshaping. We recorded $GRAB_{DA}$ signals **in a subset of rats (N = 4, ST = 2, GT/INT = 2)** during PLA after i.p injection of **synthetic μ-opioid agonist, fentanyl,** 5μg/kg (population average traces for saline and ip fentanyl injection in Figure 5A). We observed main effects of Treatment (vehicle, fentanyl) and CS (CS+,CS-), but the interaction was not significant, indicating that cue discrimination is maintained with systemic fentanyl injections, which generally potentiate DA signaling in the dBNST (Figure 5B , CS: $F_{(1,6)} = 24.42$, p=0.003, Treatment: $F_{(1,6)} = 7.16$, p=0.037, CS x Treatment: $F_{(1,6)} = 2.8$, p=0.15).

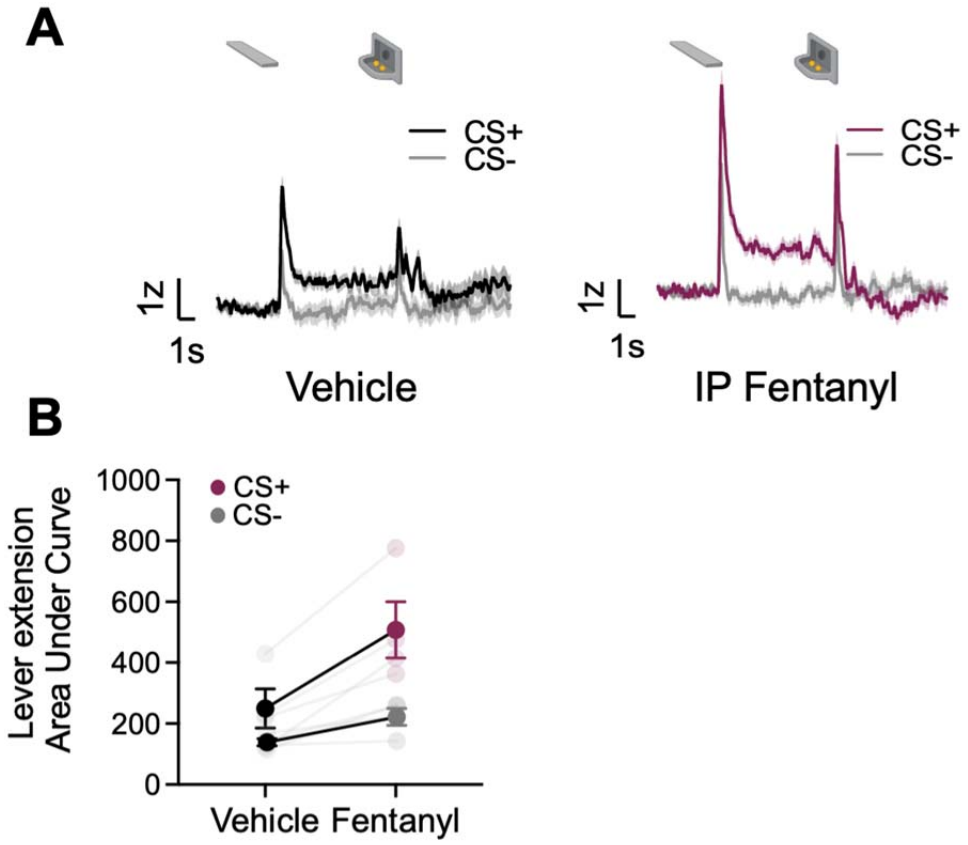

Figure 5: Systemic administration of fentanyl results in potentiation of dBNST dopamine. A) Trial-averaged $GRAB_{DA}$ signal change (z-scored $\Delta F/F$) when rats were injected with vehicle (left) or fentanyl (right) during PLA **C)** Trial average quantification of area under $GRAB_{DA}$ z-scored curve (AUC) during CS+ and CS- lever extension (2s) between vehicle and fentanyl conditions. Data are mean±SEM, *p<0.05.

Sex as a biological variable:

We use both male and female rats and have analyzed our photometry data from Pavlovian autoshaping, RPE, satiety and fentanyl test sessions using Sex instead of Tracking as a factor. We observed no main effects of Sex or interaction between Sex and any other factor.

Discussion

Using fluorescent dopamine sensor, $GRAB_{DA}$, we characterized phasic dBNST dopamine signals during a range of appetitive Pavlovian and instrumental conditions including lever autoshaping, reward violations, specific satiety, and cue-induced fentanyl-seeking. We found that dBNST dopamine signals are enhanced in STs compared to GT/INTs during cue presentation and shift from reward to cue across conditioning in STs but not in GT/INTs. Further, dBNST dopamine signals encode bidirectional reward prediction error and are greater in GT/INTs compared to STs following reward violations. Additionally, dBNST dopamine signals decrease to cue when rats are sated on food pellets associated with the cue but not when sated on homecage chow. Systemic fentanyl injections do not disrupt dBNST cue discrimination but generally potentiates dBNST dopamine signals.

Pharmacological studies establish that dopamine signaling in the dBNST maintains responding for sucrose and ethanol rewards and regulates the reinforcing properties of cocaine (Eiler et al., 2003; Epping-Jordan et al., 1998). Microdialysis and voltammetry studies show that natural and drug rewards, including opioids, increase DA in the BNST (Carboni et al. 2000; Park et al., 2012, 2013). Although dBNST dopamine is important for a variety of appetitive motivated behaviors, little is known about cue-evoked dopamine signaling and its role in cue-triggered motivation. A recent study showed BNST $GRAB_{DA}$ signals associated with both cues and

448 rewards (Lin et al., 2020). Our data extend these findings by showing individual differences in
449 CS- and US-evoked BNST dopamine signaling during Pavlovian conditioning. We also
450 demonstrate that CS-evoked BNST DA signals are state dependent and outcome-specific.

451 Consistent with prior studies, we observed individual differences in sign- and goal-
452 tracking behaviors elicited by the CS (Boakes R.A, 1977; Hearst & Jenkins, 1974; Nasser et al.,
453 2015; Robinson et al., 2014). Accompanied with this behavioral variation, we observed tracking
454 differences in $GRAB_{DA}$ signals to CS onset and differences in dopamine signal transfer from US
455 to CS, both of which were stronger in sign-tracking compared to goal-tracking and intermediate
456 rats. We observed a relationship between CS maintained $GRAB_{DA}$ signal and PCA scores,
457 indicating sign-tracking approach and interaction with the lever cue is associated with
458 heightened dBNST $GRAB_{DA}$ signaling. These findings for the dBNST dopamine signal are
459 consistent with prior tracking differences in nucleus accumbens (NAc) dopamine signals during
460 Pavlovian lever autoshaping (Flagel et al., 2011). We also find that only in ST rats did $GRAB_{DA}$
461 signals adhere to Sutton & Barto (2018) reinforcement learning algorithm, which states that after
462 learning, reward-evoked signals are temporally transferred back to antecedent cues predicting
463 reward delivery (Nasser et al., 2017; Sutton & Barto, 2018). Consistent with this, we observed
464 an increase in sustained $GRAB_{DA}$ signal during the entire 10s CS interaction period on Day 5 of
465 PLA training compared to Day 1. Sustained BNST $GRAB_{DA}$ signals during cue interaction period
466 could reflect a number of processes, including 1) ongoing lever interaction, 2) the incentive
467 value gain of the CS, 3) the strength of CS-US association, and/or 4) the back propagating US
468 to CS signal. Our results suggest that dopamine signaling differences between STs and GTs is
469 not just limited to NAc and could be present across a distributed network receiving
470 dopaminergic projections.

471 To adapt to environmental changes and learn about future rewards, dopaminergic
472 neurons calculate reward prediction errors (RPE) (Nasser et al., 2017; Schultz et al., 1997;

473 Watabe-Uchida et al., 2017). Here, we examined if BNST $GRAB_{DA}$ signals encode RPE and
474 whether there are individual differences in dBNST $GRAB_{DA}$ signals and behavioral strategies
475 following violations of reward expectations. We found that dBNST $GRAB_{DA}$ signals follow the
476 classical bidirectional prediction error signal such that the signals increased following
477 unexpected reward delivery and decreased following unexpected reward omission. Consistent
478 with attention for learning theories and empirical studies, we observed that rats increase their
479 food cup checking behavior on a trial after a positive or negative reward violation (Calu et al.,
480 2010; Pearce & Hall, 1980; Roesch et al., 2010). Sign-tracking rats increase foodcup checking
481 on trials after both unexpected reward delivery and omission, whereas GT/INTs increase
482 foodcup checking only after reward omission. Behaviorally this suggests GT/INT rats may be
483 more sensitive to negative reward violations than positive, which is consistent with their
484 sensitivity to outcome devaluation and their insensitivity to conditioned reinforcement (Keefer et
485 al., 2020; Keefer et al., 2022; Kochli et al., 2020; Morrison et al., 2015; Nasser et al., 2015;
486 Robinson and Flagel 2009; Smedley and Smith 2018). Such excitatory behavioral responses
487 (more checking for both increases and decreases in reward) before the trial are evidence for an
488 incremental attentional processes, which reflect enhanced attention to environmental predictors
489 for the purpose of increasing the rate of learning for either excitatory or inhibitory associations
490 (Calu et al., 2010; Holland and Gallagher 1993; Pearce & Hall, 1980; Roesch et al., 2007;
491 Roesch et al., 2010). Notably, reward prediction errors are critical for such enhanced attentional
492 processes, and the theoretical instantiation of incremental attention for learning about positive
493 and negative reward violations takes the absolute value of RPE signals into account (Pearce
494 and Hall 1980). Prior work establishes the involvement of other amygdala nuclei, namely the
495 basolateral and central nuclei of the amygdala for encoding unidirectional prediction error
496 signals that track enhanced attention after reward violations (Calu et al., 2010; Roesch et al.,
497 2010). Midbrain dopamine signaling is required for such attentional encoding in the basolateral
498 amygdala (Esber et al., 2012). Here we identify bidirectional dopamine encoding of positive and

499 negative reward violations in an extended amygdala nuclei, the dBNST. GT/INTs showed
500 evidence for bidirectional RPE in the dBNST DA signal, which may be important for enhancing
501 attention signals in downstream areas.

