Prefrontal D1 dopamine signaling is necessary for temporal expectation during reaction time performance

Organizing behavior in time is a key feature of goal-directed behavior and humans suffering from a variety of diseases, such as schizophrenia and Parkinson’s disease, can develop difficulties organizing their movements in time (3; 19). While frontostriatal regions are known to be involved in organizing behavior (4), the precise neural circuits remain unclear. Characterizing these circuits could be important in the development of therapies for diseases involving the frontal cortex and striatum. One paradigm that has been used to study temporal expectation is the simple reaction time task (11; Fig. 1A). In this task, subjects learn to anticipate a stimulus at long waiting times and exhibit delay-dependent speeding of reaction times (13). That is, with long delay times subjects exhibit faster reaction times as temporal expectation is high, meaning that subjects are better prepared to respond. On the other hand, with short delay times, temporal expectation is low and subjects are less prepared to respond, making reaction times slower.

In rodents, disrupting the medial prefrontal cortex consistently impairs temporal expectation during a simple reaction time task and the ability to inhibit responding (14). While prefrontal networks are modulated by several ascending projection systems, dopamine projections exert unique and cognitively specific modulation of prefrontal networks (10). However, the role of prefrontal dopamine signaling in reaction time performance remains unclear. Thus far, impairments of dopamine signaling in the striatum, such as those seen in Parkinson’s disease or by disrupting striatal dopamine have led to motor impairments, evidenced by slowed reaction time without influencing temporal expectation (Fig. 1B; 1; 8). However, manipulations of prefrontal dopamine signaling selectively impaired timing behavior in timing tasks (16).
These data lead to the hypothesis that prefrontal dopamine signaling is involved in temporal expectation during simple reaction time performance (Fig. 1B). Specifically, disrupting prefrontal dopamine signaling should impair an animal’s ability to anticipate stimuli close to the response deadline and attenuate delay-dependent speeding. Prior work has suggested that disrupting mesolimbic projections from the ventral tegmental area (VTA) does not influence temporal expectation (1; 8). However, in the present study, we tested the hypothesis that disruption of prefrontal dopamine would affect an animal’s temporal expectancy with two experiments.

**Experimental Procedures**

*Animals:* Long Evans rats (*Rattus norvegicus*, N=27), weighing 250-300 grams were used in this study. All animal procedures were performed in accordance with the protocol approved by the University of Iowa Institutional Animal Care and Use Committee (IACUC). Animals were housed individually following surgery with food *ad libitum*, a 12 hour light-on light-off schedule, and they were deprived of water 24 hours prior to behavioral testing.

*Behavioral apparatus:* Operant sound-attenuating behavioral chambers were equipped with a lever, a drinking tube, and a speaker driven to produce a tone. Water was delivered via a pump connected to a standard metal drinking tube. Each correct response in the task activated the pump at 0.03 mL/s for 1000–3000 ms, depending on training stage.

*Behavioral training:* Well-handled animals were trained via successive approximation to depress the response lever. Each lever press activated the pump for 3000 ms, during which time additional lever presses were not rewarded. Animals that successfully learned the lever press protocol were trained to ‘wait’ for the stimulus using a simple reaction time task with a fixed delay of 1000 ms (Fig. 1A). On each training day, the pump time was decreased by 500 ms until
it reached 1000 ms and the response window was shortened until it reached 600 ms. Lever presses shorter than the 1000 ms delay or longer than the 600 ms response window were followed by a timeout period (4000-8000 ms). Once animals performed over 70% correct responses in a session, they were trained on the simple reaction time task with two delays (400 ms and 1000 ms; Fig. 1A).

