Nucleus accumbens dopamine encodes the trace period during appetitive Pavlovian conditioning

Matthew J. Wanat[1], Erika T. Camacho[1,2], and Paul E.M. Phillips[3]

Author Affiliations:

[1]Department of Neuroscience and Developmental Biology, University of Texas at San Antonio, San Antonio, TX, USA.

[2]Department of Mathematics, University of Texas at San Antonio, San Antonio, TX, USA.

[3]Department of Psychiatry & Behavioral Sciences, Department of Pharmacology, Center for Neurobiology of Addiction, Pain & Emotion, University of Washington, Seattle, WA, USA.

Corresponding Author:

Matthew J. Wanat

Department of Neuroscience, Developmental and Regenerative Biology

University of Texas at San Antonio

Matthew.wanat@utsa.edu

Author Contributions: MJW and PEMP designed the experiments, MJW collected the data, MJW and ETC analyzed the data, and all authors assisted with writing the manuscript.

Funding and Disclosure:

This work was supported by National Institutes of Health grants DA033386 (MJW), DA042362 (MJW), MH127466 (MJW) and DA027858 (PEMP). The authors declare no conflict of interest.

Abstract:

Pavlovian conditioning tasks have been used to identify the neural systems involved with learning cue-outcome relationships. In delay conditioning, the presentation of a conditioned stimulus (CS) overlaps or co-terminates with the delivery of the unconditioned stimulus (US). Prior studies demonstrate that behavioral responding during delay conditioning is regulated by dopamine signaling in the nucleus accumbens (NAc). In particular, the dopamine response to the CS reflects the relative value of the upcoming reward in these tasks. In contrast to delay conditioning, trace conditioning involves a 'trace' period that separates the end of the CS and the delivery of the US. While dopamine has been implicated in trace conditioning, no studies to date have examined how NAc dopamine responds to reward-related stimuli in these tasks. To address this, we developed a within-subject trace conditioning task where distinct audio CSs signaled either a short trace period (5s) or a long trace period (55s) prior to the delivery of a food reward. Male rats exhibited higher levels of conditioned responding and a faster latency to respond to the Short Trace CS relative to the Long Trace CS. Voltammetry recordings in the NAc found that dopamine levels to the CS increased on Short Trace trials but decreased on Long Trace trials. Conversely, US-evoked dopamine responses were greater on Long Trace trials relative to Short Trace trials. We found that the CS dopamine response correlated with the latency to respond and not to the level of conditioned responding. Furthermore, the relationship between CS dopamine and latency was best explained by an exponential function. Taken together, our results illustrate how CS-evoked dopamine signals in the NAc relate to behavioral outcomes. Furthermore, we find that trace period is encoded by the bidirectional NAc dopamine response to the CS during Pavlovian conditioning.

Significance statement:

Learning how to associate a given cue with an outcome is fundamental process underlying reward seeking behavior. Dopamine in the ventral striatum is important for establishing the link between cues and rewarding outcomes in Pavlovian conditioning tasks. However, it is unclear how striatal dopamine release responds to cues during trace conditioning when there is temporal gap between the cue and the rewarding outcome. To address this, we performed voltammetry recordings of dopamine release in male rats trained on trace conditioning task. We demonstrate that cue-evoked dopamine signals encode the trace period and is related to the latency to respond. While prior reports find that the relative reward value is signaled by increases in dopamine neuron activity to cues, the current study highlights the that dopamine response to reward-predictive cues can signal the relative reward value through bidirectional changes in dopamine transmission.

Introduction:

Associating a cue with a given outcome is a fundamental process critical for both reward learning and aversive learning. These learning processes are commonly studied in the context of Pavlovian conditioning tasks in which a conditioned stimulus (CS) predicts the delivery of an unconditioned stimulus (US). Delay conditioning is a category of Pavlovian conditioning in which the presentation of the CS overlaps or co-terminates with the delivery of the US. Pharmacological and optogenetic studies illustrate that dopamine signaling facilitates learning and the expression of conditioned responses during delay conditioning tasks with food rewards (Di Ciano et al., 2001; Flagel et al., 2011; Saunders and Robinson, 2012; Lopez et al., 2015; Fraser et al., 2016; Fraser and Janak, 2017; Roughley and Killcross, 2019; Heymann et al., 2020; Lee et al., 2020; Morrens et al., 2020; Stelly et al., 2020; Khoo et al., 2021; Stelly et al., 2021; Lefner et al., 2022b). Recordings from midbrain dopamine neurons as well as dopamine release in the nucleus accumbens (NAc) demonstrate that the CS-evoked dopamine response signals the relative value of the upcoming reward in delay conditioning tasks (Fiorillo et al., 2003; Tobler et al., 2005; Fonzi et al., 2017; Lefner et al., 2022a). Furthermore, the direction of the CS-evoked dopamine response signals the valence of the outcome, as NAc dopamine levels decrease in response to the CS in delay conditioning tasks with an aversive US (Oleson and Cheer, 2013; de Jong et al., 2019; Stelly et al., 2019).

In contrast to delay conditioning where there is no temporal separation between the CS and US, trace conditioning tasks involve a ‘trace’ period following the end of the CS and the delivery of the US. While many trace conditioning tasks commonly use an aversive stimulus for the US (e.g. eyeblink conditioning, conditioned taste aversion, and fear conditioning), trace conditioning tasks can also use food rewards for the US (Reilly and Bornovalova, 2005; Woodruff-Pak and Disterhoft, 2008; Raybuck and Lattal, 2014; Pezze et al., 2015; Sias et al., 2024). Pharmacological and genetic studies indicate that dopamine signaling regulates behavioral responding during appetitive and aversive trace conditioning tasks (Fenu et al., 2001; Fenu and Di Chiara, 2003; Deng et al., 2015; Pezze et al., 2015). However, despite the evidence for dopamine’s involvement, no studies to date have examined how NAc dopamine signals relate to behavioral outcomes during appetitive trace conditioning.

In this study, male rats were trained on a trace conditioning task where distinct audio CSs signaled either a Short Trace period (5s) or a Long Trace period (55s) prior to the delivery of a food reward. Voltammetry recordings of NAc dopamine release were performed in well-trained animals. This experimental approach allows for the within-subject analysis of NAc dopamine signals to two distinct cue-outcome pairs during trace conditioning. If CS-evoked dopamine response reflects reward value, one would anticipate a larger dopamine response to the Short Trace CS (greater value for an imminent reward) relative to the Long Trace CS (lower value for a delayed reward). Consistent with this prediction, we observed a larger CS-evoked dopamine response on Short Trace trials, though notably there was a decrease in dopamine levels on Long Trace trials. Subsequent analyses found that CS-evoked changes in dopamine transmission were related to response latency according to an exponential decay function. Overall, these data illustrate notable differences in the CS-evoked NAc dopamine response between delay and trace conditioning tasks. In delay conditioning tasks, the increase in CS-evoked dopamine

response scales with reward value (Fiorillo et al., 2003; Tobler et al., 2005; Fonzi et al., 2017; Lefner et al., 2022a). In contrast, we find that NAc dopamine levels decrease in response to a reward-predicting CS associated with a Long Trace period. Therefore, dopamine encoding of reward value is not solely limited to positive changes in dopamine levels, but rather can encompass both positive and negative changes in the dopamine response to the CS.