502 BNST receives heavy dopaminergic afferents from the A10 Ventral Tegmental Area
503 (VTA) and A10dc ventral periaqueductal grey/dorsal raphe (vPAG/DR) dopaminergic cell
504 groups, and to a lesser extent from the substantia nigra pars compacta and the retrorubral
505 nucleus (Daniel & Rainnie, 2016; Hasue & Shammah-Lagnado, 2002; Melchior et al., 2021;
506 Meloni et al., 2006; Vranjkovic et al., 2017). While VTA and SNc dopamine neurons classically
507 encode bidirectional reward prediction error signals, vPAG dopamine and its projections
508 unidirectionally encode rewarding and aversive outcomes, suggesting salient event detection
509 (Berg et al., 2014; García-García et al., 2017; Lin et al., 2020; Nasser et al., 2017; Schultz,
510 1997; Walker et al., 2020; Watabe-Uchida et al., 2017). Different aspects of the dBNST DA
511 signaling we observed leads us to postulate both dopamine projections may be contributing. For
512 the bidirectional RPE we observed in dBNST, we predict that VTA dopaminergic projections are
513 the source of dopamine during reward violations. In contrast, for the greater CS signaling in ST
514 compared to GT/INT rats may reflect salient features of the CS that support the attracting and
515 reinforcing properties of cues in sign-tracking rats, which may also be supported by
516 vPAG/DR→BNST dopamine. **Future studies are needed to identify the extent to which VTA
517 and PAG/DR dopaminergic inputs contribute to the BNST DA signals observed here.
518 Dissecting the role of each dopaminergic input in driving cue and reward related
519 signaling and behavior will inform whether these circuits work synergistically or in
520 competition to influence appetitive behaviors (Lin et al., 2020; Park et al., 2012, 2013).
521 Anatomical studies indicate that a substantial proportion of putative dopaminergic
522 projections to BNST originate in the vPAG/DR (Hasue and Shammah-Lagnado, 2002;
523 Meloni et al., 2006). Prior work has established glutamate and dopamine co-release from**

524 **the vPAG/DR projection to BNST, positioning this input to directly influence BNST**
525 **synaptic plasticity and associated behaviors** (Li et al., 2016)**. Regardless, the dopamine**
526 **dynamics reported here for BNST resemble those previously reported for nucleus**
527 **accumbens in related behaviors** (Clark et al., 2013; Flagel et al., 2011; Hart et al., 2014;
528 Saddoris et al., 2015, 2016)**, suggesting a potential role for VTA DA in shaping BNST DA**
529 **signaling. Notably, NAc DA also shows greater CS-evoked, and a greater shift from US to**
530 **CS- evoked DA in ST compared to GT** (Flagel et al., 2011)**. To our knowledge, tracking-**
531 **related differences in bidirectional RPE signaling in the NAc have not been**
532 **systematically tested, however the bidirectional error encoding we observe across all**
533 **rats is consistent with prior NAc voltammetry study** (Hart et al., 2014)**. Here we report that**
534 **GT/INT, but not ST, show evidence for bidirectional RPE DA signaling in the BNST.**
535 **Whether this is also the case for NAc DA signaling remains an open question. Consistent**
536 **with our findings, short inter-trial-intervals (ITI, similar to what we employ here) during**
537 **autoshaping promote both classic NAc DA RPE signaling and goal-tracking, whereas**
538 **longer ITIs promote NAc DA CS-salience signaling and sign-tracking** (Lee et al., 2018)**.**
539 **Pharmacology studies show D1 receptors and NAc DA signaling drive CS-salience in**
540 **sign-trackers** (Chow et al., 2016; Saunders and Robinson 2012)**. The potentiating effects of**
541 **hunger and systemic fentanyl injections on BNST DA signals observed here are in line**
542 **with effects observed for NAc DA** (Bassareo et al., 2013; Castro and Berridge 2014; Cone et
543 al., 2014; Mahler et al., 2007; Pecina and Berridge 2005; Wilson et al., 1995)**. Notably, the NAc**
544 **primarily receives input from the VTA, whereas the BNST receives DA inputs from VTA**
545 **and vPAG/DR. The tracking specific differences in BNST dopamine signaling during**
546 **simple appetitive approach and reward violations observed here suggest either 1)**
547 **distinct contributions of VTA and vPAG/DR to dopamine signaling observed in BNST**
548 **and/or 2) individual differences in the engagement of DA systems that bias towards CS-**
549 **salience or RPE processes** (Chow et al., 2016; Lee et al., 2018)**. Consideration of tracking-**

550 **specific dopamine signaling differences in future studies that employ projection-specific**
551 **manipulations will aid in interpreting each projection's contribution to BNST dopamine**
552 **signaling and behavior.**

553 **A methodological limitation of the current approach is that variations in**
554 **expression of fluorescent sensor and/or fiber placement along a gradient of DA input to**
555 **BNST could potentially influence the magnitude of $GRAB_{DA}$ measurements. Our fiber**
556 **placements were largely consistent (~73% at level of bregma) and overlapped with the**
557 **densest area of viral expression of the fluorescent sensor. At this level of BNST where**
558 **we measured the majority of $GRAB_{DA}$ signals, there is heavy vPAG/DR DA input and to a**
559 **lesser extent VTA input** (Hasue and Shammah-Lagnado 2002)**. Other anatomical and/or**
560 **functional studies that target BNST up to 0.2 mm anterior or posterior to bregma also**
561 **observe substantial putative dopaminergic input from vPAG/DR** (Meloni et al. 2006; Yu et
562 al. 2021)**. Regardless, some rats presented in Figure 2 - Figure supplement 3 were**
563 **excluded that had sufficient viral expression and fiber placement, but that did not show**
564 **evidence of dopamine binding in the dBNST during these Pavlovian tasks. While we are**
565 **limited from drawing conclusions from negative data, such individual differences in**
566 **extended amygdala dopamine signaling may be important for interpreting differences in**
567 **appetitive behaviors.**

568 The BNST is a sexually dimorphic brain region (Hisasue et al., 2010; Shah et al., 2004;
569 Tsuneoka et al., 2017), highlighting the necessity of studying both sexes to fully understand the
570 contribution of BNST DA to motivated behavior. **Dopaminergic projections from**
571 **vPAG/DR→BNST play sex-specific roles, with pathway activation associated with distinct**
572 **pain and locomotor behavioral changes for males and females, respectively** (Yu et al.,
573 2021). We used both male and female rats in the present study and analyzed our **BNST DA**
574 photometry data from Pavlovian autoshaping, RPE, satiety test sessions using Sex instead of

575 Tracking as a factor. While we observed no sex effects here, prior studies establish BNST-
576 mediated sex differences in **pain and locomotor behaviors as well as in** opioid withdrawal
577 (Luster et al., 2020; Yu et al. 2021a; Yu et al., 2021b). While there is limited evidence for sex
578 differences in incubation of fentanyl seeking (a form of relapse), we find this effect to be
579 dependent on dBNST CRFR1 receptor signaling (Gyawali et al., 2020; Reiner et al., 2019,
580 2020). Drug-induced synaptic plasticity in the dBNST requires both dopamine and CRF and
581 molecular and electrophysiology studies suggest that DA increases CRF release in the dBNST
582 (Day et al., 2002; Kash et al., 2008). Given the known role for sex differences in CRF-induced
583 relapse and opioid withdrawal, it is critical to include both sexes when studying BNST DA and
584 CRF systems (Buffalari et al., 2012; Luster et al., 2020).

585 To our surprise, we found evidence for outcome-specific state dependent BNST $GRAB_{DA}$
586 signaling. Consistent with our prior studies, we found that rats decreased their lever responding
587 only when they were sated on food pellets specifically associated with the lever cue, but not
588 when sated on homecage chow (Keefer et al., 2020; Kochli et al., 2020). Similarly, we observed
589 decreased cue-evoked BNST $GRAB_{DA}$ when rats were sated on food pellets but not when they
590 were sated on chow. All rats ate all their pellets during these reinforced sessions, and we did
591 not see any change in $GRAB_{DA}$ signals during reward consumption when sated on either food
592 pellets or chow. Prior studies report a similar decrease in cue-evoked dopamine signals in the
593 basolateral amygdala and dopaminergic neuron activity in the DRN during satiety (Cho et al.,
594 2021; Lutas et al., 2019). Based on these studies that manipulated state using hunger or satiety,
595 we expected dopamine signals to generally decrease to cues both when sated on chow or
596 training pellets, but we found BNST dopamine signals only decreased when sated on the
597 training pellet associated with the cue. However, other studies find evidence for sensory specific
598 signaling in dopamine function and signaling (Sharpe et al., 2017; Takahashi et al., 2017). This
599 suggests BNST DA signals may carry sensory specific information that is critical for higher order

600 learning processes (Burke et al., 2007, 2008; Keefer et al., 2021; Lichtenberg et al., 2017;
601 Lichtenberg et al., 2021; Malvaez et al., 2015; Malvaez et al., 2019; Sias et al., 2021; Sharpe et
602 al., 2017; Takahashi et al., 2017).