_Surgery:_ Animals were initially anesthetized with 4% isoflurane followed by intraperitoneal injections of ketamine (100 mg/kg) and xylazine (10 mg/kg). A surgical level of anesthesia was maintained over the course of surgery with supplements (30 mg/kg) of ketamine every 45-60 minutes if necessary. Under aseptic conditions, the scalp was retracted, and the skull was leveled between bregma and lambda. **Experiment 1:** Bilateral craniotomies were made above target sites (AP -5.6, ML ±2.3, DV -8.0 at 12 degrees laterally). A Hamilton syringe was lowered into the target site and 1 µL of either 6-OHDA or saline was slowly infused over 2 minutes at 15 µL/hour. Syringes were kept in place for 2 minutes to allow drug diffusion. Craniotomies were sealed and scalps sutured closed. After 1 week recovery, animals were trained in the simple reaction time task. **Experiment 2:** Trained rats were implanted bilaterally with 22-gauge guide cannulae in the medial prefrontal cortex using coordinates (AP +3.2, ML ±1.2, DV -3.6 at 10 degrees laterally). Following one week of recovery, animals were acclimated to infusion procedures and briefly anesthetized with isoflurane. A 33-gauge injector cannula was infused with vehicle or drug (0.5 µL of sulpiride (1 µg/µL), 0.5 µL of SCH23390 (0.1 µg/µL or 1 µg/µL) dissolved in PBS (pH ~ 7.4). The injector was inserted into the guide cannula and 0.5 µL of infusion fluid was delivered per site at a rate of 0.5 µL/min via an infusion pump. After the injection was completed, the injector was left in place for 2 minutes to allow diffusion. Thirty minutes following infusion, animals underwent the simple reaction time task.
Histology: Animals were anesthetized with 100 mg/kg sodium pentobarbital, perfused with 4% paraformaldehyde, and brains were coronally sectioned on a sliding microtome. **Experiment 1:** Slices were free-floated in 1xPBS with 0.01% sodium azide and immunofluorescent staining was done for tyrosine hydroxylase (TH) (Rabbit anti-TH; Millipore; 1:2000), with secondary antibodies (Alexa Flour 568 goat anti-rabbit IgG, 1:400, Invitrogen) was done with normal goat serum and tissues were mounted on slides coated with Superfrost Plus and coverslipped with DAPI staining. Tissue was visualized using a fluorescent microscope using standard FITC and TRITC filters. TH was quantified by counting TH+ cell bodies in the VTA and axons in the prefrontal cortex for both 6-OHDA and control animals. **Experiment 2:** Guide cannulae locations were noted during histology, slices were mounted on slides coated with Superfrost Plus, and visualized under a microscope following DAPI staining. Their placement was translated to a coronal brain atlas.

**Data analysis:** All behavioral data were loaded into MATLAB for exploratory data analysis and plotting. Reaction times were compared via two-tailed t-tests between groups. For trial-by-trial analysis, reaction time data were loaded into R and a linear-mixed effects model with trials and animals as a random effect was performed.

**Results**

**Experiment 1: VTA dopamine depletion and temporal expectation**

To test the hypothesis that prefrontal dopamine influences temporal expectation, we depleted the source of prefrontal dopamine from the VTA using stereotaxic 6-OHDA injections into the VTA. These injections significantly decreased the number of TH+ cell bodies in the VTA ($t_{(12)}=2.78$, $p=0.02$; Fig. 2A-B). Notably, VTA dopamine depletion also significantly
decreased the number of axons stained with TH in the prefrontal cortex \( (t_{11}=3.31, p=0.007; \text{Fig. 2A-B}) \). These data suggest that we successfully depleted dopamine in mesocortical circuits.

To ensure that our dopamine depletions did not influence movement, we measured two movement parameters, open field and rotarod (data not shown). We found that while classic unilateral (right) median-forebrain-bundle dopamine depletions decreased movement \( (t_{18}=4.2, p=0.0006) \), depleting VTA dopamine had no effect on open-field movement \( (t_{12}=0.28, p=0.78; \text{median-forebrain bundle: } t_{12}=5.0, p=0.0003) \). Furthermore, we found that depleting VTA dopamine did not influence rotarod performance \( (t_{13}=-0.06, p=0.93) \). Lack of impairment on these gross measures of movement suggests that VTA dopamine depletion does not impair movement.

