Cognitive, Affective, & Behavioral Neuroscience (2024) 24:304–321
https://doi.org/10.3758/s13415-023-01139-8

SPECIAL ISSUE/PRECLINICAL ASSAYS

Dopaminergic modulation of sensitivity to immediate and delayed punishment during decision-making

Grace L. Minnes[1] · Anna J. Wiener[1] · Anna E. Liley[1] · Nicholas W. Simon[1]

Accepted: 1 November 2023 / Published online: 5 December 2023

Abstract

Effective decision-making involves careful consideration of all rewarding and aversive outcomes. Importantly, negative outcomes often occur later in time, leading to underestimation, or "discounting," of these consequences. Despite the frequent occurrence of delayed outcomes, little is known about the neurobiology underlying sensitivity to delayed punishment during decision-making. The Delayed Punishment Decision-making Task (DPDT) addresses this by assessing sensitivity to delayed versus immediate punishment in rats. Rats initially avoid punished reinforcers, then select this option more frequently when delay precedes punishment. We used DPDT to examine effects of acute systemic administration of catecholaminergic drugs on sensitivity to delayed punishment in male and female adult rats. Cocaine did not affect choice of rewards with immediate punishment but caused a dose-dependent reduction in choice of delayed punishment. Neither activation nor blockade of D1-like dopamine receptor affected decision-making, but activation of D2-like dopamine receptors reduced choice of delayed punishment. D2 blockade did not attenuate cocaine's effects on decision-making, suggesting that cocaine's effects are not dependent on D2 receptor activation. Increasing synaptic norepinephrine via atomoxetine also reduced choice of delayed (but not immediate) punishment. Notably, when DPDT was modified from ascending to descending pre-punishment delays, these drugs did not affect choice of delayed or immediate punishment, although high-dose quinpirole impaired behavioral flexibility. In summary, sensitivity to delayed punishment is regulated by both dopamine and norepinephrine transmission in task-specific fashion. Understanding the neurochemical modulation of decision-making with delayed punishment is a critical step toward treating disorders characterized by aberrant sensitivity to negative consequences.

Keywords Dopamine · Decision-making · Punishment · Delay discounting · Norepinephrine

Abbreviations

DPDT	Delayed Punishment Decision-making Task
SUD	Substance Use Disorder
ANOVA	Analysis of Variance
IP	Intraperitoneal
OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex
BLA	Basolateral Amygdala
NAcc	Nucleus Accumbens
ITI	Intertrial Interval

This work was supported by R15DA046797 (NWS). The authors have no conflicts of interest to disclose.

✉ Nicholas W. Simon
nwsimon@memphis.edu

[1] Department of Psychology, University of Memphis, Memphis, TN, USA

Introduction

Effective decision making requires assessment and integration of the costs and benefits associated with each option. The ability to evaluate negative outcomes of a decision is impaired in several psychiatric illnesses, with punishment carrying less salience in substance use disorder (SUD) and excessive salience in affective disorders, such as major depressive disorder (Jean-Richard-Dit-Bressel et al., 2018; Nestadt et al., 2016; Pushkarskaya et al., 2015; Starcke et al., 2010). Accordingly, investigating the neurobiology of cost/benefit decision-making has been a staple of preclinical research. However, an aspect of cost/benefit decision-making that is often overlooked is that punishment does not always manifest immediately after a choice, instead occurring after a time delay. Delays reduce the salience of an impending outcome during decision-making, a phenomenon known as "delay discounting" (Fischhoff & Broomell, 2020; Murphy et al., 2001; Shead & Hodgins, 2009). Previous

research has focused primarily on the neurobiology of discounting delayed rewards (Cardinal, 2006; de Whit et al., 2002; Owens et al., 2019; Winstanley et al., 2004), with minimal investigation of discounting of delayed punishment. Furthermore, preclinical research on punishment has almost exclusively used punishment that occurs immediately after a choice (Jacobs & Moghaddam, 2020; Jean-Richard-Dit-Bressel et al., 2018; Simon et al., 2009). Understanding how the brain engenders discounting of delayed punishment is a significant gap in the literature that must be addressed to enable behavioral and biological treatments for maladaptive decision-making.

There are relatively few preclinical models that capture sensitivity to delayed punishment (Rodríguez et al., 2018, González-Barriga & Orduña, 2022, Woolverton et al., 2012, and Zech et al., 2022), and little is known about the neuronal and pharmacological substrates of delayed punishment discounting. We developed the Delayed Punishment Decision-making Task (DPDT; Liley et al., 2019), which was similar to the temporal discounting of shock procedure reported previously by Rodríguez et al. (2018). During DPDT, rats choose between a single-pellet reward and a three-pellet reward accompanied by a mild foot shock punishment (Liley et al., 2019; Orsini & Simon, 2020). As the session progresses, a delay is inserted between the larger reward and shock that systematically increases throughout the session (0, 4, 8, 12, and 16 s). As predicted, rats favor the smaller, safe reward when punishment is immediate but shift preference toward the larger punished reward when shock is delayed. This preference for delayed over immediate punishment indicates that rats discount the negative motivational value of delayed punishment. Liley et al. (2019) determined that males are more likely than females to choose options associated with delayed (but not immediate) punishment, indicative of greater delay discounting of punishment. Finally, delay discounting of punishment is not correlated with discounting of delayed rewards, suggesting that these discounting processes may rely on separate neural mechanisms.