603 Studies show elevated BNST dopamine, dopamine induced plasticity, and dopamine
604 mediated seeking behavior during and after drug administration (Carboni et al., 2000; Eiler et
605 al., 2003; Epping-Jordan et al., 1998; Kash et al., 2008; Krawczyk et al., 2013; Krawczyk et al.,
606 2011a; Krawczyk et al., 2011b; Melchior et al., 2021; Stamatakis et al., 2014). We extend these
607 findings by reporting that systemic fentanyl injections do not disrupt dBNST cue discrimination
608 and generally potentiate dBNST dopamine signals. The present study supports the need for
609 future work aimed at fully characterizing drug-induced changes to dBNST DA cue and reward
610 encoding during natural and opioid reward seeking.

611 Dopamine projections to the BNST are concentrated in the dBNST and synapse
612 specifically onto the CRFergic neurons (Meloni et al., 2006; Phelix et al., 1994). Molecular and
613 electrophysiology studies suggest that dopamine increases local CRF release in the dBNST and
614 drug-induced synaptic plasticity in the dBNST requires both dopamine and CRF (Day et al.,
615 2002; Kash et al., 2008; Silberman et al., 2013). These anatomical and *ex vivo* physiology
616 studies suggest dopamine and CRF are critically interacting to drive reward and stress-related
617 behaviors. Indeed, our prior work indicates that CRF receptor activation in the dBNST is
618 necessary for CS-triggered opioid relapse (Gyawali et al., 2020). Further, dBNST dopamine
619 receptor activation decreases blood corticosterone levels in mice suggesting that an increased
620 dopamine response in the dBNST could serve as an anxiolytic signal, which could promote
621 continued drug seeking (Daniel & Rainnie, 2016; Kash et al., 2008; Melchior et al., 2021; Meloni
622 et al., 2006).

623 The present findings add substantially to the role of dBNST dopamine in motivated
624 behaviors, providing a comprehensive characterization of endogenous dBNST dopamine

625 dynamics in cue-induced behaviors under several natural and drug reward conditions. The
626 fluorescent dopamine sensor $GRAB_{DA}$ is a useful tool for studying real-time BNST DA dynamics
627 in the context of motivated behaviors (Lin et al., 2020; Sun et al., 2020).

628

629 **Materials and Methods**

Key Resources Table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Transfected construct (*H. Sapiens*)	AAV9.hsyn.DA4.4.eyfp	WZ Biosciences	h-D03	Titer>1.0X10E13GC/mL
Chemical compound, drug	Fentanyl	Cayman Chemicals	Cat: 22659	
Chemical compound, drug	Reboxetine Mesylate	MedChemExpress	Cat: HY-14560C	
Chemical compound, drug	TCS	Access Technologies	Cat: TCS-04	
Chemical compound, drug	Paraformaldehyde	Sigma	Cat: P6148	
Software, algorithm	MED-PC IV	Med Associates	RRID: SCR_012156	Version: IV
Software, algorithm	Excel	Microsoft	RRID: SCR_016137	
Software, algorithm	SPSS	IBM	RRID: SCR_019096	Version: 26
Software, algorithm	Matlab	Mathworks	RRID: SCR_001622	Version: 2020a
Software, algorithm	Graphpad Prism	Graphpad Software	RRID: SCR_002798	Version: 9

Software, algorithm	Synapse Software	Tucker-Davis Technologies	RRID: SCR_006495	
Other	LED Driver	ThorLabs	Cat: DC4100	LED Driver capable of driving high-power four-wavelength LED sources simultaneously with a current range between 0 and 1000 mA
Other	Fluorescence Minicube	Doric	Cat: iIFMC4-G2_IE(400-410)_E(460-490)_F(500-550)_S	Fluorescence Mini Cube with 4 ports: one port for the functional fluorescence excitation light, one for the isosbestic excitation, one for the fluorescence detection, and one for the sample
Other	Fiber optic patchcord	Doric	D202-4094-3	MFP_400/430/LWMJ-0.48_3m_FCM-MF2.5
Other	Fiber optical cannula	ThorLabs	Cat: CFMC54L10	Ceramic ferrule Ø400 µm core, 0.50 NA fiber that is flat cleaved to a length 10 mm
Other	Metabond powder	Parkell	Cat: S396	See *Virus and fiber optic implantation surgery* for more details
Other	Metabond quick base	Parkell	Cat: S398	See *Virus and fiber optic implantation surgery* for more details
Other	Metabond catalyst	Parkell	Cat: S371	See *Virus and fiber optic implantation surgery* for more details
Other	Dental Cement	DenMat	Cat: 034524101	See *Virus and fiber optic implantation surgery* for more details
Other	Dental Cement Catalyst	DenMat	Cat: 4506	See *Virus and fiber optic implantation surgery* for more details

Other	Sucrose pellets	Test Diet	5TUL; Cat: 1811155	Purified ingredient rodent tablet, protein: 20.6%, fat: 12.7%, carbohydrate: 66.7%

Subjects: We used 8 weeks old male and female Sprague Dawley rats (Charles River, N = 42) weighing >250g before surgery. After surgery, we individually housed the rats and maintained them under a reversed 12:12 h light/dark cycle (lights off at 9 AM). We estimated the sample size based on prior studies (Bacharach et al., 2018; Kochli et al., 2020) and pilot experiments. Each primary experiment was replicated in at least one additional cohort. Investigators were blinded to the tracking phenotype until the end of the experiments. We performed all experiments in accordance with the "Guide for the care and use of laboratory animals" (8th edition, 2011, US National Research Council) and the University of Maryland Institutional Animal Care and Use Committee approved all experimental procedures. We excluded rats because of lack of viral expression **(N = 4)**, incorrect fiber optic placements **(N = 6)**, **and headcap loss (N = 4). Additionally, we excluded rats (N = 8) that showed food cup entry artifacts before we optimized our photometry setup. The artifacts resulted in loss of signal due to the patch cord hitting the wall of the foodcup. Finally, rats (N = 4) presented in Figure 2 - Figure supplement 3 were excluded that had sufficient viral expression and fiber placement but did not show robust photometry signals in the dBNST during CS+ presentation by day five of PLA training (see *Photometry Analysis* subsection for more details).**

Virus and fiber optic implantation surgery: We anesthetized 9-week-old rats with isoflurane (4.5% induction, 2-3% maintenance) and placed them in a stereotaxic frame. We maintained stable body temperature with a heating pad and administered pre-operative analgesic carprofen (5 mg/kg, s.c) and lidocaine (10mg/mL at the site of incision). We made a scalp incision and drilled a hole above left dBNST AP = 0.0 or -0.1 from bregma, ML = +3.5, DV = - 6.75 or -6.8 at 16° from midline for viral injection and DV = -6.6 or -6.7 relative to skull for fiber implantation. In

addition, we also drilled 3 holes anterior and posterior to attach anchor screws. We lowered 5μL Hamilton syringe unilaterally into the dBNST and injected AAV9.hsyn.DA4.4.eyfp ($1.14x10^{14}$ GC/mL; WZ Biosciences) via a micropump at a volume of 0.7 - 1μL over 10 minutes. We implanted the fiber optic (ThorLabs CFMC54L10, 400 μm, 0.50 NA, 10 mm) 0.1 mm or 0.15 mm above the virus injection site. We anchored the fiber optic to the skull using dental cement (Metabond and Denmat) and jeweler screws. We handled the rats at least three times a week after surgery before starting behavioral and photometry sessions.

Apparatus: We conducted behavioral experiments in operant chambers housed in sound attenuating cabinets (Med Associates). Each chamber had one white house light that was illuminated during the entire session. On the opposite wall, two retractable levers (CS+ and CS-, right or left location counterbalanced) were located on either side of the foodcup. The foodcup was attached to a programmed pellet dispenser that delivered 45mg training pellets (Testdiet, 5TUL, protein 20.6%, fat 12.7%, carbohydrate 66.7%).

Pavlovian Lever Autoshaping (PLA): We conducted all training sessions during the dark phase. Schematic of our behavioral design can be found in Figure 1A. Five weeks after viral injection surgery, we maintained rats at 90% of *ad libitum* body weight and during all behavioral sessions unless noted otherwise. Prior to the PLA training, we exposed rats to 25 magazine training trials divided into three sessions to acclimatize rats to the operant box and fiber optic cables. The three sessions consisted of 7,8, and 10 trials respectively in which two food pellets (US) were delivered, 0.5s apart using a variable interval (VI) 60s (50-70s) schedule. After magazine training sessions, we trained rats in five 46-minute PLA sessions. Each session consisted of 25 reinforced (CS+) and 25 non-reinforced (CS-) lever presentation trials on a mean VI 45s (35-55s) schedule (Fig. 1B). Each CS+ trial consisted of the insertion and retraction of a lever for 10s followed by delivery of two food pellets, 0.5s apart. CS- trials consisted of insertion/retraction of another lever, but no US delivery. We recorded food-cup and lever

approach during the ten seconds CS interaction and calculated a Pavlovian Conditioned Approach (PCA) score (Meyer et al., 2012). We use PCA score as a comprehensive measure of individual differences in PLA that account for contact, latency, and probability differences. We used each rat's Days 4 and 5 average PCA score to determine whether they are sign-trackers (avg PCA score ≥ 0.5, ST) or goal-trackers/intermediates (avg PCA score < 0.5, GT/INT).