Methods:

Subjects and surgery

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington. Male Sprague-Dawley rats (Charles River, CA) aged P60-65 (300-350 g) were pair-housed upon arrival and given ad libitum access to water and chow and maintained on a 12-hour light/dark cycle (n = 23). We acknowledge the limitation of only including male subjects. Though we note that the data in this study were collected in 2011-2013, which was prior to the NIH policy regarding sex as a biological variable, when it was regrettably commonplace to perform single sex studies. Rats underwent surgery to implant voltammetry electrodes in the NAc core (relative to bregma: 1.3 mm anterior; ± 1.3 mm lateral; 7.0 mm ventral) and a Ag/AgCl reference electrode. Carbon fiber voltammetry electrodes consisted of a carbon fiber housed in silica tubing and cut to a length of ~150 µm (Clark et al., 2010). Rats were single-housed following surgery.

Behavioral procedures

All procedures were performed during the light cycle. After >1 week of recovery from surgery, rats were placed and maintained on mild food restriction (~15 g/day of standard lab chow) to target 90% free-feeding weight, allowing for an increase of 1.5% per week. Behavioral sessions were performed in chambers (Med Associates) that had a house light, a food tray, and auditory stimulus generators (whitenoise and 2.9 kHz tone). To familiarize rats with the chamber and food retrieval, rats underwent a single magazine training session in which 20 food pellets (45 mg, BioServ) were non-contingently delivered at a 90 ± 15 s variable interval. Trace conditioning sessions consisted 25 Short Trace trials and 25 Long Trace trials that were presented in a pseudorandom manner. Short Trace trials consisted of a 5 s audio CS followed by a 5 s trace period after which a single food pellet was delivered. Long Trace trials consisted of a 5 s audio CS followed by a 55s trace period after which a single food pellet was delivered. The food tray light illuminated for 4.5 s after the delivery of the food pellet US and trials were separated by a 5 -15 s intertrial interval (ITI). The audio CSs used for Short and Long Trace trials (tone and whitenoise) were counterbalanced across subjects. Rats underwent a total of 30 trace conditioning sessions. Behavioral metrics monitored include the head entry latency following the onset of the CS presentation, the head entry latency following the US delivery, and conditioned responding. We calculated conditioned responding as the rate of head entries during the 5 s CS relative to the rate of head entries during the 5 s preceding the CS, consistent with prior studies (Fonzi et al., 2017; Stelly et al., 2020; Stelly et al., 2021; Lefner et al., 2022a; Lefner et al., 2022b).

Voltammetry recordings

Voltammetry recordings of NAc dopamine release were performed in well-trained subjects (sessions 25-30). Chronically-implanted electrodes were connected to a head-mounted amplifier to monitor changes in dopamine release in behaving rats using fast-scan cyclic voltammetry as described previously (Clark et al., 2010; Fonzi et al., 2017; Oliva and Wanat, 2019; Stelly et al., 2019; Stelly et al., 2020; Oliva et al., 2021; Stelly et al., 2021; Lefner et al., 2022a). The carbon fiber electrodes were held at -0.4 V (vs. Ag/AgCl) with voltammetric scans applied at 10 Hz in which the potential was ramped in a triangular waveform to +1.3 V and back to -0.4 V at a rate of 400 V/s. A principal component regression analysis was performed on the voltammetry signal using a standard training set (Heien et al., 2005). The average post-implantation sensitivity of electrodes (34 nA/μM) was used to estimate dopamine concentration (Clark et al., 2010). Chemical verification of dopamine was achieved by obtaining a high correlation of the cyclic voltammogram during a reward-related event to that of a dopamine standard (correlation coefficient $r^2 \geq 0.75$ by linear regression). Voltammetry data for a session were excluded from analysis if the detected voltammetry signal did not satisfy this chemical verification criteria (Fonzi et al., 2017; Oliva and Wanat, 2019; Stelly et al., 2019; Stelly et al., 2020; Oliva et al., 2021; Stelly et al., 2021; Lefner et al., 2022a). Voltammetry data from given electrode was included provided there were two or more recording sessions that satisfied the above stated inclusion criteria (n = 10 electrodes). The CS-evoked dopamine response was quantified as the average dopamine signal during the 5 s CS relative to the 5 s prior to the CS. The US-evoked dopamine response was quantified as the average dopamine signal during the 3 s following the US delivery relative to the average dopamine signal during the 1 s preceding the US delivery.

Modeling and data analysis

We examined the relationship between the CS dopamine response and latency as a function of the trace period. Data from the full trace conditioning data set were fit to a linear function with 2 parameters:

Linear: $Dopamine = A*Latency + B$

Prior research indicates that exponential and hyperbolic functions can account for the relationship between cue-evoked dopamine neuron firing and reward delay in primates (Kobayashi and Schultz, 2008). To perform non-linear curve fits on the current data set, we reexamined data collected from a related delay conditioning task where the trace period was 0 s (Fonzi et al., 2017). This delay conditioning task used the same audio CSs and delivered the same number of rewards per session as the trace conditioning task. Delay conditioning rats also underwent the same training procedure with voltammetry recordings performed only in well-trained animals (>24 conditioning sessions). We solely focused on trials with the same ITI duration in both the delay and trace conditioning tasks (10 and 15 s ITI). In this manner we examined how the trace period (0, 5, 55 s) influences the CS dopamine response and its relationship to response latency. We note that hyperbolic functions are not suitable for our data

set which includes negative values for the CS dopamine response on Long Trace trials. We therefore initially fit the data from trace and delay conditioning rats to an exponential function with 3 parameters:

Exponential 1: $Dopamine = A*e^{(B*Latency)} + C$

This Exponential 1 function gave a better fit and smaller error than single exponential function with 2 parameters. In all cases the parameters were optimized to give the best fit without restriction on the parameters. When examining the individual data for each trace period (0, 5, 55 s) utilizing the Exponential 1 function, we found (i) the fit for the delay conditioning / 0 s trace data to be concave up and increasing, (ii) the fit for the 5 s trace data to be concave down and decreasing, and (iii) the fit for the 55 s trace data to be concave up and decreasing. Taken together, this dopamine-latency relationship suggested a combined behavior similar to a Gaussian function. Thus, the data were also fit to an exponential function with 4 parameters (specifically, a Gaussian function) that was optimized to account for the dopamine-latency relationship within each trace period (0, 5, 55 s):

Exponential 2: $Dopamine = A*e^{(-\left(\frac{(Latency - B)}{C}\right)^2)} + D$

The performance of these functions was evaluated on the full data set from the trace conditioning rats. The deviation of the predicted dopamine versus actual dopamine values were quantified for each equation.