The neurochemical mechanisms underlying sensitivity to delayed punishment remain unclear; however, there is evidence that this aspect of cognition is regulated by dopamine transmission. Dopamine is a neurotransmitter involved with motivation, learning, and cost/benefit decision-making (Floresco & Magyar, 2006; Robbins, 2003). In addition to reinforcing and psychostimulant properties, dopaminergic drugs modulate cost/benefit decision making (Hori et al., 2021; Phillips et al., 2007; Westbrook et al., 2020). Acute cocaine exposure reduces choice of large, delayed rewards, indicative of increased reward delay discounting (Evenden & Ryan, 1996; Smethells & Carroll, 2015). During punishment-based risky decision-making, cocaine causes inflexibility in response to changes in punishment risk (Simon et al., 2009). Amphetamine, another drug that increases synaptic dopamine (dela Peña et al., 2015; Faraone, 2018), exerts effects on decision-making that differ from cocaine. Acute amphetamine exposure reduced risky decision making in both punishment and probabilistic based risk-taking tasks (Simon et al., 2011; Simon et al., 2009; Zeeb et al., 2009). Interestingly, amphetamine reduced risky choices in the rat gambling task (Zeeb et al., 2009) but increased risky choices in the probabilistic risky decision-making task (St Onge & Floresco, 2009). Additionally, amphetamine use was shown to decrease discounting of delayed rewards, as well as show a decrease in impulsivity in both humans (de Wit et al., 2002; Pattij & Vanderschuren, 2008) and rat models (Cardinal et al., 2000; Pattij & Vanderschuren, 2008). Critically, the decision-making constructs affected by dopaminergic drugs across these tasks are two critical components of DPDT: delayed outcomes, and reward/punishment conflict. Therefore, it is probable that dopamine regulates sensitivity to delayed punishment during decision-making.

While substances, such as cocaine and amphetamine, enable investigation of the effects of broad dopamine receptor activation, there are multiple dopamine receptor subtypes. Dopamine receptors are divided into two categories: D1-like excitatory receptors, and D2-like inhibitory receptors (Beaulieu et al., 2015; Mishra et al., 2018). There is convincing evidence that D1 and D2 receptors subserve different functions in cost/benefit decision-making. Both activation and blockade of the D1 receptor appears to have no effect on risky decision-making (Oinio et al., 2017; Simon et al., 2011, Zeeb et al., 2009), whereas D2 receptor activation attenuates risky decision-making (Blaes et al., 2018; Mitchell et al., 2014; Simon et al., 2011). D2 receptor blockade had no effect on risky decision-making (Blaes et al., 2018; Simon et al., 2011).

Investigation of the role of D1 and D2 receptors in the discounting of delayed rewards has yielded conflicting results. Early research found that blockade of the D1 receptor did not affect delay discounting (Wade et al., 2000). In contrast, more recent studies observed that blockade of the D1 receptor resulted in reduced choice of delayed rewards, reflective of increased discounting (Koffarnus et al., 2011; Li et al., 2015). However, these differing results may be due to procedural and methodological differences. While D1 receptor activation decreased delay discounting in humans (Soutschek et al., 2020), it did not affect preference for delayed gratification in rats, although it did appear to impair reward magnitude discrimination (Koffarnus et al., 2011). Additionally, while blockade of the D2 receptor was found to increase delay discounting (Wade et al., 2000), it was more recently found that D2 receptor blockade did not lead to any change in behavior (Li et al., 2015). A comparable lack of effect was observed with D2 receptor activation not affecting delay discounting (Castrellon et al., 2021; Koffarnus et al., 2011).

Therefore, while results vary based on task parameters, both D1 and D2 receptors seem to be involved with reward delay discounting, suggesting that they also may modulate sensitivity to delayed punishment.

The current study was the first to explore whether sensitivity to delayed punishment during cost/benefit decision-making is regulated by dopamine receptor activation. We first assessed effects of multiple doses of acute systemic cocaine on sensitivity to delayed punishment during DPDT. We then observed effects of D1 and D2 receptor agonists and antagonists on DPDT performance. Cocaine and a D2 receptor agonist both reduced choice of delayed (but nor immediate) punishment; to determine if cocaine's effects were dependent on D2 activation, we next co-administered cocaine and a D2 antagonist. Finally, we tested the acute effects of atomoxetine, a norepinephrine transporter inhibitor, on decision-making in DPDT.

Methods

Subjects

Male (n = 9) and female (n = 9), Long-Evans rats, aged ~100 days old, obtained from Envigo were used for all drug experiments. All rats were individually housed in a 12-hr reverse light/dark cycle, and food was restricted to 85% free feeding weight to increase motivation and task engagement.