Reward Prediction Error (RPE) Probe sessions: After five PLA sessions, we gave rats (N = 13) one session in which we violated rats' reward expectations to probe for reward prediction error signaling. During this session, only CS+ lever was presented, and rats received 48 trials divided into three different trial types presented in pseudorandom order. In the 'expected reward' condition, we gave 24 reinforced CS+ → US trials (50% of total trials). In the 'unexpected reward' condition, we delivered two food pellets (US) randomly during the intertrial interval period without the predictive CS+ (12 trials, 25% of total trials). Finally, in the 'unexpected reward omission' condition, we delivered the CS+, but omitted the US (12 trials, 25% of total trials) (Patriarchi et al., 2018).

Satiety test: After the RPE session, we trained a subset of rats (N = 11) in PLA for two more days when rats were either sated on food pellets or hungry. On the first day, we gave half the rats 30g of the training food pellets in a ramekin for 30 minutes (pellet sated condition) in their home cage after rats had completed 25 out of 50 trials. For the other half of the rats, we gave empty ramekins in their home cage (control condition). After 30 minutes, we placed the rats back into the operant chamber where they completed the remaining 25 trials in PLA. The next day, we gave training pellets to rats that received empty ramekins on the first day and vice versa. We ran the chow satiety test in a subset of rats (N = 7) using the same experimental design as pellet satiety test but replaced the food pellets in the ramekins with homecage chow instead.

Fentanyl i.p injections: We injected 5μg/kg i.p fentanyl (Cayman Chemical) or vehicle in rats (N = 4) 5 minutes before PLA sessions. We selected this dose based on pilot experiments. In two counterbalanced PLA sessions, we gave the rats either i.p injection of fentanyl or saline.

Fiber Photometry: We used LEDs (ThorLabs) to deliver 465 nm (wavelength to excite $GRAB_{DA}$) and 405 nm (isosbestic control) and measure dopamine activity. The isosbestic signal is used as a control for fiber bleaching and motion artifacts as it is subtracted from the 465 nm signal during analysis. We sinusoidally modulated the intensity of the 465 nm and 405 nm light at 210 and 337Hz respectively and connected the LEDs to a four-port fluorescence mini cube (Doric Lenses). The combined LED output passed through a fiber optic cable (1 m long; 400 μm core; 0.48 NA; Doric Lenses) which was connected to the implanted fiber optics with sleeves. We maintained the light intensity at the tip of the fiber optic cable at 10-15 μW across behavioral sessions. We collected the $GRAB_{DA}$ and isosbestic control channel emission using the same fiber optic cable and focused the emission light onto a photoreceiver (Newport). We low pass filtered and digitized the emission light at 3Hz and 5 KHz respectively by a digital processor controlled by Synapse software suite (RZ5P, Tucker Davis Technologies (TDT)). We time-stamped the behavioral events including lever insertion/retraction, lever press, food cup entry etc. by sending them as TTL (transistor-transistor logic) pulses to Synapse software.

Histology: After all behavioral testing, we deeply anesthetized rats with isoflurane and transcardially perfused them with 200 mL of 0.1 M PBS followed by 400 mL of 4% paraformaldehyde (PFA) in distilled H_2O. We quickly removed the brains and post-fixed them in 4% PFA for at least two hours before we transferred them to 30% sucrose in PBS for 48 hours at 4°C. We subsequently froze the brains using dry ice and stored them in −20°C until sectioning. We collected 50 μm coronal sections containing BNST on a cryostat (Leica Microsystems) and preserved them in a cryopreservant. We mounted the sections on slides and coverslipped them with Vectashield mounting medium with DAPI (Vector Laboratories). We

728 verified fiber optic placements and viral expression in the dBNST using anatomical boundaries
729 defined by Paxinos and Watson (Paxinos & Watson, 2006) under a confocal microscope. A
730 representative example and summary of $GRAB_{DA}$ expression and fiber placements are shown in
731 Fig. 1C.

732 *Photometry Analysis*: We analyzed the signals using custom-written MATLAB (Mathworks)
733 scripts. We calculated ΔF/F (z score) by smoothing signals from the isosbestic control channel
734 (Lerner et al., 2015; Root et al., 2020). We regressed the isosbestic signal onto the $GRAB_{DA}$-
735 dependent signal to create a fitted isosbestic signal by using the linear model generated during
736 the regression. We then calculated z scores by subtracting the fitted isosbestic signal from
737 $GRAB_{DA}$-dependent signal and dividing by the fitted isosbestic signal. This resulted in $GRAB_{DA}$
738 signal devoid of artifacts created by photobleaching, fiber bending, or movements. We collected
739 z scores in the behavioral window of interest defined as 5s before cue onset to 10s after pellet
740 delivery. We quantified area under the curve (AUC) in the 2s following cue onset and pellet
741 delivery and independently calculated these parameters for CS+ and CS- trials. In all dopamine
742 signal analyses, unless otherwise noted, we subtract CS- signal from the CS+ signal. We
743 defined significant transients in our behavioral window if the peak amplitude **during the trials (0**
744 **to +20 s relative to cue onset)** was 2 z-score (p=0.05) above baseline (5s prior to cue onset)
745 **during the entire behavioral window on Day 1 or Day 5 of PLA**. **Furthermore, to ensure**
746 **these signals were time-locked to cues and not spurious, we calculated 95% confidence**
747 **intervals using bootstrapped resampling (1000 resamples) of all trials' photometry data**
748 **for each rat across CS+ trials of Day 5 of PLA. Most rats displayed a consistent, robust**
749 **increase in signal reaching significantly above baseline within 70 milliseconds of CS+**
750 **onset, that stayed above baseline for a minimum of 40 milliseconds, consecutively. 4 rats**
751 **did not meet either of these criteria (greater than 2 z-score peak signal or 40 ms of**
752 **consecutive time with significantly elevated signal at CS+) and were excluded. Their data**

is in Figure 2 - Figure supplement 3. All included rats met both criteria. We also removed trials where the patch cord disconnected from further signal processing.

Statistical Analysis: We analyzed the data using SPSS, Graphpad Prism, and Matlab. We used mixed design repeated measures ANOVAs to analyze PLA behavioral and $GRAB_{DA}$ signal data. Whenever ANOVAs revealed significant interactions between groups, we ran t-tests with Bonferroni corrections for multiple comparisons to guard against Type I error. We define dependent measures, within/between-subject factors, and report significant effects and interactions in the corresponding results section.

Data availability

The data used in this manuscript are available on Zenodo (DOI: 10.5281/zenodo.7017848).

Code availability

Modified TDT-supplied MATLAB code (https://www.tdt.com/docs/sdk/offline-data-analysis/offline-data-matlab/fiber-photometry-epoch-averaging-example/) is available on Github (https://github.com/ugyawali/photometry).

Ethics Statement

The animal study was reviewed and approved by University of Maryland, School of Medicine Institutional Animal Care and Use Committee.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

We thank Jessie Feng for technical assistance and the Animal Care Facility for colony maintenance. We thank Asaf Keller and the Department of Anatomy and Neurobiology for sharing photometry equipment.

References cited:

Bacharach, Sam Z., Helen M. Nasser, Natalie E. Zlebnik, Hannah M. Dantrassy, Daniel E. Kochli, Utsav Gyawali, Joseph F. Cheer, and Donna J. Calu. “Cannabinoid Receptor-1 Signaling Contributions to Sign-Tracking and Conditioned Reinforcement in Rats.” *Psychopharmacology* 235, no. 10 (October 2018): 3031–43. https://doi.org/10.1007/s00213-018-4993-6.

Bassareo, V., F. Cucca, C. Cadoni, P. Musio, and G. Di Chiara. “Differential Influence of Morphine Sensitization on Accumbens Shell and Core Dopamine Responses to Morphine- and Food-Conditioned Stimuli.” *Psychopharmacology (Berl)* 225, no. 3 (February 2013): 697–706. https://doi.org/10.1007/s00213-012-2856-0.

Berg, B. A., G. Schoenbaum, and M. A. McDannald. “The Dorsal Raphe Nucleus Is Integral to Negative Prediction Errors in Pavlovian Fear.” *Eur J Neurosci* 40, no. 7 (October 2014): 3096–3101. https://doi.org/10.1111/ejn.12676.

Boakes, Robert A. “Performance on Learning to Associate a Stimulus with Positive Reinforcement.” In *Operant-Pavlovian Interactions*, 67–101. Routledge, 1977.

Buffalari, Deanne M., Chelsey K. Baldwin, Matthew W. Feltenstein, and Ronald E. See. “Corticotrophin Releasing Factor (CRF) Induced Reinstatement of Cocaine Seeking in Male and Female Rats.” *Physiology & Behavior* 105, no. 2 (January 18, 2012): 209–14. https://doi.org/10.1016/j.physbeh.2011.08.020.