Statistical analyses were performed in GraphPad Prism. Significant effects were determined by performing repeated measures ANOVAs with post-hoc Sidak's tests where appropriate, paired t-tests, and linear regressions. The Geisser-Greenhouse correction was applied to address unequal variances between groups. A Friedman test followed by a post-hoc Dunn's test was used to examine data that did not conform to a Gaussian distribution.

Results:

Rats were trained on a trace conditioning task that contained two distinct trial types (**Fig. 1A**). During Short Trace trials, the presentation of a 5 s audio CS (tone or whitenoise) was followed by a 5 s trace period after which the US was delivered (food pellet). During Long Trace trials, the presentation of a 5 s audio CS (whitenoise or tone) was followed by a 55 s trace period after which the US was delivered. The assignment of the audio CS to a given trial type was counterbalanced between animals. Sessions consisted of a 25 Short Trace trials and 25 Long Trace trials that were presented in a pseudorandom pattern and separated by a 5 - 15 s ITI. Rats underwent 24 behavior-only training sessions to ensure they were well-trained prior to performing voltammetry recordings. Animals exhibited higher levels of conditioned responding on Short Trace trials relative to Long Trace trials (two-way repeated measures ANOVA, effect of trial type $F_{(1,22)} = 6.5$, $p = 0.018$; interaction effect $F_{(4.4, 97.4)} = 3.0$, $p = 0.018$, $n = 23$; **Fig. 1B**). The latency to initiate a head entry following the CS onset was longer on Long Trace trials relative to Short Trace trials (two-way repeated measures ANOVA; effect of trial type $F_{(1,22)} = 26.4$, *** $p < 0.0001$; interaction effect $F_{(6.0, 132.1)} = 2.6$, $p = 0.021$; **Fig. 1C**). Interestingly, this effect was

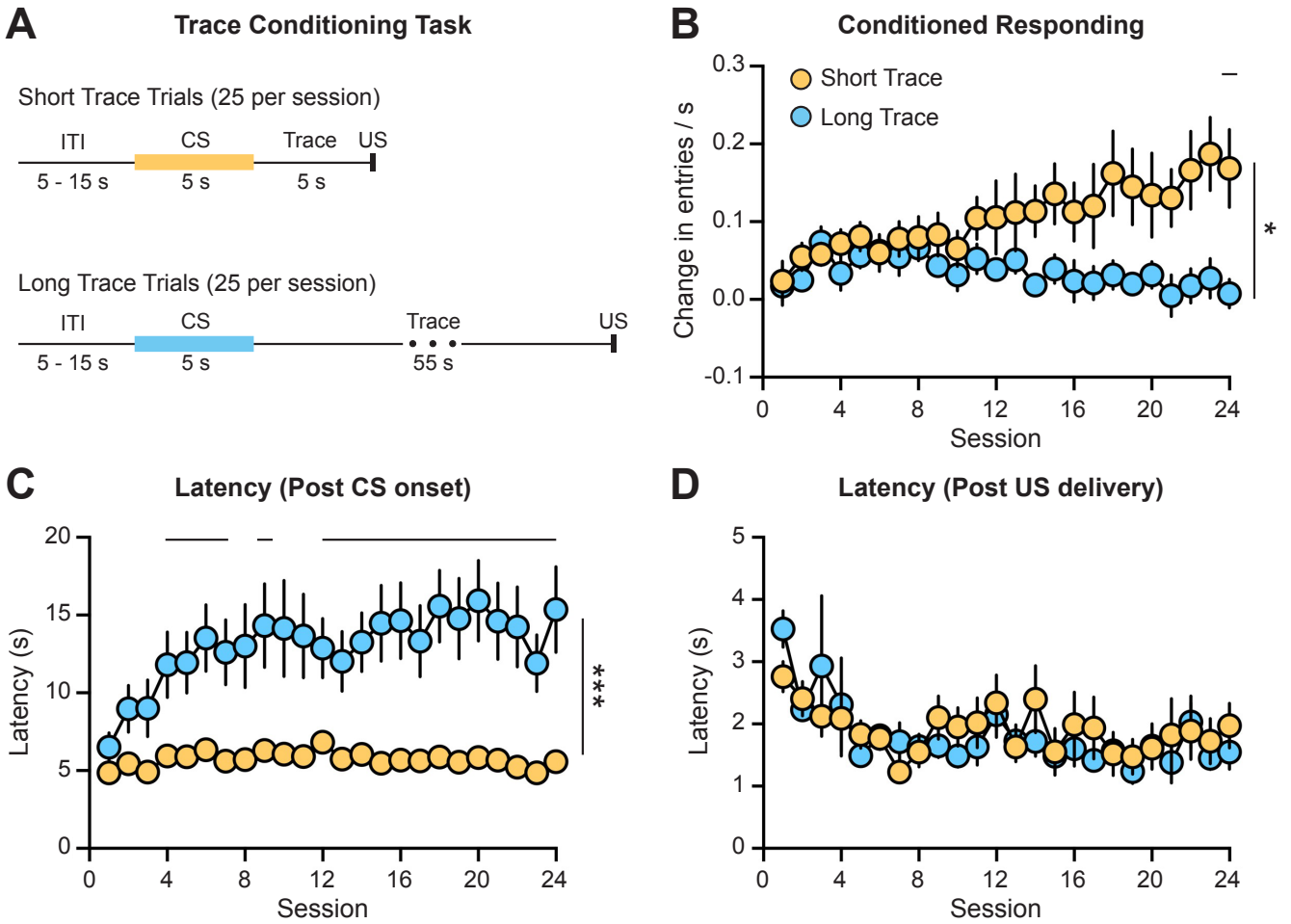

Figure 1. Trace conditioning task. (A) Schematic of Short and Long Trace trials, which are presented in a pseudorandom pattern within a session. (B) Higher levels of conditioned responding on Short Trace trials relative to Long Trace trials (effect of trial type, * $p = 0.018$). (C) Longer latency to perform a head entry following the CS onset on Long Trace trials relative to Short Trace trials (effect of trial type, *** $p < 0.0001$). (D) No difference in the latency to retrieve the reward between Short and Long Trace trials. Horizontal lines on graphs denote post hoc Sidak's test, $p < 0.05$.

driven by a slowing of the latency to respond on Long Trace trials. Finally, we examined the latency to the first head entry following the US delivery and found no difference between trial types (two-way repeated measures ANOVA; effect of trial type $F_{(1,22)} = 1.0$, $p = 0.32$; **Fig. 1D**). Collectively, these data illustrate that the presentation of the Short Trace CS enhances conditioned responding, whereas the presentation of the Long Trace CS delays the latency to perform a head entry.