Apparatus

Testing was conducted in standard rat behavioral test chambers (Med Associates) housed within sound attenuating cubicles. Each chamber was equipped with a recessed food pellet delivery trough fitted with a photo beam to detect head entries and a 1.12-W lamp to illuminate the food trough. Sucrose food pellets were delivered into the food trough, 2 cm above the floor centered in the side wall. Two retractable levers were located on the left and right side of the food trough, 11 cm above the floor, with cue lights located directly above each lever. A 1.12-W house light was mounted on the opposing side wall of the chamber. Beneath the house light was a circular nose-poke port equipped with a light and photo beam to detect entry. The floor of the test chamber was composed of steel rods connected to a shock generator that delivered scrambled foot shocks. Locomotor activity was assessed throughout each session with infrared activity monitors located on either side of the chamber just above the floor. Test chambers were interfaced with a computer running custom-written codes through MedPC software (Med Associates), which controlled all external cues and behavioral events.

Behavior

Shaping

Rats were first shaped to perform the basic components of DPDT. In the first behavior-shaping sessions (magazine training), rats learned to associate the sound of food delivery with food availability in the food trough in a single session. Following magazine training, rats were trained to press levers for the delivery of a single food pellet, which was accompanied by the illumination of the light in the food trough**.** After learning lever pressing, rats learned to initiate trials with a nosepoke. The nosepoke resulted in the extension of either lever. By pressing on the extended lever, a single food pellet was delivered. The final task was reward discrimination training, in which rats learned that a pressing on one lever yielded a large (3 pellets) food reward, whereas a press on the other lever yielded a small (1 pellet) food reward. See Orsini & Simon (2020) for detailed shaping procedures.

Delayed Punishment Decision-making Task (DPDT)

The delayed punishment task measures the influence of delayed versus immediate punishment on reward magnitude-based decision-making. Rats chose between one and three food pellet reinforcers; the larger option accompanied by a foot shock that occurs systematically later in time as the task progresses. Sessions consisted of six blocks with 12 trials each. Each trial began with illumination of the house light and food trough, after which rats were required to nose poke into the lit trough within a 10-s period to initiate the trial. A nose poke extinguished the trough light and then caused either a single lever or two levers on both sides of the trough to extend. The first two trials of each block were forced choice trials, with only a single lever available to establish the reward/punishment parameters of each lever individually within the current block. After forced choice trials, the following ten trials were free-choice trials in which both levers extend simultaneously, allowing rats to choose a preferred lever/reinforcement schedule.

The choice of one lever resulted in immediate delivery of a single pellet, and the other caused immediate delivery of three pellets (spaced out over a 3-s period), in addition to a mild foot shock. Identity of levers (left vs. right) were fixed across all sessions and counterbalanced between subjects. During the first block, the shock occurred immediately after lever press; the blocks following had a delay preceding shock that was progressively extended to 4, 8, 12, and 16 s across blocks. If the unpunished lever was chosen, the intertrial interval (ITI) was increased by a period equivalent to the delay preceding shock (4, 8, 12, or 16 s) to maintain consistency of trial length regardless of choice. After food

delivery, delay, and shock (when large reward was chosen), the house light extinguished, and an ITI of 10 ± 2 s preceded the next trial. Figure 1 displays the progression of a single DPDT free-choice trial. If rats did not choose an extended lever within the allotted 10 s, the trial was marked as an omission and was followed by the ITI. After completion of all five blocks, rats performed a sixth block in which the large reward was no longer followed by a foot shock to confirm a preference for the large reward in the absence of punishment (see Fig. 1 for a full schematic of the task).

Foot shock amplitude began at 0.1 mA and increased by 0.03 mA in the following session if rats completed >85% of trails. This incremental increase in shock intensity limited omissions and allowed all rats to acquire task parameters. Final shock intensity varied for each rat—determined by lack of omissions and a positive sloping curve shown by their individual data. Once final shock intensity was finalized, subjects trained for a minimum of 20 consecutive sessions, or until stable choice performance was achieved, defined as no significance in a repeated-measures day by block analysis of variance (ANOVA) over the final 5 days of behavior. Rats that were unable to achieve stability or consistently displayed excessive omissions were removed from the experiment.

A separate group of drug naïve rats (n = 10; 6 females, 4 males) also were tested on a reversed version of DPDT (RevDPDT) to determine whether the behavioral effects of quinpirole, cocaine, or atomoxetine were task-specific or altered behavioral flexibility. RevDPDT was comparable to DPDT, except the blocks were presented in descending order (no shock, 16-, 12-, 8-, 4-s delay preceding punishment).