Buffalari, Deanne M., and Ronald E. See. “Footshock Stress Potentiates Cue-Induced Cocaine-Seeking in an Animal Model of Relapse.” *Physiology & Behavior* 98, no. 5 (December 7, 2009): 614–17. https://doi.org/10.1016/j.physbeh.2009.09.013.

Buhusi, Catalin V., and Warren H. Meck. “What Makes Us Tick? Functional and Neural Mechanisms of Interval Timing.” *Nat Rev Neurosci* 6, no. 10 (2005): 755–65. https://doi.org/10.1038/nrn1764.

Burk, J. A., and R. G. Mair. “Effects of Dorsal and Ventral Striatal Lesions on Delayed Matching Trained with Retractable Levers.” *Behavioural Brain Research* 122, no. 1 (2001): 67–78. https://doi.org/10.1016/s0166-4328(01)00169-3.

Burke, Kathryn A., Theresa M. Franz, Danielle N. Miller, and Geoffrey Schoenbaum. “Conditioned Reinforcement Can Be Mediated by Either Outcome-Specific or General Affective Representations.” *Frontiers in Integrative Neuroscience* 1 (November 2, 2007). https://doi.org/10.3389/neuro.07.002.2007.

———. “The Role of the Orbitofrontal Cortex in the Pursuit of Happiness and More Specific Rewards.” *Nature* 454, no. 7202 (July 17, 2008): 340–44. https://doi.org/10.1038/nature06993.

Calipari, E. S., R. C. Bagot, I. Purushothaman, T. J. Davidson, J. T. Yorgason, C. J. Pena, D. M. Walker, et al. “In Vivo Imaging Identifies Temporal Signature of D1 and D2 Medium Spiny Neurons in Cocaine Reward.” *Proc Natl Acad Sci U S A* 113, no. 10 (March 8, 2016): 2726–31. https://doi.org/10.1073/pnas.1521238113.

Calu, Donna J., Matthew R. Roesch, Richard Z. Haney, Peter C. Holland, and Geoffrey Schoenbaum. “Neural Correlates of Variations in Event Processing during Learning in Central Nucleus of Amygdala.” *Neuron* 68, no. 5 (December 9, 2010): 991–1001. https://doi.org/10.1016/j.neuron.2010.11.019.

Carboni, E., A. Silvagni, M. T. Rolando, and G. Di Chiara. “Stimulation of in Vivo Dopamine Transmission in the Bed Nucleus of Stria Terminalis by Reinforcing Drugs.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 20, no. 20 (October 15, 2000): RC102.

Castro, Daniel C., and Kent C. Berridge. “Opioid Hedonic Hotspot in Nucleus Accumbens Shell: Mu, Delta, and Kappa Maps for Enhancement of Sweetness ‘Liking’ and ‘Wanting.’” *J Neurosci* 34, no. 12 (March 19, 2014): 4239–50. https://doi.org/10.1523/JNEUROSCI.4458-13.2014.

Chang, Stephen E., Lauren D. Krueger, and Shelly B. Flagel. “Investigating Individual Differences in Opioid-Taking and Opioid-Seeking Behavior in Male Rats.” *Psychopharmacology* 239, no. 4 (April 2022): 1065–80. https://doi.org/10.1007/s00213-021-06023-2.

Cho, Jounhong Ryan, Xinhong Chen, Anat Kahan, J. Elliott Robinson, Daniel A. Wagenaar, and Viviana Gradinaru. “Dorsal Raphe Dopamine Neurons Signal Motivational Salience Dependent on Internal State, Expectation, and Behavioral Context.” *The Journal of Neuroscience* 41, no. 12 (March 24, 2021): 2645–55. https://doi.org/10.1523/JNEUROSCI.2690-20.2021.

Chow, J. J., J. R. Nickell, M. Darna, and J. S. Beckmann. “Toward Isolating the Role of Dopamine in the Acquisition of Incentive Salience Attribution.” *Neuropharmacology* 109 (October 2016): 320–31. https://doi.org/10.1016/j.neuropharm.2016.06.028.

Clark, J. J., A. L. Collins, C. A. Sanford, and P. E. Phillips. “Dopamine Encoding of Pavlovian Incentive Stimuli Diminishes with Extended Training.” *J Neurosci* 33, no. 8 (February 20, 2013): 3526–32. https://doi.org/10.1523/JNEUROSCI.5119-12.2013.

Cone, J. J., J. E. McCutcheon, and M. F. Roitman. “Ghrelin Acts as an Interface between Physiological State and Phasic Dopamine Signaling.” *J Neurosci* 34, no. 14 (April 2, 2014): 4905–13. https://doi.org/10.1523/JNEUROSCI.4404-13.2014.

Day, Heidi E. W., Nicole M. Vittoz, Matthew M. Oates, Aldo Badiani, Stanley J. Watson, Terry E. Robinson, and Huda Akil. “A 6-Hydroxydopamine Lesion of the Mesostriatal Dopamine System Decreases the Expression of Corticotropin Releasing Hormone and Neurotensin MRNAs in the Amygdala and Bed Nucleus of the Stria Terminalis.” *Brain Research* 945, no. 2 (August 2, 2002): 151–59. https://doi.org/10.1016/s0006-8993(02)02747-6.

Egli, Regula E., Thomas L. Kash, Kevin Choo, Valentina Savchenko, Robert T. Matthews, Randy D. Blakely, and Danny G. Winder. “Norepinephrine Modulates Glutamatergic Transmission in the Bed Nucleus of the Stria Terminalis.” *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 30, no. 4 (April 2005): 657–68. https://doi.org/10.1038/sj.npp.1300639.

Eiler, William J. A., Regat Seyoum, Katrina L. Foster, Chaz Mailey, and Harry L. June. “D1 Dopamine Receptor Regulates Alcohol-Motivated Behaviors in the Bed Nucleus of the Stria Terminalis in Alcohol-Preferring (P) Rats.” *Synapse (New York, N.Y.)* 48, no. 1 (April 2003): 45–56. https://doi.org/10.1002/syn.10181.

Epping-Jordan, M. P., A. Markou, and G. F. Koob. “The Dopamine D-1 Receptor Antagonist SCH 23390 Injected into the Dorsolateral Bed Nucleus of the Stria Terminalis Decreased Cocaine Reinforcement in the Rat.” *Brain Research* 784, no. 1–2 (February 16, 1998): 105–15. https://doi.org/10.1016/s0006-8993(97)01190-6.

Esber, G. R., M. R. Roesch, S. Bali, J. Trageser, G. B. Bissonette, A. C. Puche, P. C. Holland, and G. Schoenbaum. “Attention-Related Pearce-Kaye-Hall Signals in Basolateral Amygdala Require the Midbrain Dopaminergic System.” *Biol Psychiatry* 72, no. 12 (December 15, 2012): 1012–19. https://doi.org/10.1016/j.biopsych.2012.05.023.

Flagel, Shelly B., Jeremy J. Clark, Terry E. Robinson, Leah Mayo, Alayna Czuj, Ingo Willuhn, Christina A. Akers, Sarah M. Clinton, Paul E. M. Phillips, and Huda Akil. “A Selective Role for Dopamine in Stimulus-Reward Learning.” *Nature* 469, no. 7328 (January 6, 2011): 53–57. https://doi.org/10.1038/nature09588.

Flagel, Shelly B., Stanley J. Watson, Terry E. Robinson, and Huda Akil. “Individual Differences in the Propensity to Approach Signals vs Goals Promote Different Adaptations in the Dopamine System of Rats.” *Psychopharmacology* 191, no. 3 (April 2007): 599–607. https://doi.org/10.1007/s00213-006-0535-8.

Flavin, Stephanie A., and Danny G. Winder. “Noradrenergic Control of the Bed Nucleus of the Stria Terminalis in Stress and Reward.” *Neuropharmacology* 70 (July 2013): 324–30. https://doi.org/10.1016/j.neuropharm.2013.02.013.

Fragale, Jennifer E., Morgan H. James, and Gary Aston-Jones. “Intermittent Self-Administration of Fentanyl Induces a Multifaceted Addiction State Associated with Persistent Changes in the Orexin System.” *Addiction Biology* 26, no. 3 (May 2021): e12946. https://doi.org/10.1111/adb.12946.

Gyawali, Utsav, David A. Martin, Agnieszka Sulima, Kenner C. Rice, and Donna J. Calu. “Role of BNST CRFR1 Receptors in Incubation of Fentanyl Seeking.” *Frontiers in Behavioral Neuroscience* 14 (2020): 153. https://doi.org/10.3389/fnbeh.2020.00153.

Hasue, Renata H., and Sara J. Shammah-Lagnado. “Origin of the Dopaminergic Innervation of the Central Extended Amygdala and Accumbens Shell: A Combined Retrograde Tracing and

Immunohistochemical Study in the Rat." *The Journal of Comparative Neurology* 454, no. 1 (December 2, 2002): 15–33. https://doi.org/10.1002/cne.10420.

Hearst, Eliot, and Herbert M. Jenkins. *Sign-Tracking: The Stimulus-Reinforcer Relation and Directed Action*. Austin, Tex.: Psychonomic Society, 1974.

Hisasue, S., M. L. Seney, E. Immerman, and N. G. Forger. "Control of Cell Number in the Bed Nucleus of the Stria Terminalis of Mice: Role of Testosterone Metabolites and Estrogen Receptor Subtypes." *J Sex Med* 7, no. 4 Pt 1 (April 2010): 1401–9. https://doi.org/10.1111/j.1743-6109.2009.01669.x.