Voltammetry recordings of NAc dopamine levels were performed in rats that were well-trained on the trace conditioning task (>24 sessions). Data were analyzed from animals with electrodes in the NAc core with at least 2 viable voltammetry recording sessions (n = 15 electrodes from 10 rats; **Fig. 2A**). Dopamine levels increased in response to the Short Trace CS but decreased to the Long Trace CS (**Fig. 2B-C**). We quantified CS-evoked dopamine response as the average dopamine signal during the 5 s CS relative to the 5 s prior to the CS presentation. Dopamine release to the Short Trace CS was greater than the dopamine response to the Long Trace CS (paired t-test, $t_{14} = 4.0$, ** $p = 0.001$, **Fig. 2E**). Therefore, the magnitude of the CS-evoked dopamine response is inversely related to the trace period before the

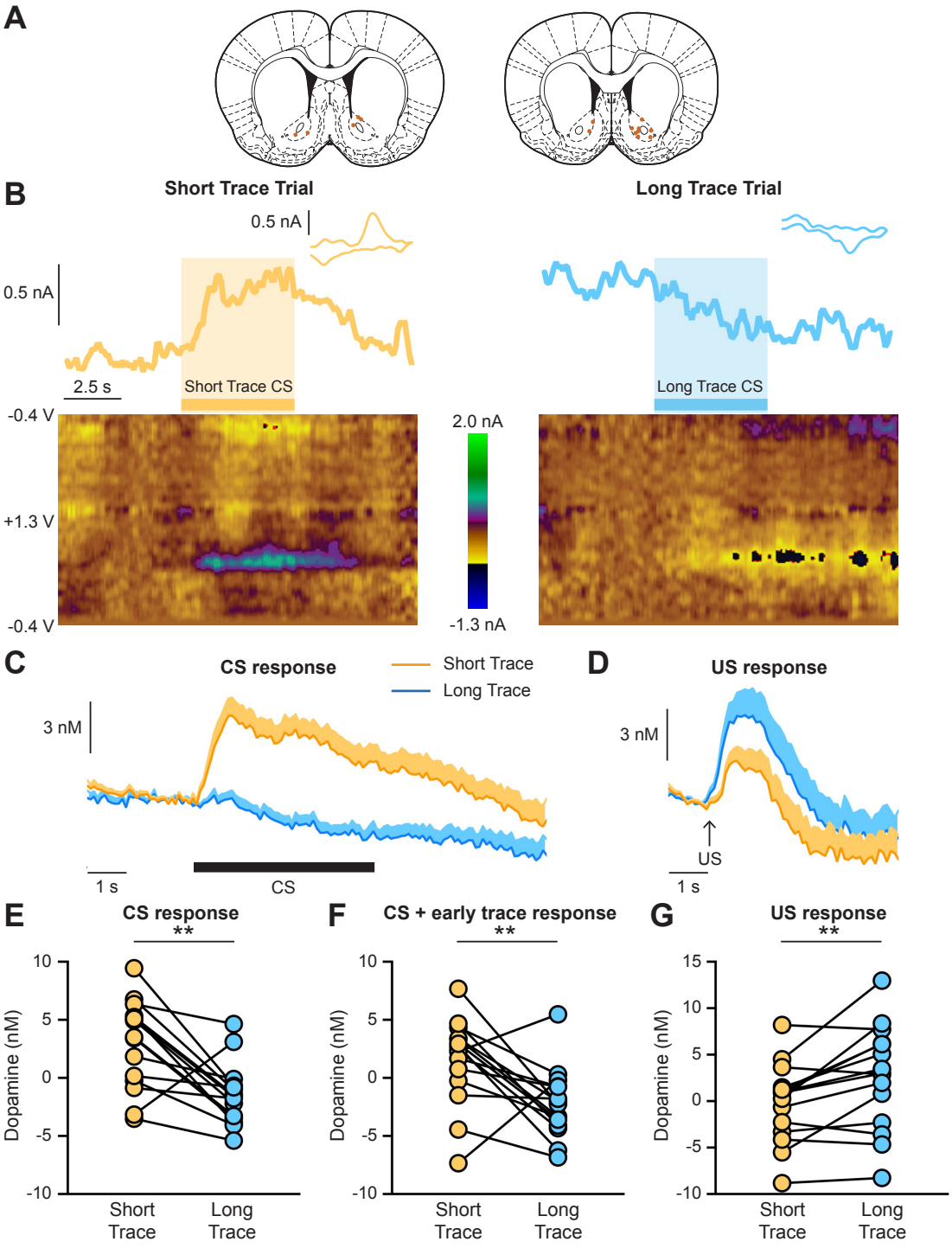

Figure 2. NAc dopamine release during trace conditioning. (A) Voltammery electrode placement. Voltammetry recordings were performed in well-trained rats (> 24 sessions) (B) Representative voltammetry recordings illustrating the dopamine current (top), cyclic voltammogram (top, inset), and the voltammetry color plot (bottom) from a Short Trace and Long Trace trial. (C) Average dopamine response to the CS and the initial trace period. (D) Average dopamine response to the US delivery. (E) Larger dopamine response to the Short Trace CS relative to the Long Trace CS (paired t-test, t_{14} = 4.0, ** p = 0.001). Dopamine to the Short Trace CS was greater than null values (one sample t-test relative to 0, t_{14} = 3.0, ** p = 0.009). (F) Larger dopamine response to the CS and the first 5 s of the trace period on Short Trace trials relative to Long Trace trials (paired t-test, t_{14} = 3.1, ** p = 0.008). Dopamine on Long Trace trials decreased below null values (one sample t-test relative to 0, t_{14} = 2.9, p = 0.01). (G) Larger dopamine response to the US delivery on Long Trace trials relative to Short Trace trials (paired t-test, t_{14} = 3.4, ** p = 0.004).