Shock threshold testing

To determine whether quinpirole exposure caused an overall increase in shock sensitivity (leading to reduced choice of punished reward), a subset of rats (n = 10, 6 females/4 males) performed shock threshold testing modified from Jacobs and Moghaddam (2020). In brief, rats were placed into an operant chamber with a shock-generating grating, then scrambled foot shocks were presented for 5-s intervals. The shock intensity began at 0.05 mA and was increased 0.02 mA every 10 s. The session culminated when the rat physically responded to the shock, defined as movement of all four limbs or vocalization. The shock intensity that elicited this response was labeled the shock threshold.

Drug injection schedules

Drug treatments were administered via intraperitoneal (IP) injection over an 8-day testing period, with the injections alternating every other day (Fig. 2). Injections were performed before running DPDT, depending on the drug (see Table 1 for doses and absorption times). Drugs were each administered at an injection volume of 1 ml/kg based on weight taken the morning of injections. Each experiment had its own four injection dosages that were counterbalanced throughout the 8-day injection period. Testing took place 10–30 minutes after injections (Table 1), which was sufficient to allow drug absorption while producing effects on behavior in tasks of comparable length (St Onge & Floresco, 2009; Simon et al., 2009, 2011).

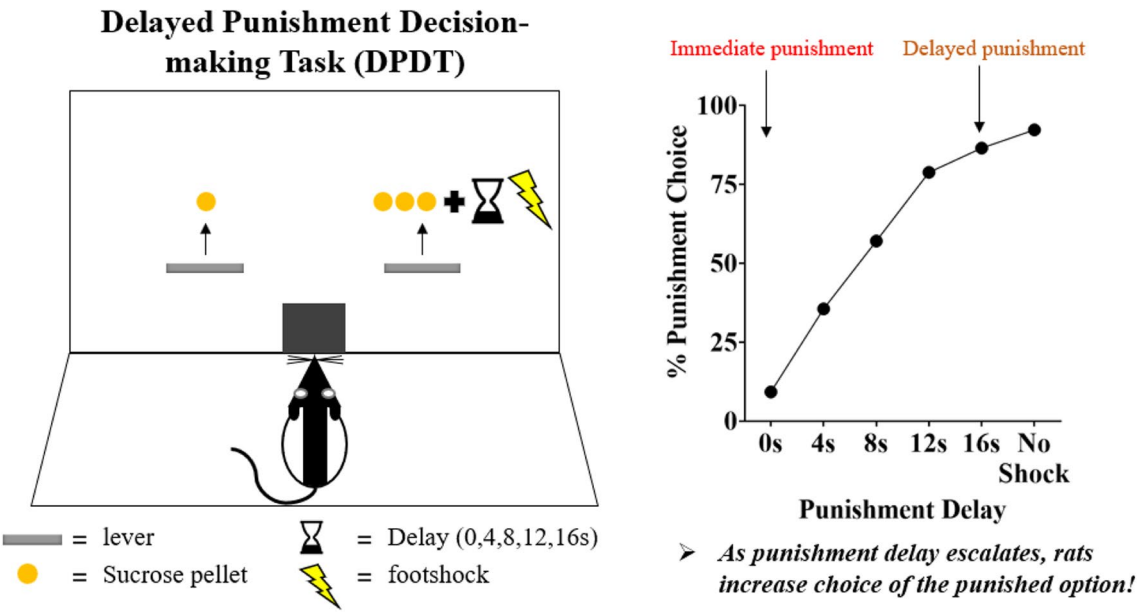

Fig 1 DPDT schematic and baseline curve. Location of task stimuli (left panel). Pattern of choice typically observed during DPDT (right panel)

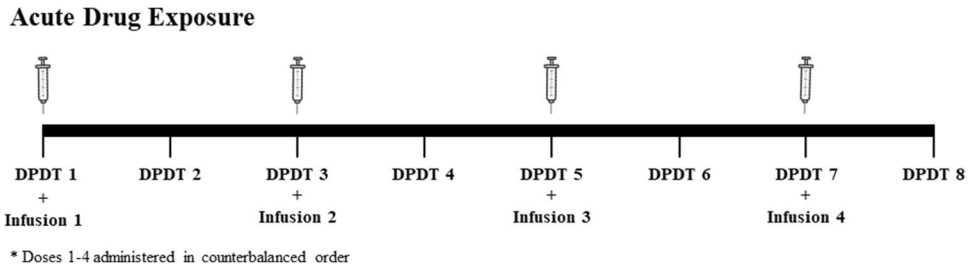

Fig. 2 Acute drug treatment protocol. Intraperitoneal injections were given before DPDT every other day over an 8-day period (represented by the syringes above). Drug-free baseline DPDT sessions occurred between drug sessions to confirm that behavior remained stable throughout the protocol. Doses 1–4 were administered in counterbalanced order for each drug

Table 1 Summary of acute pharmacology experiments. Quinpirole (saline/mid/high; 6 days), cocaine (saline/high; 4 days), and atomoxetine (saline/high; 4 days) were retested with abbreviated schedules in RevDPDT