Holland, P. C., and M. Gallagher. "Amygdala Central Nucleus Lesions Disrupt Increments, but Not Decrements, in Conditioned Stimulus Processing." *Behav Neurosci* 107, no. 2 (April 1993): 246–53. https://doi.org/10.1037//0735-7044.107.2.246.

———. "Effects of Amygdala Central Nucleus Lesions on Blocking and Unblocking." *Behav Neurosci* 107, no. 2 (April 1993): 235–45. https://doi.org/10.1037//0735-7044.107.2.235.

Hsu, T. M., J. E. McCutcheon, and M. F. Roitman. "Parallels and Overlap: The Integration of Homeostatic Signals by Mesolimbic Dopamine Neurons." *Front Psychiatry* 9 (2018): 410. https://doi.org/10.3389/fpsyt.2018.00410.

Kash, Thomas L., William P. Nobis, Robert T. Matthews, and Danny G. Winder. "Dopamine Enhances Fast Excitatory Synaptic Transmission in the Extended Amygdala by a CRF-R1-Dependent Process." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 28, no. 51 (December 17, 2008): 13856–65. https://doi.org/10.1523/JNEUROSCI.4715-08.2008.

Kawa, Alex B., Brandon S. Bentzley, and Terry E. Robinson. "Less Is More: Prolonged Intermittent Access Cocaine Self-Administration Produces Incentive-Sensitization and Addiction-like Behavior." *Psychopharmacology* 233, no. 19–20 (October 2016): 3587–3602. https://doi.org/10.1007/s00213-016-4393-8.

Keefer, S. E., D. E. Kochli, and D. Calu. "Basolateral Amygdala to Insular Cortex Activity Makes Sign-Tracking Behavior Insensitive to Outcome Value." *BioRxiv* 2022.02.24.481881; (2022). https://doi.org/10.1101/2022.02.24.481881.

Keefer, Sara E., Sam Z. Bacharach, Daniel E. Kochli, Jules M. Chabot, and Donna J. Calu. "Effects of Limited and Extended Pavlovian Training on Devaluation Sensitivity of Sign- and Goal-Tracking Rats." *Frontiers in Behavioral Neuroscience* 14 (2020): 3. https://doi.org/10.3389/fnbeh.2020.00003.

Keefer, Sara E., Utsav Gyawali, and Donna J. Calu. "Choose Your Path: Divergent Basolateral Amygdala Efferents Differentially Mediate Incentive Motivation, Flexibility and Decision-Making." *Behavioural Brain Research* 409 (July 9, 2021): 113306. https://doi.org/10.1016/j.bbr.2021.113306.

Kochli, D. E., S. E. Keefer, U. Gyawali, and D. J. Calu. "Basolateral Amygdala to Nucleus Accumbens Communication Differentially Mediates Devaluation Sensitivity of Sign- and Goal-Tracking Rats." *Front Behav Neurosci* 14 (2020): 593645. https://doi.org/10.3389/fnbeh.2020.593645.

Krawczyk, Michal, François Georges, Robyn Sharma, Xenos Mason, Amandine Berthet, Erwan Bézard, and Eric C. Dumont. "Double-Dissociation of the Catecholaminergic Modulation of Synaptic Transmission in the Oval Bed Nucleus of the Stria Terminalis." *Journal of Neurophysiology* 105, no. 1 (January 2011): 145–53. https://doi.org/10.1152/jn.00710.2010.

Krawczyk, Michal, Xenos Mason, Julian DeBacker, Robyn Sharma, Catherine P. Normandeau, Emily R. Hawken, Cynthia Di Prospero, et al. "D1 Dopamine Receptor-Mediated LTP at GABA Synapses Encodes Motivation to Self-Administer Cocaine in Rats." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33, no. 29 (July 17, 2013): 11960–71. https://doi.org/10.1523/JNEUROSCI.1784-13.2013.

Krawczyk, Michal, Robyn Sharma, Xenos Mason, Julian Debacker, Andrea A. Jones, and Eric C. Dumont. "A Switch in the Neuromodulatory Effects of Dopamine in the Oval Bed Nucleus of the

Stria Terminalis Associated with Cocaine Self-Administration in Rats." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 31, no. 24 (June 15, 2011): 8928–35. https://doi.org/10.1523/JNEUROSCI.0377-11.2011.

Labouesse, Marie A., Reto B. Cola, and Tommaso Patriarchi. "GPCR-Based Dopamine Sensors—A Detailed Guide to Inform Sensor Choice for In Vivo Imaging." *International Journal of Molecular Sciences* 21, no. 21 (January 2020): 8048. https://doi.org/10.3390/ijms21218048.

Langdon, Angela J., Melissa J. Sharpe, Geoffrey Schoenbaum, and Yael Niv. "Model-Based Predictions for Dopamine." *Current Opinion in Neurobiology* 49 (April 2018): 1–7. https://doi.org/10.1016/j.conb.2017.10.006.

Lee, B., R. N. Gentry, G. B. Bissonette, R. J. Herman, J. J. Mallon, D. W. Bryden, D. J. Calu, et al. "Manipulating the Revision of Reward Value during the Intertrial Interval Increases Sign Tracking and Dopamine Release." *PLoS Biol* 16, no. 9 (September 2018): e2004015. https://doi.org/10.1371/journal.pbio.2004015.

Lee, Rachel S., Ben Engelhard, Ilana B. Witten, and Nathaniel D. Daw. "A Vector Reward Prediction Error Model Explains Dopaminergic Heterogeneity." Preprint. Neuroscience, March 2, 2022. https://doi.org/10.1101/2022.02.28.482379.

Li, C., J. A. Sugam, E. G. Lowery-Gionta, Z. A. McElligott, N. M. McCall, A. J. Lopez, J. M. McKlveen, K. E. Pleil, and T. L. Kash. "Mu Opioid Receptor Modulation of Dopamine Neurons in the Periaqueductal Gray/Dorsal Raphe: A Role in Regulation of Pain." *Neuropsychopharmacology* 41, no. 8 (July 2016): 2122–32. https://doi.org/10.1038/npp.2016.12.

Lichtenberg, N. T., Z. T. Pennington, S. M. Holley, V. Y. Greenfield, C. Cepeda, M. S. Levine, and K. M. Wassum. "Basolateral Amygdala to Orbitofrontal Cortex Projections Enable Cue-Triggered Reward Expectations." *J Neurosci* 37, no. 35 (August 30, 2017): 8374–84. https://doi.org/10.1523/JNEUROSCI.0486-17.2017.

Lichtenberg, N. T., L. Sepe-Forrest, Z. T. Pennington, A. C. Lamparelli, V. Y. Greenfield, and K. M. Wassum. "The Medial Orbitofrontal Cortex-Basolateral Amygdala Circuit Regulates the Influence of Reward Cues on Adaptive Behavior and Choice." *J Neurosci* 41, no. 34 (August 25, 2021): 7267–77. https://doi.org/10.1523/JNEUROSCI.0901-21.2021.

Lin, Rui, Jingwen Liang, Ruiyu Wang, Ting Yan, Youtong Zhou, Yang Liu, Qiru Feng, et al. "The Raphe Dopamine System Controls the Expression of Incentive Memory." *Neuron* 106, no. 3 (May 6, 2020): 498-514.e8. https://doi.org/10.1016/j.neuron.2020.02.009.

Luster, Brennon R., Elizabeth S. Cogan, Karl T. Schmidt, Dipanwita Pati, Melanie M. Pina, Kedar Dange, and Zoé A. McElligott. "Inhibitory Transmission in the Bed Nucleus of the Stria Terminalis in Male and Female Mice Following Morphine Withdrawal." *Addiction Biology* 25, no. 3 (May 2020): e12748. https://doi.org/10.1111/adb.12748.

Lutas, Andrew, Hakan Kucukdereli, Osama Alturkistani, Crista Carty, Arthur U. Sugden, Kayla Fernando, Veronica Diaz, Vanessa Flores-Maldonado, and Mark L. Andermann. "State-Specific Gating of Salient Cues by Midbrain Dopaminergic Input to Basal Amygdala." *Nature Neuroscience* 22, no. 11 (November 2019): 1820–33. https://doi.org/10.1038/s41593-019-0506-0.

Madayag, A. C., S. J. Stringfield, K. J. Reissner, C. A. Boettiger, and D. L. Robinson. "Sex and Adolescent Ethanol Exposure Influence Pavlovian Conditioned Approach." *Alcohol Clin Exp Res* 41, no. 4 (April 2017): 846–56. https://doi.org/10.1111/acer.13354.

Mahler, Stephen V., Kyle S. Smith, and Kent C. Berridge. "Endocannabinoid Hedonic Hotspot for Sensory Pleasure: Anandamide in Nucleus Accumbens Shell Enhances 'liking' of a Sweet Reward." *Neuropsychopharmacology* 32, no. 11 (2007): 2267–78. https://doi.org/10.1038/sj.npp.1301376.

Malvaez, Melissa, Venuz Y. Greenfield, Alice S. Wang, Allison M. Yorita, Lili Feng, Kay E. Linker, Harold G. Monbouquette, and Kate M. Wassum. "Basolateral Amygdala Rapid Glutamate Release Encodes an Outcome-Specific Representation Vital for Reward-Predictive Cues to

Selectively Invigorate Reward-Seeking Actions." *Sci Rep* 5 (July 27, 2015): 12511. https://doi.org/10.1038/srep12511.