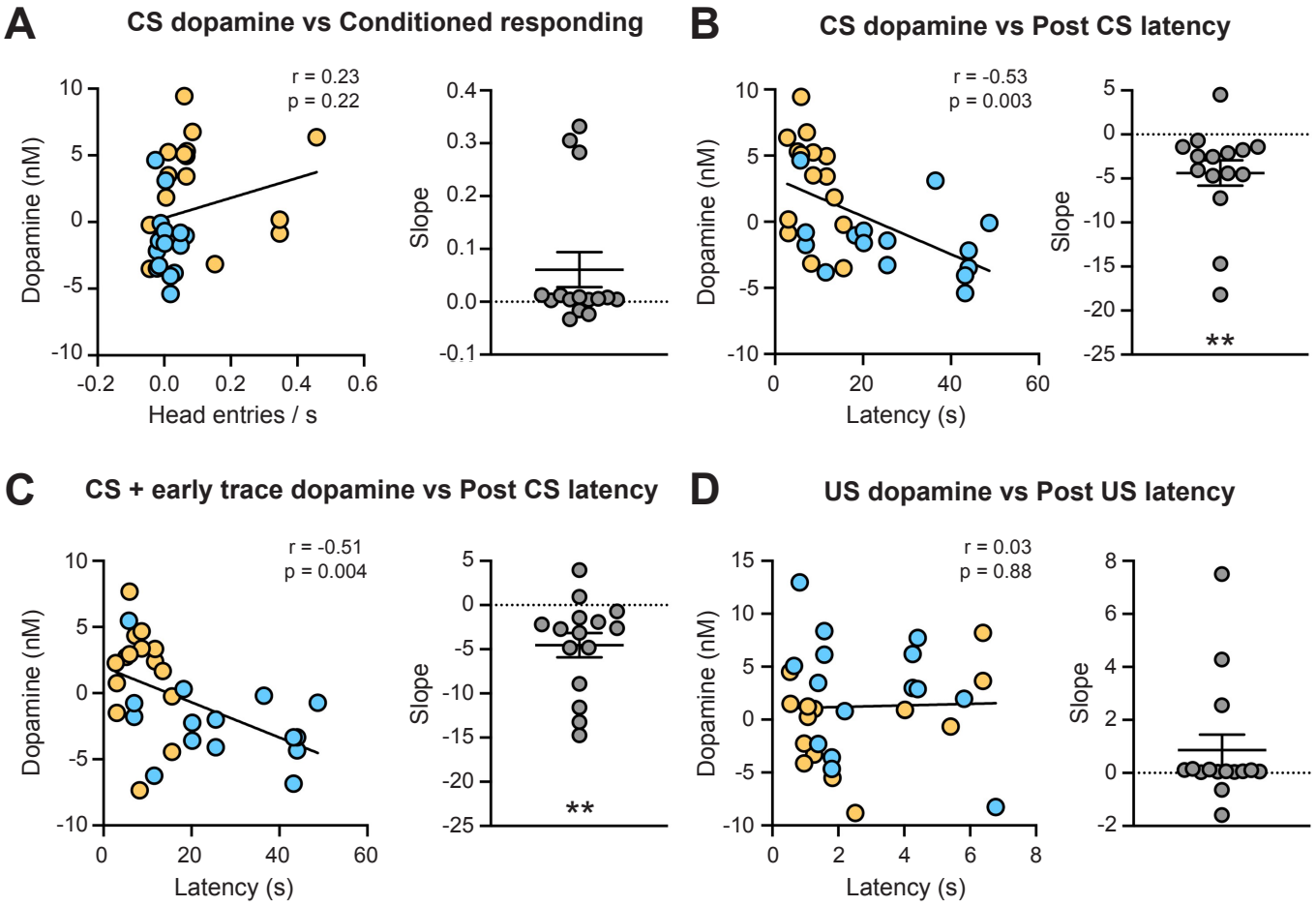

Figure 3. Correlating NAc dopamine signals to behavioral responding. Subpanels present the correlation between dopamine and behavior (left) and the slope of the relationship across electrodes (right). (A) The CS dopamine response was not correlated with conditioned responding. (B) The CS dopamine response was inversely related to the head entry latency (r = -0.53, p = 0.003). The slope of this relationship was significant across electrodes (one sample t-test relative to 0, t_{14} = 3.1, ** p = 0.009). (C) The dopamine response to the CS and the first 5 s of the trace period was inversely related to the head entry latency (r = -0.51, p = 0.004). The slope of this relationship was significant across electrodes (one sample t-test relative to 0, t_{14} = 3.3, ** p = 0.005). (D) The US dopamine response was not correlated with latency to retrieve the reward.

US is delivered. We also examined the CS dopamine response along with the first 5 s of the trace period and found a similar relationship with greater dopamine levels on the Short Trace trials relative to Long Trace trials (paired t-test, t_{14} = 3.1, ** p = 0.008, **Fig. 2F**). In contrast, there was a larger dopamine response to the US delivery on Long Trace trials relative to Short Trace trials (paired t-test, t_{14} = 3.4, ** p = 0.004). These findings are consistent with prior studies illustrating a greater dopamine response to delayed or unexpected rewards (Fiorillo et al., 2003; Wanat et al., 2010).

We next determined how the dopamine signals correlated with behavioral outcomes. Although Short Trace trials produced higher levels of conditioned responding and larger CS-evoked dopamine release relative to Long Trace trials (**Figs. 1-2**), there was no correlation between CS dopamine levels and conditioned responding (**Fig. 3A, left**). We also calculated the slope of this relationship for each electrode and found that the average slope was not significantly different from 0 (**Fig. 3A, right**). These data collectively illustrate the lack of a relationship between conditioned responding and CS dopamine release during trace conditioning. In contrast, we found that CS dopamine levels were inversely related