In addition, we investigated whether VTA dopamine signaling can influence motor learning and motivation in the learning rates of our animals. We used a 3-way analysis of variance modeling the effect of learning on task performance, trials, and reaction time as a function of VTA dopamine depletion (data not shown). There was a main effect of learning on performance \( (F_{(104)}=26.5, p<10^{-5}) \), rewards collected \( (F_{(104)}=16.1, p=0.0001) \), and reaction time \( (F_{(104)}=29.0, p<10^{-6}) \). We found that VTA dopamine depletion did not significantly interact with performance \( (F_{(104)}=0.01, p=0.76) \), rewards collected \( (F_{(104)}=1.2, p=0.28) \) or reaction time \( (F_{(104)}=0.8, p=0.37) \). These data suggest that VTA dopamine depletion does not impair learning or performance of the simple reaction time task, as animals with depleted VTA dopamine learned similarly when compared to controls.

We then investigated the hypothesis that mesocortical dopamine projections are involved in temporal expectation by testing animals with VTA dopamine depletion in a simple reaction time task with two delays. VTA dopamine-depleted animals had similar correct responses (Short:
77±2%, Long: 79±2% - mean±SEM; t_{(13)}=1, p=0.33; Fig. 3A) and similar overall reaction times when compared to controls (Short: 275±19 ms, Long: 259±17 ms; t_{(13)}=1.21, p=0.25; Fig. 3B). However, whereas control animals demonstrated robust delay-dependent speeding in their first session (Short: 312±27 ms, Long: 230±17 ms; paired t_{(7)}=4.37, p=0.003), animals with VTA dopamine depletion did not (Short: 300±25 ms, Long: 261±18 ms; paired t_{(7)}=1.62; p=0.16; Fig. 3B). Furthermore, a linear mixed effects model with animals and trials as random effects revealed that there was a main effect of delay length on reaction time (F_{(15,1496)}=5.69, p<10^{-10}) and no main effect of VTA dopamine depletion. Crucially, there was a significant interaction between VTA dopamine depletion, delay, and reaction time (F_{(15,1496)}=3.10, p<10^{-5}) suggesting that VTA dopamine depletion attenuates delay-dependent speeding and impairs temporal expectation.

Next, we trained the animals for 5 days to investigate if VTA dopamine depleted animals learned temporal expectancy. VTA dopamine depleted animals did not learn delay-dependent speeding with training (Short: 304±31 ms, Long: 260±27 ms; paired t_{(7)}=1.1; p=0.30), whereas control animals consistently demonstrated delay-dependent speeding (Short: 313±27 ms, Long: 223±15 ms; paired t_{(7)}=2.7; p=0.02). While VTA dopamine depleted animals learned to wait for an imperative stimulus in the simple reaction time task, they did not learn to speed their reaction times during longer delays. These data provide evidence that mesocortical dopamine signaling is required for temporal expectation.

To confirm that these effects were truly dopamine dependent, we administered levodopa (15 mg/kg intraperitoneal) to VTA dopamine-depleted animals. We found that levodopa administration to VTA dopamine-depleted animals restored delay-dependent speeding (Short: 294±21 ms, Long: 239±22 ms; paired t_{(6)}=3.8; p=0.009; linear mixed-effects interaction between
delay and levodopa: $F_{(7,1435)}=2.3$, $p=0.004$; Fig. 3C). Taken together, these data support the idea that VTA dopamine projections are necessary for temporal expectation during simple reaction time performance.

**Experiment 2: Prefrontal dopamine and temporal expectation**

As described above, VTA dopamine projections are involved in temporal expectation. The VTA projects to two major targets: 1) the medial prefrontal cortex, and 2) the nucleus accumbens. Previous work has demonstrated that dopamine disruption in the nucleus accumbens does not influence reaction time performance (1). However, the role of the VTA projection to the medial prefrontal cortex is unknown. In experiment 2 we tested the hypothesis that dopamine signaling within the medial prefrontal cortex is also necessary for temporal expectation.