Drug	Injection dose	Absorption time (before testing)
Acute cocaine	Saline Low (5 mg/kg) Mid (10 mg/kg) High (15 mg/kg)	15 min
SCH23390	Saline Low (.005 mg/kg) Mid (0.01 mg/kg) High (0.03 mg/kg)	20 min
SKF81297	Saline Low (0.1 mg/kg) Mid (0.3 mg/kg) High (1.0 mg/kg)	15 min
Eticlopride	Saline Low (0.01 mg/kg) Mid (0.03 mg/kg)	15 min
Quinpirole	High (0.05 mg/kg) Saline Low (0.0375 mg/kg) Mid (0.125 mg/kg) High (0.25 mg/kg)	10 min
Eticlopride + cocaine	Saline – Saline Saline – Eticlopride Cocaine – Saline Cocaine – Eticlopride Saline	15 min
Atomoxetine	Low (0.03 mg/kg) Mid (1.00 mg/kg) High (3.00 mg/kg)	30 min

Data Analysis

To minimize the effects of trial omissions on decision-making, punished reward choice was measured as % choice of the large reinforcer from all completed trials in each individual block (i.e., a block with 6 omissions and 4 completed trials consisting of 2 punished and 2 safe choices would be scored as 50% punished reward choice). The effects of drug dose on choice during DPDT and RevDPDT for all drug schedules were analyzed using a sex × drug × delay mixed ANOVA. Trial omissions during DPDT were analyzed using a sex × drug × delay mixed ANOVA. Locomotor activity during DPDT was analyzed by using a sex × delay × drug mixed ANOVA. Shock threshold has analyzed using a sex X drug mixed ANOVA.

Results

Effects of acute cocaine on sensitivity to delayed punishment during DPDT

After acquisition of DPDT, rats (n = 18; 9F/9M) were administered saline or cocaine (5, 10, 15 mg/kg) via IP injection before testing. Each rat received all four doses across an 8-day counterbalanced schedule. One female was not included in data analyses because of excessive omissions. First, we assessed choice of punished rewards as a function of punishment delay to confirm that rats discounted delayed punishment. As expected, there was a significant effect of delay ($F(1.789, 26.835) = 38.431$, $p < .001$, establishing that rats chose the punished lever more frequently as punishment delay increased (Fig. 3a). We next determined cocaine effects on choice of the punished reward. A significant drug effect was obtained ($F(3, 45) = 7.007$, $p = .001$), indicating that acute cocaine reduced overall punished choice (Fig. 3a). Additionally, there was a significant drug × delay interaction ($F(5.210, 78.154) = 3.431$, $p = .007$), revealing that cocaine did not affect choice when punishment was immediate but reduced punished choice when punishment was delayed. There also was a significant sex difference ($F(1, 15) = 16.068$, $p = .001$), such that females chose the punished option less frequently than males (Fig. 3b-c). There was no significant drug × sex interaction ($F(3, 45) = 2.307$, $p = .089$) or sex × delay × drug interaction ($F(15, 78.154) = 1.260$, $p = .229$). In summary, acute cocaine caused a dose-dependent reduction in choice of rewards associated