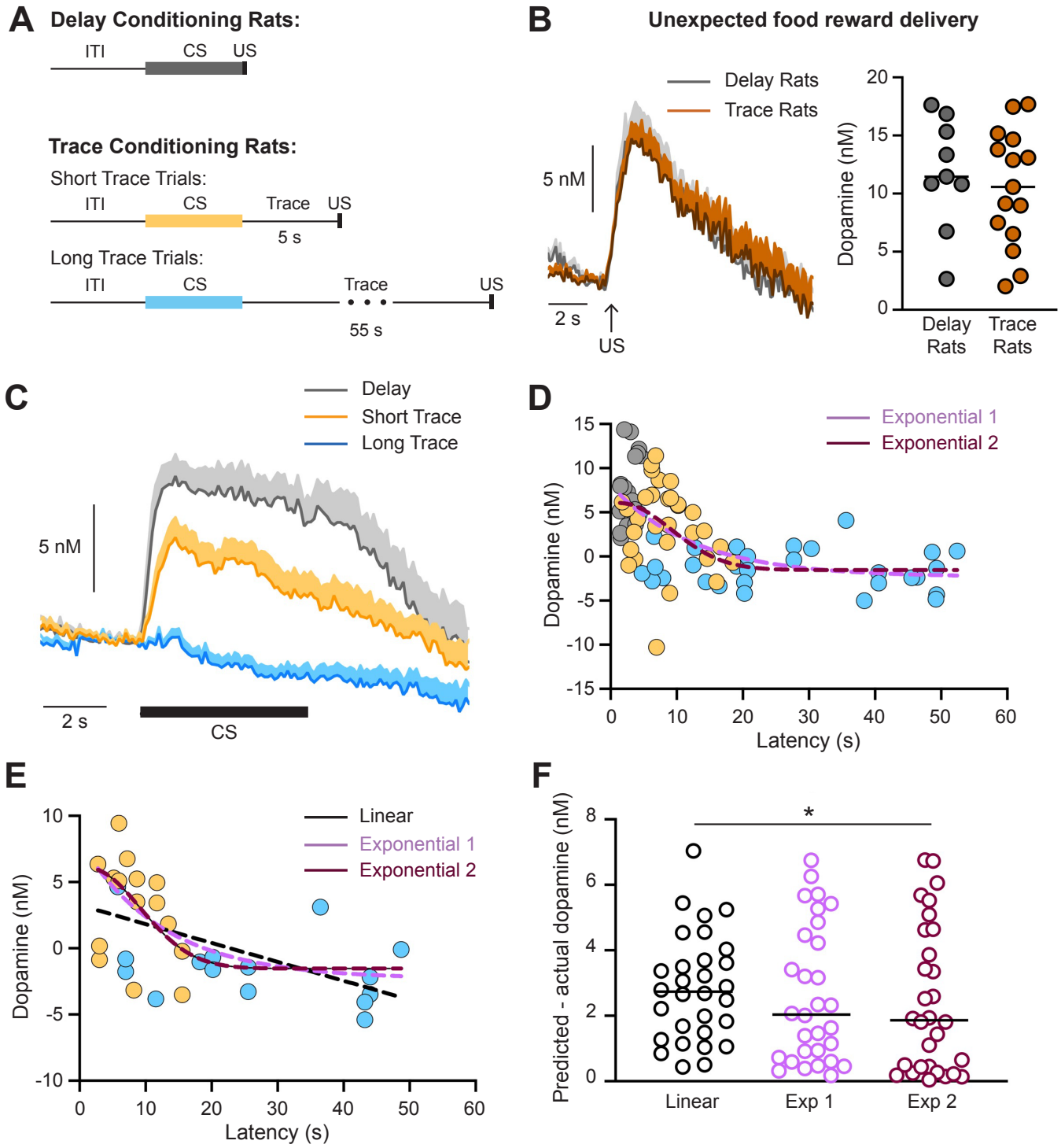

Figure 4. The relationship between CS dopamine and the latency to respond across different trace period durations. (A) Schematic of delay conditioning trials (with no trace period) and trace conditioning trials. (B) No difference in the dopamine response to unpredicted reward presentations between rats trained on the delay conditioning task or the trace conditioning task. (C) Average NAc dopamine release to the CS. (D) Data from 10 and 15 s ITI trials from rats trained on the delay and trace conditioning tasks. Overlayed dashed lines illustrate the the best fit exponential functions based on the delay and trace conditioning data using three variables (Exponential 1: A = 10.43, B = 0.07954, C = -2.32; pink) or four variables (Exponential 2: A = 7.568, B = -1.328, C = 11.06, D = -1.531; red). (E) Best fit exponential and linear functions overlayed on the average responses from the full data set (see also **Fig. 3B**; Linear function: A = -0.1429, B = 3.262; black). (F). Difference between the predicted and actual dopamine values for the linear and exponential functions (Friedman test, F = 6.07, p = 0.04; post-hoc Dunn's test, * p = 0.04).

to the head entry latency (r = -0.51, p = 0.004) and the slope of this relationship was significantly different from 0 across electrodes (one sample t-test relative to 0, t_{14} = 3.3, ** p = 0.005; **Fig. 3B**). Similar results were observed when examining the relationship between head entry latency and the dopamine response during the CS and the first 5 s of the trace period (r = -0.51, p = 0.004; **Fig. 3C**). While there was a larger dopamine response to the US on Long Trace trials, this was not related to the latency to retrieve the food reward (**Fig. 3D**).

Prior studies indicate that linear and non-linear functions can account for the relationship between dopamine transmission and behavioral responding in timing-related tasks. The subjective hazard rate, or rather the expectation than an event will occur, is inversely related behavioral responding and dopamine neuron firing activity according to a linear function over rapid timescales (< 2 s) (Pasquereau and Turner, 2015). However, recordings from dopamine neurons during a delay discounting task using longer timescales (1 – 16s) demonstrated that the CS dopamine response decreases in a non-linear manner as the reward delay increases (Kobayashi and Schultz, 2008). Specifically, the decay in the CS dopamine response is steep for more immediate rewards and shallow for more delayed rewards (Kobayashi and Schultz, 2008). While there was a significant correlation between CS dopamine levels and head entry latency (**Fig. 3B**), the nature of this relationship may not be linear given the longer timescales used in the trace conditioning task. Determining how the trace period influences the CS dopamine response and its relationship to behavior therefore requires additional data from short trace periods when the decay in the dopamine response is steepest. To address this, we reexamined data collected from a related delay conditioning task where the trace period is 0 s (Fonzi et al., 2017). This delay conditioning task used the same audio CSs and delivered the same number of rewards per session as the trace conditioning task. Delay conditioning rats also underwent the same training procedure with voltammetry recordings performed only in well-trained animals (>24 conditioning sessions). We solely focused on trials with the same ITI duration in both the delay and trace conditioning tasks (10 and 15 s ITI). In this manner we can establish how the trace period (0, 5, 55 s) influences the CS dopamine response and its relationship to response latency (**Fig. 4A**).

It is important to demonstrate that a given stimulus produces an equivalent dopamine response in both trace and delay conditioned rats before one compares the dopamine signals between these experiments. To address this, we evaluated the dopamine response to an unexpected food reward that was delivered outside of the training sessions. We found no difference in the NAc dopamine response to unexpected food rewards between trace and delay conditioned rats (**Fig. 4B**). As would be expected, CS-evoked dopamine levels in the NAc were greatest for delay conditioned rats with no trace period (**Fig. 4C**). We fit the data from 10 and 15s ITI trials to best-fit exponential functions with either three parameters (Exponential 1) or four parameters (Exponential 2; **Fig. 4D**). We then assessed the performance of these functions on the full data set from the trace conditioning rats (**Figs. 3B, 4E**). Both exponential equations performed better than a linear function based on the full data set, with the four parameter Exponential 2 equation providing a significant improvement relative to the linear function (**Fig. 4F**). Taken together, these data coupled with prior reports illustrate how exponential functions best

account for the relationship between dopamine and behavioral responding on the order of 10s of seconds.

Discussion:

We demonstrate that the CS dopamine response in the NAc encodes the trace period between the end of the cue and the delivery of the reward. These results are consistent with the findings from Pavlovian delay conditioning and operant delay discounting tasks where the CS dopamine response signals an estimate of reward value. Specifically, these studies demonstrate that the CS-evoked *increase* in dopamine signaling is inversely related to the time until the reward (Fiorillo et al., 2003; Kobayashi and Schultz, 2008; Day et al., 2010; Gan et al., 2010). However, we find that a reward predictive cue produces a *decrease* in NAc dopamine levels when it signals a long trace period. This cue-evoked decrease in dopamine signaling is unexpected, as the presentation of a CS^- during delay conditioning can still enhance dopamine release due to response generalization (Day et al., 2007). Furthermore, a recent study found no change in dopamine neuron activity to cues that are reliably delivered after the reward delivery in animals well-trained on a backward conditioning task (Taira et al., 2024). Based on these findings from delay and backward conditioning tasks, one would expect either no dopamine response or a modest increase in dopamine levels to a cue that is never paired with a reward delivery. However, by examining the dopamine response during a trace conditioning task, we uncovered that dopamine encoding of reward value is not solely limited to positive changes in dopamine levels, but rather can encompass both positive and negative changes in the dopamine response to the CS.