We found that infusing saline into the prefrontal cortex did not appreciably influence behavior; animals performed at established high levels of accuracy (75±5%) and exhibited delay-dependent speeding (Short: 321±67 ms, Long: 274±29 ms; paired $t_{(7)}=2.4$, $p=0.04$). Animals infused with the D1-type dopamine receptor blocker SCH23390 into the prefrontal cortex retained accurate simple reaction-timing (10 mg/mL SCH23390; 77±7%; paired $t_{(5)}=0.3$, $p=0.81$; Fig. 4A); however, they did not exhibit delay-dependent speeding at low doses (0.5 μg SCH23390: Short: 288±37 ms, Long: 244±29 ms; paired $t_{(4)}=1.3$, $p=0.28$) or high doses (5 μg SCH23390: Short: 258±22 ms, Long: 271±16 ms; paired $t_{(4)}=0.5$, $p=0.68$; Fig. 4B). Interestingly, high doses of SCH23390 resulted in a slight speeding of reaction time at short delays (258±22 ms, 321±67 ms in saline sessions; paired $t_{(4)}=2.6$, $p=0.05$). At 5.0 μg of SCH23390, animals performed less trials (63±15 rewards, 45.7±14 rewards in saline sessions; paired $t_{(5)}=3.65$, $p=0.02$); however, at lower doses they performed a similar number of trials when compared to control sessions (0.5 μg SCH23390: paired $t_{(4)}=0.12$, $p=0.91$).
A linear mixed-effects model with trials and animals as random effects revealed main effects of SCH23390 dose ($F_{(5,662)}=3.4$, $p=0.004$) as well as delay ($F_{(5,662)}=3.2$, $p=0.007$) on reaction time. Notably, there was a significant interaction between dose and delay-dependent speeding ($F_{(5,662)}=4.0$, $p=0.001$; Fig. 4B-C). To confirm specificity of this effect, we infused a D2-type dopamine receptor blocker, sulpiride, and found no effect on temporal expectation. That is, animals with prefrontal D2 blockade had similar accuracy (73.5±9.6%) and exhibited delay-dependent speeding similar to saline sessions (7 animals; Short: 294 ±67 ms, Long: 274±29 ms; paired $t_{(6)}=2.61$, $p=0.04$). These data suggest that prefrontal D1 blockade attenuates reaction time delay-dependent. Combined with the results from experiment 1, this study provides specific evidence that mesocortical dopamine signaling is selectively involved in temporal expectation during simple reaction time performance.

**Discussion**

The present study examined the hypothesis that prefrontal dopamine signaling is involved in temporal expectation during simple reaction time performance. We tested this idea using neurotoxin-induced depletion of dopamine input from the VTA to the medial prefrontal cortex and local pharmacological blockade of prefrontal dopamine receptors. We report that VTA dopamine depletion specifically impaired delay-dependent speeding without affecting learning or performance of the simple reaction time task or motor behavior. Furthermore, we found that prefrontal D1 but not D2-type receptor blockade selectively impaired delay-dependent speeding. Taken together, these data provide novel evidence that prefrontal D1 dopamine signaling is involved in temporal expectation during simple reaction time tasks.

These findings are novel as the medial prefrontal cortex has been shown to be involved in temporal processing during a simple reaction time task (14), and prefrontal D1 dopamine
signaling is required perceptual timing (16). These results expand the scope of prefrontal D1 signaling in temporal processing to temporal expectation during a simple reaction time task (11; 13), and provide a pharmacological window into how prefrontal networks organize behavior in time.

Although the simple-reaction time task can be used to study temporal processing, in this study, temporal expectation is confounded with attention, arousal, and motor preparation (13; 14). As such, we cannot rule out a role for attention or motor preparation in our study. Future studies will independently manipulate temporal expectation using a cue to present stimuli at the same time but with different temporal expectation. Such studies require extensive training in rats, but could further illuminate the neural circuitry of temporal processing.