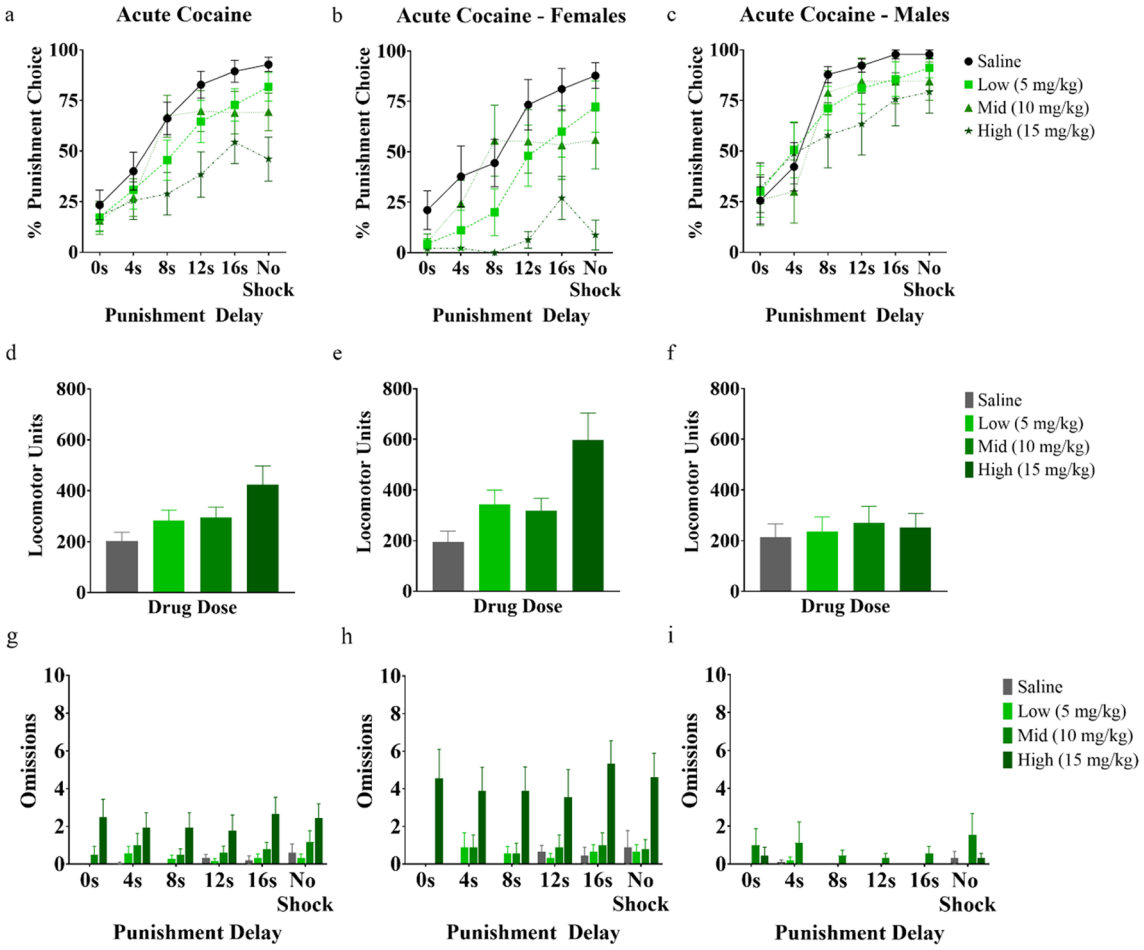

Fig. 3 Effects of cocaine on decision-making, locomotion, and omissions during DPDT. Cocaine caused a dose-dependent reduction in choice of the delayed (but not immediate) punishment (**a**). This pattern was observed in both female (**b**) and male (**c**) rats. Cocaine caused a dose-dependent increase in total locomotor units, with the highest dose causing the greatest increase in locomotion (**d**). This effect was more pronounced in females (**e**) than males (**f**). Cocaine increased omissions across the session (**g**). This effect was more prominent in females (**h**) than males (**i**). Data are depicted as mean ± standard error of the mean

with delayed but not immediate punishment in both male and female rats.

Next, we assessed the effects of acute cocaine on locomotion during DPDT. As expected, cocaine increased locomotor activity ($F(1.727, 24.173) = 8.544$, $p = .002$; Fig. 3d). There was no significant sex difference in cocaine induced locomotion ($F(1, 14) = 3.005$, $p = .105$; Fig. 3e-f). However, there was also a significant drug × sex interaction ($F(3, 24.173) = 6.407$, $p = .001$), indicating that the locomotor-activating effects of cocaine (particularly at high dose of 15 mg/kg) were greater in females than males.

Finally, we assessed the effects of acute cocaine on trial omissions. There was no effect of delay ($F(1.740, 27.844) = 1.100$, $p = .339$) or drug × delay interaction ($F(80, 240) = .754$, $p = .727$), indicating that rate of omissions was consistent across all delays (Fig. 3g). There was a significant drug effect ($F(1.956, 31.301) = 10.051$, $p < .001$, indicating that acute cocaine increased omitted trials. There also was a significant sex difference ($F(1, 16) = 6.471$, $p = .022$), such that females omitted more trials than their male counterparts (Fig. 3h-i). A significant drug × sex interaction was also detected ($F(3, 31.301) = 11.570$, $p < .001$), with cocaine increasing omissions in females more than males, particularly at the high dose (15 mg/kg). There was no sex × delay × drug interaction ($F(15, 240) = .634$, $p = .845$).

Effects of D1-like receptor manipulation on sensitivity to delayed punishment during DPDT

We next investigated the role of D1-like receptors in decision-making with delayed punishment by testing the effects of the agonist SKF81297 and the antagonist SCH23390 on DPDT. Rats (n = 10; 5 females/5 males) were administered saline, SKF81297(0.1, 0.3, 1.0 mg/kg), or SCH23390 (0.005, 0.01, 0.03 mg/kg) via IP injection before testing. For each drug, each rat received all four doses across an 8-day

counterbalanced schedule. One male and one female were not included in final analyses for SKF81297 due to excessive omissions. Following SKF81297 exposure, there was a significant effect of delay ($F(1.634, 9.806) = 31.775$, $p < .001$) with rats choosing the punished lever more frequently with increased punishment delay (Fig. 4a). There was no drug effect ($F(3, 9.806) = .505$, $p = .684$) or drug × delay interaction ($F(15, 90) = .983$, $p = .479$), meaning that D1 activation did not affect choice of delayed or immediate punishment. There also was no significant sex difference ($F(1, 6) = .947$, $p = .368$), drug × sex interaction ($F(3, 18) = .303$, $p = .823$), or sex × delay × drug interaction ($F(15, 90) = .622$, $p = .850$; Fig. 4b-c). Finally, there were no significant effects of drug or sex on locomotion (Fig. 4d-f) or omissions ($ps > .141$; Fig. 4g-i).