Animals reliably prefer cues associated with immediate rewards relative to cues associated with delayed rewards in operant tasks (Day et al., 2010; Gan et al., 2010). In agreement, rats exhibited a higher level of conditioned responding on Short Trace trials relative to Long Trace trials. We note that the magnitude of conditioned responding during trace conditioning was ~5 times lower relative to similarly designed delay conditioning tasks, which indicates that the temporal separation between the CS and US during Pavlovian conditioning influences the intensity of responding during the cue (Fonzi et al., 2017; Stelly et al., 2020; Stelly et al., 2021; Lefner et al., 2022a; Lefner et al., 2022b). The difference in conditioned responding between trial types emerged after ~10 training sessions and was driven by an increase in responding to the Short Trace CS. In contrast, a difference in the latency to respond between trial types rapidly emerged during early training sessions and was driven by a slowing in the response to Long Trace CS. These data underscore how distinct cue-evoked behavioral responses can develop independently of one another during Pavlovian conditioning. In support, we previously found that systemically antagonizing dopamine receptors can differentially regulate conditioned responding and the latency to respond during delay conditioning (Lefner et al., 2022b). Future studies are needed to determine how cue-driven behaviors during trace conditioning are controlled by dopamine transmission.

Short Trace trials produced higher levels of conditioned responding and greater CS-evoked dopamine release in the NAc relative to Long Trace trials. However, we found no correlation between conditioned responding and the CS dopamine response during trace conditioning. These results are

consistent with our prior work using well-trained animals undergoing delay conditioning (Fonzi et al., 2017). In this study distinct cues signaled the time since the previous reward delivery. One audio cue always followed a short ITI and signaled a higher rate of reward while another audio cue always followed a long ITI and signaled a lower rate of reward. While there was greater dopamine release to the short ITI cue, this was not accompanied by a higher level of conditioned responding in well-trained subjects under static conditions. Rather, CS-evoked dopamine release in this delay conditioning task correlated with conditioned responding during early learning and when the training context was changed (Fonzi et al., 2017; Stelly et al., 2021). Taken together, the absence of a relationship between NAc dopamine levels and conditioned responding is to be expected given that dopamine recordings during this trace conditioning task were performed in well-trained animals under static conditions.

Whereas there was no link between dopamine and conditioned responding, we instead found that CS dopamine levels in the NAc were related to the response latency. Our initial analysis illustrated a significant linear correlation between dopamine levels and the latency. However prior research indicates that the relationship between dopamine and behavior over longer time scales may be better explained by an exponential function (Fiorillo et al., 2003; Kobayashi and Schultz, 2008). Indeed, we found that best fit exponential functions were more accurate at predicting dopamine levels relative to a standard linear function in well-trained animals. Future studies are needed to determine how CS-evoked dopamine relates to the response latency during early training sessions.

A regrettable limitation of this current study was the use of only male subjects, though we note that the data were collected prior to the implementation of the sex as a biological variable policy from the NIH. Sex differences have been observed in a number of dopamine-dependent behaviors (Lynch and Carroll, 1999; Zachry et al., 2019; Kutlu et al., 2020; Bishnoi et al., 2021; Chen et al., 2021; George et al., 2021). In particular, females exhibit higher levels of conditioned responding and a faster response latency than males during a delay conditioning task (Lefner et al., 2022a). Increasing evidence illustrates how cycling hormones can modulate dopamine transmission, which can subsequently impact dopamine-dependent behaviors (Calipari et al., 2017; Yoest et al., 2019; Zachry et al., 2021; Zhang et al., 2021). Therefore, future research is needed to determine how NAc dopamine signaling relates to behavioral responding during trace conditioning in females across the estrus cycle.

Prior research highlights that midbrain dopamine neurons contribute to evaluating differences in time on rapid timescales (< 2s) (Pasquereau and Turner, 2015; Soares et al., 2016). For example, dopamine neuron activity can reflect the expectation that an event will occur (Pasquereau and Turner, 2015). Additionally, optogenetic manipulations of dopamine neurons can bidirectionally alter the perceived temporal interval between two cues (Soares et al., 2016). Our current results extend these findings to illustrate that the CS-evoked dopamine response in NAc core can encode the trace period over long temporal intervals (0-55 s). Collectively, these highlight a role for dopamine in timing-related calculations over a range of timescales.

References:

Bishnoi IR, Ossenkopp KP, Kavaliers M (2021) Sex and age differences in locomotor and anxiety-like behaviors in rats: From adolescence to adulthood. Dev Psychobiol 63:496-511.

Calipari ES, Juarez B, Morel C, Walker DM, Cahill ME, Ribeiro E, Roman-Ortiz C, Ramakrishnan C, Deisseroth K, Han MH, Nestler EJ (2017) Dopaminergic dynamics underlying sex-specific cocaine reward. Nature communications 8:13877.

Chen CS, Ebitz RB, Bindas SR, Redish AD, Hayden BY, Grissom NM (2021) Divergent Strategies for Learning in Males and Females. Current biology : CB 31:39-50 e34.

Clark JJ, Sandberg SG, Wanat MJ, Gan JO, Horne EA, Hart AS, Akers CA, Parker JG, Willuhn I, Martinez V, Evans SB, Stella N, Phillips PE (2010) Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals. Nature methods 7:126-129.

Day JJ, Roitman MF, Wightman RM, Carelli RM (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10:1020-1028.

Day JJ, Jones JL, Wightman RM, Carelli RM (2010) Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. Biol Psychiatry 68:306-309.

de Jong JW, Afjei SA, Pollak Dorocic I, Peck JR, Liu C, Kim CK, Tian L, Deisseroth K, Lammel S (2019) A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System. Neuron 101:133-151 e137.

Deng S, Zhang L, Zhu T, Liu YM, Zhang H, Shen Y, Li WG, Li F (2015) A behavioral defect of temporal association memory in mice that partly lack dopamine reuptake transporter. Sci Rep 5:17461.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J Neurosci 21:9471-9477.

Fenu S, Di Chiara G (2003) Facilitation of conditioned taste aversion learning by systemic amphetamine: role of nucleus accumbens shell dopamine D1 receptors. Eur J Neurosci 18:2025-2030.

Fenu S, Bassareo V, Di Chiara G (2001) A role for dopamine D1 receptors of the nucleus accumbens shell in conditioned taste aversion learning. J Neurosci 21:6897-6904.

Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299:1898-1902.

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011) A selective role for dopamine in stimulus-reward learning. Nature 469:53-57.

Fonzi KM, Lefner MJ, Phillips PEM, Wanat MJ (2017) Dopamine Encodes Retrospective Temporal Information in a Context-Independent Manner. Cell reports 20:1765-1774.

Fraser KM, Janak PH (2017) Long-lasting contribution of dopamine in the nucleus accumbens core, but not dorsal lateral striatum, to sign-tracking. Eur J Neurosci 46:2047-2055.