Malvaez, Melissa, Christine Shieh, Michael D. Murphy, Venuz Y. Greenfield, and Kate M. Wassum. "Distinct Cortical–Amygdala Projections Drive Reward Value Encoding and Retrieval." *Nature Neuroscience* 22, no. 5 (2019): 762–69. https://doi.org/10.1038/s41593-019-0374-7.

Maracle, Amanda C., Catherine P. Normandeau, Éric C. Dumont, and Mary C. Olmstead. "Dopamine in the Oval Bed Nucleus of the Stria Terminalis Contributes to Compulsive Responding for Sucrose in Rats." *Neuropsychopharmacology* 44, no. 2 (January 2019): 381–89. https://doi.org/10.1038/s41386-018-0149-y.

Martin, D. A., U. Gyawali, and D. J. Calu. "Effects of 5-HT2A Receptor Stimulation on Economic Demand for Fentanyl after Intermittent and Continuous Access Self-Administration in Male Rats." *Addict Biol*, May 26, 2020, e12926. https://doi.org/10.1111/adb.12926.

Martin, D. A., S. E. Keefer, and D. J. Calu. "Investigating Discriminative Stimulus Modulation of Opioid Seeking after Conflict-Induced Abstinence in Sign- and Goal-Tracking Rats." *Psychopharmacology (Berl)* 239, no. 10 (October 2022): 3223–36. https://doi.org/10.1007/s00213-022-06204-7.

Melchior, James R., Rafael E. Perez, Gregory J. Salimando, Joseph R. Luchsinger, Aakash Basu, and Danny G. Winder. "Cocaine Augments Dopamine-Mediated Inhibition of Neuronal Activity in the Dorsal Bed Nucleus of the Stria Terminalis." *Journal of Neuroscience* 41, no. 27 (July 7, 2021): 5876–93. https://doi.org/10.1523/JNEUROSCI.0284-21.2021.

Meloni, Edward G., Lyle P. Gerety, Allison T. Knoll, Bruce M. Cohen, and William A. Carlezon. "Behavioral and Anatomical Interactions between Dopamine and Corticotropin-Releasing Factor in the Rat." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 26, no. 14 (April 5, 2006): 3855–63. https://doi.org/10.1523/JNEUROSCI.4957-05.2006.

Montague, P. R., P. Dayan, and T. J. Sejnowski. "A Framework for Mesencephalic Dopamine Systems Based on Predictive Hebbian Learning." *Journal of Neuroscience* 16, no. 5 (March 1, 1996): 1936–47. https://doi.org/10.1523/JNEUROSCI.16-05-01936.1996.

Morrison, Sara E., Michael A. Bamkole, and Saleem M. Nicola. "Sign Tracking, but Not Goal Tracking, Is Resistant to Outcome Devaluation." *Front Neurosci* 9 (2015): 468. https://doi.org/10.3389/fnins.2015.00468.

Nasser, Helen M., Donna J. Calu, Geoffrey Schoenbaum, and Melissa J. Sharpe. "The Dopamine Prediction Error: Contributions to Associative Models of Reward Learning." *Frontiers in Psychology* 8 (2017): 244. https://doi.org/10.3389/fpsyg.2017.00244.

Nasser, Helen M., Yu-Wei Chen, Kimberly Fiscella, and Donna J. Calu. "Individual Variability in Behavioral Flexibility Predicts Sign-Tracking Tendency." *Frontiers in Behavioral Neuroscience* 9 (2015): 289. https://doi.org/10.3389/fnbeh.2015.00289.

Page, Michelle E., and Irwin Lucki. "Effects of Acute and Chronic Reboxetine Treatment on Stress-Induced Monoamine Efflux in the Rat Frontal Cortex." *Neuropsychopharmacology* 27, no. 2 (August 2002): 237–47. https://doi.org/10.1016/S0893-133X(02)00301-9.

Park, J., B. M. Kile, and R. M. Wightman. "In Vivo Voltammetric Monitoring of Norepinephrine Release in the Rat Ventral Bed Nucleus of the Stria Terminalis and Anteroventral Thalamic Nucleus." *Eur J Neurosci* 30, no. 11 (December 3, 2009): 2121–33. https://doi.org/10.1111/j.1460-9568.2009.07005.x.

Park, Jinwoo, Elizabeth S. Bucher, Khristy Fontillas, Catarina Owesson-White, Jennifer L. Ariansen, Regina M. Carelli, and R. Mark Wightman. "Opposing Catecholamine Changes in the Bed Nucleus of the Stria Terminalis during Intracranial Self-Stimulation and Its Extinction." *Biological Psychiatry* 74, no. 1 (July 1, 2013): 69–76. https://doi.org/10.1016/j.biopsych.2012.11.008.

Park, Jinwoo, Robert A. Wheeler, Khristy Fontillas, Richard B. Keithley, Regina M. Carelli, and R. Mark Wightman. "Catecholamines in the Bed Nucleus of the Stria Terminalis Reciprocally Respond to Reward and Aversion." *Biological Psychiatry*, Mechanisms of Compromised Stress

Resilience During Development and Aging, 71, no. 4 (February 15, 2012): 327–34. https://doi.org/10.1016/j.biopsych.2011.10.017.

Patriarchi, Tommaso, Jounhong Ryan Cho, Katharina Merten, Mark W. Howe, Aaron Marley, Wei-Hong Xiong, Robert W. Folk, et al. "Ultrafast Neuronal Imaging of Dopamine Dynamics with Designed Genetically Encoded Sensors." *Science* 360, no. 6396 (June 29, 2018). https://doi.org/10.1126/science.aat4422.

Paxinos, George, and Charles Watson. "The Rat Brain in Stereotaxic Coordinates - 6th Edition," November 2, 2006.

Pearce, J. M., and G. Hall. "A Model for Pavlovian Learning: Variations in the Effectiveness of Conditioned but Not of Unconditioned Stimuli." *Psychological Review* 87, no. 6 (November 1980): 532–52.

Pecina, S., and K. C. Berridge. "Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do Mu-Opioids Cause Increased Hedonic Impact of Sweetness?" *J Neurosci* 25, no. 50 (December 14, 2005): 11777–86. https://doi.org/10.1523/JNEUROSCI.2329-05.2005.

Phelix, C. F., Z. Liposits, and W. K. Paull. "Catecholamine-CRF Synaptic Interaction in a Septal Bed Nucleus: Afferents of Neurons in the Bed Nucleus of the Stria Terminalis." *Brain Research Bulletin* 33, no. 1 (1994): 109–19. https://doi.org/10.1016/0361-9230(94)90056-6.

Pitchers, Kyle K., Kyra B. Phillips, Jonte L. Jones, Terry E. Robinson, and Martin Sarter. "Diverse Roads to Relapse: A Discriminative Cue Signaling Cocaine Availability Is More Effective in Renewing Cocaine Seeking in Goal Trackers Than Sign Trackers and Depends on Basal Forebrain Cholinergic Activity." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 37, no. 30 (July 26, 2017): 7198–7208. https://doi.org/10.1523/JNEUROSCI.0990-17.2017.

Reiner, David J., Ida Fredriksson, Olivia M. Lofaro, Jennifer M. Bossert, and Yavin Shaham. "Relapse to Opioid Seeking in Rat Models: Behavior, Pharmacology and Circuits." *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 44, no. 3 (2019): 465–77. https://doi.org/10.1038/s41386-018-0234-2.

Reiner, David J., Olivia M. Lofaro, Sarah V. Applebey, Hannah Korah, Marco Venniro, Carlo Cifani, Jennifer M. Bossert, and Yavin Shaham. "Role of Projections between Piriform Cortex and Orbitofrontal Cortex in Relapse to Fentanyl Seeking after Palatable Food Choice-Induced Voluntary Abstinence." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 40, no. 12 (March 18, 2020): 2485–97. https://doi.org/10.1523/JNEUROSCI.2693-19.2020.

Robinson, Terry E., and Shelly B. Flagel. "Dissociating the Predictive and Incentive Motivational Properties of Reward-Related Cues through the Study of Individual Differences." *Biological Psychiatry* 65, no. 10 (May 15, 2009): 869–73. https://doi.org/10.1016/j.biopsych.2008.09.006.

Robinson, Terry E., Lindsay M. Yager, Elizabeth S. Cogan, and Benjamin T. Saunders. "On the Motivational Properties of Reward Cues: Individual Differences." *Neuropharmacology* 76 Pt B (January 2014): 450–59. https://doi.org/10.1016/j.neuropharm.2013.05.040.

Roesch, M. R., D. J. Calu, and G. Schoenbaum. "Dopamine Neurons Encode the Better Option in Rats Deciding between Differently Delayed or Sized Rewards." *Nat Neurosci* 10, no. 12 (December 2007): 1615–24. https://doi.org/10.1038/nn2013.

Roesch, Matthew R., Donna J. Calu, Guillem R. Esber, and Geoffrey Schoenbaum. "Neural Correlates of Variations in Event Processing during Learning in Basolateral Amygdala." *Journal of Neuroscience* 30, no. 7 (February 17, 2010): 2464–71. https://doi.org/10.1523/JNEUROSCI.5781-09.2010.

Saddoris, M. P. "Terminal Dopamine Release Kinetics in the Accumbens Core and Shell Are Distinctly Altered after Withdrawal from Cocaine Self-Administration." *ENeuro* 3, no. 5 (October 2016). https://doi.org/10.1523/ENEURO.0274-16.2016.