During SCH23390 administration, there was again a significant effect of delay ($F(2.140, 17.122) = 18.982$, $p < .001$), such that rats chose the punished lever more frequently as delay increased (Fig. 5a). As with the D1 agonist, there was no effect of drug ($F(3, 17.122) = .240$, $p = .868$) and no significant drug × delay interaction ($F(15, 120) = .830$, $p = .643$). There was no significant sex difference ($F(1, 8) = .218$, $p = .658$), drug × sex effect was shown ($F(3, 24) = .138$, $p = .936$), or sex × delay × drug interaction ($F(15, 120) = .917$, $p = .547$) (Fig. 5b-c). There were no significant effects of drug or sex on locomotion (Fig. 5d-f) or omissions (Fig. 5g-i), although there was a near significant drug × delay interaction for omissions ($F(15, 120) = 1.744$, $p = .051$), such that high dose SCH23390 (0.03 mg/kg) increased omissions across the session. In summary, neither activation nor blockade of D1-like receptors affected choice of delayed or immediate punishment.

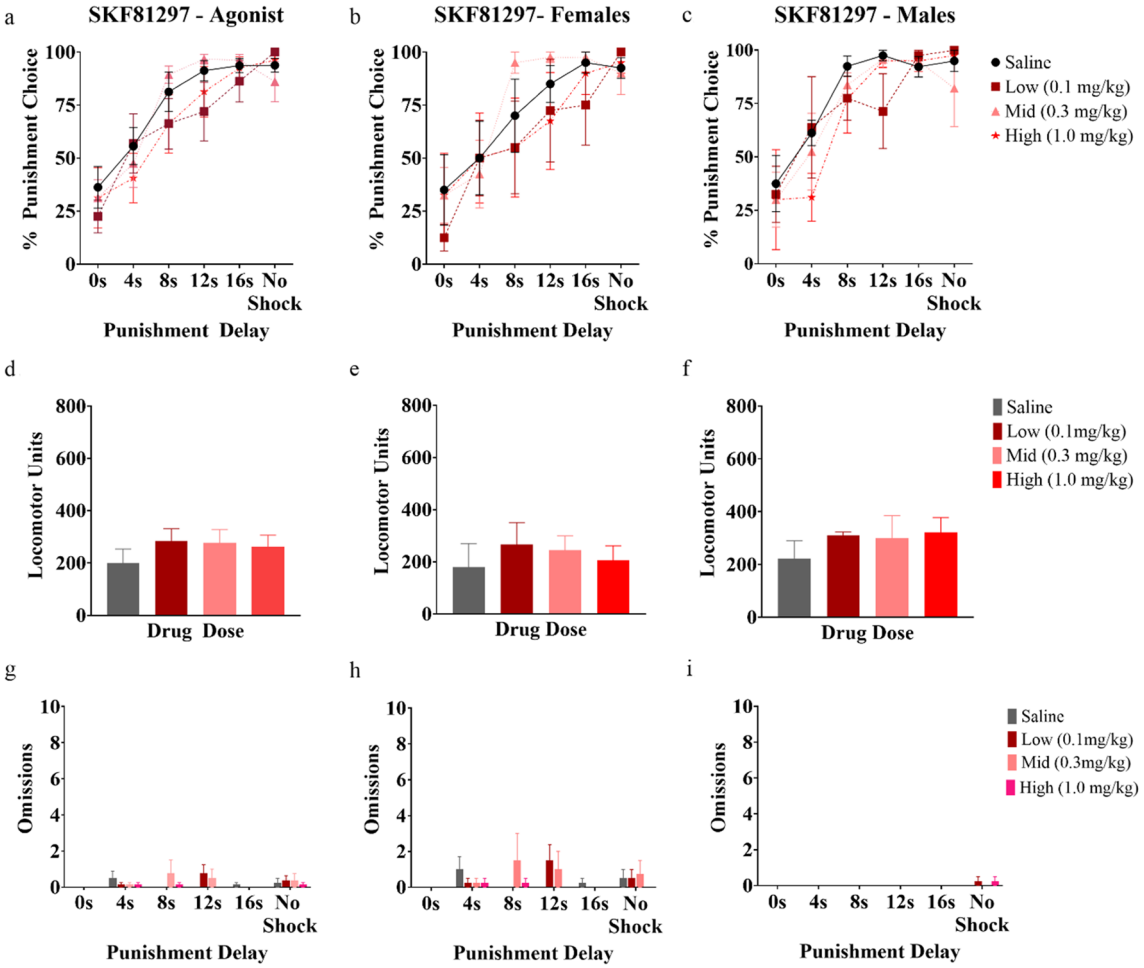

Fig. 4 Effects of SKF81297 on decision-making, locomotion, and omissions during DPDT. SKF81297 did not lead to changes in choice of the delayed (or immediate) punishment (**a**). This was observed in both female (**b**) and male (**c**) rats. SKF81297 did not lead to a difference in locomotor units (**d**). There was no difference in locomotion between females (**e**) or males (**f**). SKF81297 did not lead to a difference in omitted trials (**g**). There were no differences between females (**h**) or males (**i**). Data are depicted as mean ± standard error of the mean