Fraser KM, Haight JL, Gardner EL, Flagel SB (2016) Examining the role of dopamine D2 and D3 receptors in Pavlovian conditioned approach behaviors. Behav Brain Res 305:87-99.

Gan JO, Walton ME, Phillips PE (2010) Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. Nat Neurosci 13:25-27.

George BE, Barth SH, Kuiper LB, Holleran KM, Lacy RT, Raab-Graham KF, Jones SR (2021) Enhanced heroin self-administration and distinct dopamine adaptations in female rats. Neuropsychopharmacology 46:1724-1733.

Heien ML, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Wassum KM, Wightman RM (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci U S A 102:10023-10028.

Heymann G, Jo YS, Reichard KL, McFarland N, Chavkin C, Palmiter RD, Soden ME, Zweifel LS (2020) Synergy of Distinct Dopamine Projection Populations in Behavioral Reinforcement. Neuron 105:909-920 e905.

Khoo SY, Uhrig A, Samaha AN, Chaudhri N (2021) Effects of dopamine receptor antagonism and amphetamine-induced psychomotor sensitization on sign- and goal-tracking after extended training. Behav Brain Res 407:113238.

Kobayashi S, Schultz W (2008) Influence of reward delays on responses of dopamine neurons. J Neurosci 28:7837-7846.

Kutlu MG, Zachry JE, Brady LJ, Melugin PR, Kelly SJ, Sanders C, Tat J, Johnson AR, Thibeault K, Lopez AJ, Siciliano CA, Calipari ES (2020) A novel multidimensional reinforcement task in mice elucidates sex-specific behavioral strategies. Neuropsychopharmacology 45:1463-1472.
Lee K, Claar LD, Hachisuka A, Bakhurin KI, Nguyen J, Trott JM, Gill JL, Masmanidis SC (2020) Temporally restricted dopaminergic control of reward-conditioned movements. Nat Neurosci 23:209-216.
Lefner MJ, Dejeux MI, Wanat MJ (2022a) Sex Differences in Behavioral Responding and Dopamine Release during Pavlovian Learning. eNeuro 9.
Lefner MJ, Stelly CE, Fonzi KM, Zurita H, Wanat MJ (2022b) Critical periods when dopamine controls behavioral responding during Pavlovian learning. Psychopharmacology (Berl) 239:2985-2996.
Lopez JC, Karlsson RM, O'Donnell P (2015) Dopamine D2 Modulation of Sign and Goal Tracking in Rats. Neuropsychopharmacology 40:2096-2102.
Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 144:77-82.
Morrens J, Aydin C, Janse van Rensburg A, Esquivelzeta Rabell J, Haesler S (2020) Cue-Evoked Dopamine Promotes Conditioned Responding during Learning. Neuron 106:142-153 e147.
Oleson EB, Cheer JF (2013) On the role of subsecond dopamine release in conditioned avoidance. Front Neurosci 7:96.
Oliva I, Wanat MJ (2019) Operant Costs Modulate Dopamine Release to Self-Administered Cocaine. J Neurosci 39:1249-1260.
Oliva I, Donate MM, Lefner MJ, Wanat MJ (2021) Cocaine experience abolishes the motivation suppressing effect of CRF in the ventral midbrain. Addict Biol 26:e12837.
Pasquereau B, Turner RS (2015) Dopamine neurons encode errors in predicting movement trigger occurrence. J Neurophysiol 113:1110-1123.
Pezze MA, Marshall HJ, Cassaday HJ (2015) Dopaminergic modulation of appetitive trace conditioning: the role of D1 receptors in medial prefrontal cortex. Psychopharmacology (Berl) 232:2669-2680.
Raybuck JD, Lattal KM (2014) Bridging the interval: theory and neurobiology of trace conditioning. Behav Processes 101:103-111.
Reilly S, Bornovalova MA (2005) Conditioned taste aversion and amygdala lesions in the rat: a critical review. Neurosci Biobehav Rev 29:1067-1088.
Roughley S, Killcross S (2019) Differential involvement of dopamine receptor subtypes in the acquisition of Pavlovian sign-tracking and goal-tracking responses. Psychopharmacology (Berl) 236:1853-1862.
Saunders BT, Robinson TE (2012) The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci 36:2521-2532.
Sias AC, Jafar Y, Goodpaster CM, Ramirez-Armenta K, Wrenn TM, Griffin NK, Patel K, Lamparelli AC, Sharpe MJ, Wassum KM (2024) Dopamine projections to the basolateral amygdala drive the encoding of identity-specific reward memories. Nat Neurosci 27:728-736.
Soares S, Atallah BV, Paton JJ (2016) Midbrain dopamine neurons control judgment of time. Science 354:1273-1277.
Stelly CE, Tritley SC, Rafati Y, Wanat MJ (2020) Acute Stress Enhances Associative Learning via Dopamine Signaling in the Ventral Lateral Striatum. J Neurosci 40:4391-4400.
Stelly CE, Girven KS, Lefner MJ, Fonzi KM, Wanat MJ (2021) Dopamine release and its control over early Pavlovian learning differs between the NAc core and medial NAc shell. Neuropsychopharmacology 46:1780-1787.
Stelly CE, Haug GC, Fonzi KM, Garcia MA, Tritley SC, Magnon AP, Ramos MAP, Wanat MJ (2019) Pattern of dopamine signaling during aversive events predicts active avoidance learning. Proc Natl Acad Sci U S A 116:13641-13650.
Taira M, Millard SJ, Verghese A, DiFazio LE, Hoang IB, Jia R, Sias A, Wikenheiser A, Sharpe MJ (2024) Dopamine Release in the Nucleus Accumbens Core Encodes the General Excitatory Components of Learning. J Neurosci 44.
Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307:1642-1645.
Wanat MJ, Kuhnen CM, Phillips PE (2010) Delays conferred by escalating costs modulate dopamine release to rewards but not their predictors. J Neurosci 30:12020-12027.
Woodruff-Pak DS, Disterhoft JF (2008) Where is the trace in trace conditioning? Trends Neurosci 31:105-112.

Yoest KE, Cummings JA, Becker JB (2019) Oestradiol influences on dopamine release from the nucleus accumbens shell: sex differences and the role of selective oestradiol receptor subtypes. Br J Pharmacol 176:4136-4148.
Zachry JE, Johnson AR, Calipari ES (2019) Sex Differences in Value-Based Decision Making Underlie Substance Use Disorders in Females. Alcohol Alcohol 54:339-341.
Zachry JE, Nolan SO, Brady LJ, Kelly SJ, Siciliano CA, Calipari ES (2021) Sex differences in dopamine release regulation in the striatum. Neuropsychopharmacology 46:491-499.
Zhang X, Guan W, Yang T, Furlan A, Xiao X, Yu K, An X, Galbavy W, Ramakrishnan C, Deisseroth K, Ritola K, Hantman A, He M, Josh Huang Z, Li B (2021) Genetically identified amygdala-striatal circuits for valence-specific behaviors. Nat Neurosci 24:1586-1600.