Saddoris, M. P., X. Wang, J. A. Sugam, and R. M. Carelli. “Cocaine Self-Administration Experience Induces Pathological Phasic Accumbens Dopamine Signals and Abnormal Incentive Behaviors in Drug-Abstinent Rats.” *J Neurosci* 36, no. 1 (January 6, 2016): 235–50. https://doi.org/10.1523/JNEUROSCI.3468-15.2016.

Saddoris, Michael P., Fabio Cacciapaglia, R. Mark Wightman, and Regina M. Carelli. “Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation.” *J Neurosci* 35, no. 33 (August 19, 2015): 11572–82. https://doi.org/10.1523/JNEUROSCI.2344-15.2015.

Saunders, B. T., J. M. Richard, E. B. Margolis, and P. H. Janak. “Dopamine Neurons Create Pavlovian Conditioned Stimuli with Circuit-Defined Motivational Properties.” *Nat Neurosci* 21, no. 8 (August 2018): 1072–83. https://doi.org/10.1038/s41593-018-0191-4.

Saunders, B. T., L. M. Yager, and T. E. Robinson. “Cue-Evoked Cocaine ‘Craving’: Role of Dopamine in the Accumbens Core.” *J Neurosci* 33, no. 35 (August 28, 2013): 13989–0. https://doi.org/10.1523/JNEUROSCI.0450-13.2013.

Saunders, Benjamin T., and Terry E. Robinson. “A Cocaine Cue Acts as an Incentive Stimulus in Some but Not Others: Implications for Addiction.” *Biological Psychiatry* 67, no. 8 (April 15, 2010): 730–36. https://doi.org/10.1016/j.biopsych.2009.11.015.

———. “The Role of Dopamine in the Accumbens Core in the Expression of Pavlovian-Conditioned Responses.” *The European Journal of Neuroscience* 36, no. 4 (August 2012): 2521–32. https://doi.org/10.1111/j.1460-9568.2012.08217.x.

Schultz, W., P. Dayan, and P. R. Montague. “A Neural Substrate of Prediction and Reward.” *Science (New York, N.Y.)* 275, no. 5306 (March 14, 1997): 1593–99. https://doi.org/10.1126/science.275.5306.1593.

Schultz, Wolfram. “Neuronal Reward and Decision Signals: From Theories to Data.” *Physiological Reviews* 95, no. 3 (July 2015): 853–951. https://doi.org/10.1152/physrev.00023.2014.

Shah, Nirao M., David J. Pisapia, Silas Maniatis, Monica M. Mendelsohn, Adriana Nemes, and Richard Axel. “Visualizing Sexual Dimorphism in the Brain.” *Neuron* 43, no. 3 (August 5, 2004): 313–19. https://doi.org/10.1016/j.neuron.2004.07.008.

Sharpe, M. J., C. Y. Chang, M. A. Liu, H. M. Batchelor, L. E. Mueller, J. L. Jones, Y. Niv, and G. Schoenbaum. “Dopamine Transients Are Sufficient and Necessary for Acquisition of Model-Based Associations.” *Nat Neurosci* 20, no. 5 (May 2017): 735–42. https://doi.org/10.1038/nn.4538.

Sias, A. C., A. K. Morse, S. Wang, V. Y. Greenfield, C. M. Goodpaster, T. M. Wrenn, A. M. Wikenheiser, et al. “A Bidirectional Corticoamygdala Circuit for the Encoding and Retrieval of Detailed Reward Memories.” *Elife* 10 (June 18, 2021). https://doi.org/10.7554/eLife.68617.

Silberman, Yuval, Robert T. Matthews, and Danny G. Winder. “A Corticotropin Releasing Factor Pathway for Ethanol Regulation of the Ventral Tegmental Area in the Bed Nucleus of the Stria Terminalis.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 33, no. 3 (January 16, 2013): 950–60. https://doi.org/10.1523/JNEUROSCI.2949-12.2013.

Silberman, Yuval, and Danny G. Winder. “Emerging Role for Corticotropin Releasing Factor Signaling in the Bed Nucleus of the Stria Terminalis at the Intersection of Stress and Reward.” *Frontiers in Psychiatry* 4 (May 29, 2013): 42. https://doi.org/10.3389/fpsyt.2013.00042.

Smedley, E. B., and K. S. Smith. “Evidence of Structure and Persistence in Motivational Attraction to Serial Pavlovian Cues.” *Learn Mem* 25, no. 2 (February 2018): 78–89. https://doi.org/10.1101/lm.046599.117.

Stamatakis, Alice M., Dennis R. Sparta, Joshua H. Jennings, Zoe A. McElligott, Heather Decot, and Garret D. Stuber. “Amygdala and Bed Nucleus of the Stria Terminalis Circuitry: Implications for Addiction-Related Behaviors.” *Neuropharmacology* 76 Pt B (January 2014): 320–28. https://doi.org/10.1016/j.neuropharm.2013.05.046.

Sun, Fangmiao, Jianzhi Zeng, Miao Jing, Jingheng Zhou, Jiesi Feng, Scott F. Owen, Yichen Luo, et al. “A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of

Dopamine in Flies, Fish, and Mice." *Cell* 174, no. 2 (July 12, 2018): 481-496.e19. https://doi.org/10.1016/j.cell.2018.06.042.

Sun, Fangmiao, Jingheng Zhou, Bing Dai, Tongrui Qian, Jianzhi Zeng, Xuelin Li, Yizhou Zhuo, et al. "Next-Generation GRAB Sensors for Monitoring Dopaminergic Activity in Vivo." *Nature Methods* 17, no. 11 (November 2020): 1156–66. https://doi.org/10.1038/s41592-020-00981-9.

Sutton, Richard S., and Andrew G. Barto. *Reinforcement Learning: An Introduction*. 2nd ed. Adaptive Computation and Machine Learning Series. Cambridge, MA, USA: A Bradford Book, 2018.

Takahashi, Y. K., H. M. Batchelor, B. Liu, A. Khanna, M. Morales, and G. Schoenbaum. "Dopamine Neurons Respond to Errors in the Prediction of Sensory Features of Expected Rewards." *Neuron* 95, no. 6 (September 13, 2017): 1395-1405 e3. https://doi.org/10.1016/j.neuron.2017.08.025.

Tsuneoka, Y., S. Tsukahara, S. Yoshida, K. Takase, S. Oda, M. Kuroda, and H. Funato. "Moxd1 Is a Marker for Sexual Dimorphism in the Medial Preoptic Area, Bed Nucleus of the Stria Terminalis and Medial Amygdala." *Front Neuroanat* 11 (2017): 26. https://doi.org/10.3389/fnana.2017.00026.

Vranjkovic, Oliver, Melanie Pina, Thomas L. Kash, and Danny G. Winder. "The Bed Nucleus of the Stria Terminalis in Drug-Associated Behavior and Affect: A Circuit-Based Perspective." *Neuropharmacology* 122 (August 1, 2017): 100–106. https://doi.org/10.1016/j.neuropharm.2017.03.028.

Waelti, P., A. Dickinson, and W. Schultz. "Dopamine Responses Comply with Basic Assumptions of Formal Learning Theory." *Nature* 412, no. 6842 (July 5, 2001): 43–48. https://doi.org/10.1038/35083500.

Wagner, Edward J. "Sex Differences in Cannabinoid-Regulated Biology: A Focus on Energy Homeostasis." *Frontiers in Neuroendocrinology* 40 (2016): 101–9. https://doi.org/10.1016/j.yfrne.2016.01.003.

Walker, R. A., K. M. Wright, T. C. Jhou, and M. A. McDannald. "The Ventrolateral Periaqueductal Grey Updates Fear via Positive Prediction Error." *Eur J Neurosci* 51, no. 3 (February 2020): 866–80. https://doi.org/10.1111/ejn.14536.

Watabe-Uchida, Mitsuko, Neir Eshel, and Naoshige Uchida. "Neural Circuitry of Reward Prediction Error." *Annual Review of Neuroscience* 40 (July 25, 2017): 373–94. https://doi.org/10.1146/annurev-neuro-072116-031109.

Willuhn, Ingo, Matthew J. Wanat, Jeremy J. Clark, and Paul E. M. Phillips. "Dopamine Signaling in the Nucleus Accumbens of Animals Self-Administering Drugs of Abuse." *Current Topics in Behavioral Neurosciences* 3 (2010): 29–71. https://doi.org/10.1007/7854_2009_27.

Yu, W., C. M. Caira, R. Rivera Sanchez N. Del, G. A. Moseley, and T. L. Kash. "Corticotropin-Releasing Factor Neurons in the Bed Nucleus of the Stria Terminalis Exhibit Sex-Specific Pain Encoding in Mice." *Sci Rep* 11, no. 1 (June 14, 2021): 12500. https://doi.org/10.1038/s41598-021-91672-8.

Yu, Waylin, Dipanwita Pati, Melanie M. Pina, Karl T. Schmidt, Kristen M. Boyt, Avery C. Hunker, Larry S. Zweifel, Zoe A. McElligott, and Thomas L. Kash. "Periaqueductal Gray/Dorsal Raphe Dopamine Neurons Contribute to Sex Differences in Pain-Related Behaviors." *Neuron* 109, no. 8 (April 21, 2021): 1365-1380.e5. https://doi.org/10.1016/j.neuron.2021.03.001.