Prior work has consistently demonstrated that striatal dopamine signaling does not influence temporal expectation. For instance, patients with Parkinson’s disease have dramatically decreased striatal dopamine and impaired response initiation (5). These patients tend to exhibit temporal expectation and delay-dependent speeding despite having slow overall reaction times. Multiple studies have demonstrated that blocking dopamine signaling within the nucleus accumbens does not influence reaction time performance or delay-dependent speeding (1; 8). Although the VTA sends strong dopaminergic projections to the nucleus accumbens (15), these studies suggest that this projection is not consistently involved reaction time performance or temporal expectation. Importantly, a subset of patients with Parkinson’s disease have impaired executive function at incident diagnosis and likely have impaired prefrontal dopamine signaling (17). Our data lead to the hypothesis that only those patients with executive dysfunction would have impaired temporal expectation.
In our study, neither dopamine manipulation (SCH23390 or sulpiride) increased premature responding or influenced waiting. Our results are quite distinct from prefrontal inactivation (14) and instead suggest that prefrontal D1 signaling is not involved in impulsivity or inhibitory control (9). We did find a specific effect of prefrontal D1 signaling on temporal expectation. In light of previous research, these data suggest that prefrontal networks responsive to D1 dopamine signaling may inhibit reaction times only during a temporally relevant window. Although there was no difference in overall reaction time following VTA dopamine depletion, animals with prefrontal D1 dopamine blockade were marginally faster, from which we infer that prefrontal influence might be inhibitory (Fig. 4B-C). A possibility for differences in reaction times between VTA dopamine depletion and prefrontal dopamine blockade could include cortical reorganization as seen in lesion studies (2). VTA dopamine depletions should affect all targets of the VTA, which include other cortical regions. A final possibility is that additional areas, such as the premotor cortex, may be affected by VTA dopamine depletion and influence reaction time performance. Future studies could systematically deplete dopamine in several motor regions to explore this issue.

Prefrontal D1 signaling has traditionally been associated with high level cognitive behaviors, such as working memory, risk-based decision making, and attention (6; 7). The present study supports previous data implicating prefrontal D1 signaling in organizing goal-directed behavior (6). Yet, our data are the first to link prefrontal D1 signaling to a simple reaction time task, supporting the idea that performance on this task can benefit from cognitive judgment of time.
Figures

**Figure 1:** (A) Simple reaction time task, where animals were trained to press and hold a lever until the onset of a tone (stimulus) at which point a lever release was rewarded with water (correct release). (B) Schematic representation of delay-dependent speeding measured by reaction time (thick black line).

**Figure 2:** (A) Representative images of TH+ (red) cells in the VTA (10X) and axons in the prefrontal cortex (PFC) (40X) in controls and VTA dopamine depleted animals (VTA 6OHDA). (B) VTA-6OHDA significantly decreased TH+ cells in the VTA and the number of TH+ projection axons in the prefrontal cortex (PFC) when compared with controls. * p<0.05.
Figure 3: While VTA dopamine depletion did not change accuracy (A), control animals developed delay-dependent speeding and (B), animals with VTA dopamine depletion did not. (C) Levodopa administration in VTA dopamine-depleted animals restored delay-dependent speeding. * p<0.05; + significant interaction between dopamine depletion and delay-length in a linear random-effects model.

Figure 4: (A) There was no effect on accuracy following saline infusions, D1 1 mg/ml, D1 10mg/ml, and D2 1 mg/ml. (B) Following saline (black line) and sulpiride (dotted line), animals exhibited delay-dependent speeding as shown by faster reaction times (ms) during the long delay period (long) when compared to the short delay (short). However, following D1 (gray lines), animals had slower reaction times during the longer delays. (C) D1 blockade via SCH23390 attenuated the slope of delay-dependent slope while D2 blockade via sulpiride did not. * p<0.05; + significant interaction between SCH23390 dose and delay-length in a linear random-effects model.
References