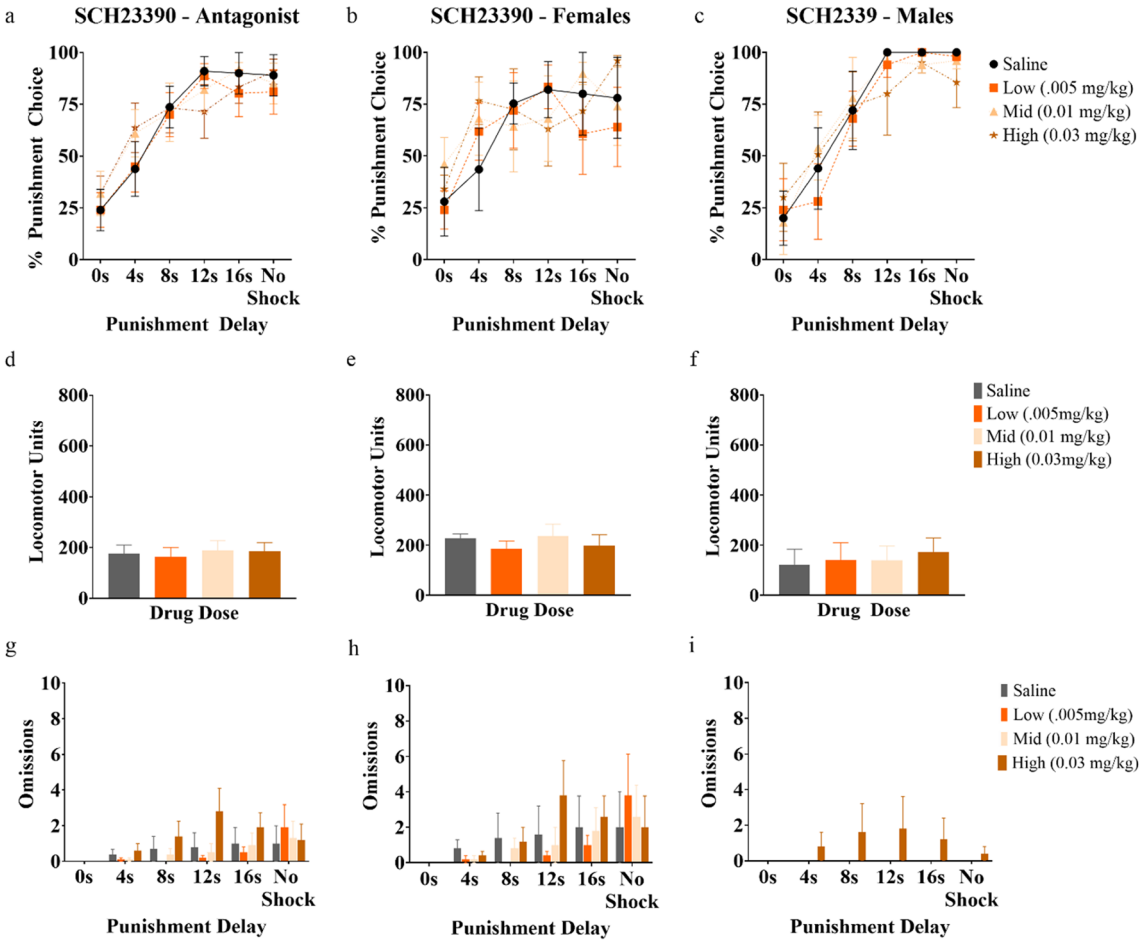

Fig. 5 Effects of SCH23390 on decision-making, locomotion, and omissions during DPDT. SCH23390 did not lead to changes in choice of the delayed (or immediate) punishment (**a**). This was observed in both female (**b**) and male (**c**) rats. SCH23390 did not lead to a difference in locomotor units (**d**). There was no difference in locomotion between females (**e**) or males (**f**). SCH23390 did not lead to differences in omissions (**g**). There were no differences between females (**h**) or males (**i**). Data are depicted as mean ± standard error of the mean

Effects of D2-receptor manipulation on sensitivity to delayed punishment during DPDT

Next, we investigated the effects of D2-like agonist quinpirole and the antagonist eticlopride on decision-making in DPDT. Rats (n = 18; 9 females/9 males) were administered saline, quinpirole (0.0375, 0.125, 0.25 mg/kg), or eticlopride (0.01, 0.03, 0.05 mg/kg) via IP injection before testing. For each drug, each rat received all four doses across an 8-day counterbalanced schedule. One female and one male were not included in final analyses for quinpirole because of excessive omission. One female and three males were not included in final analyses for eticlopride because of excessive omissions. Just as previously, there was a significant effect of delay ($F(2.007, 24.080) = 17.721$, $p < .001$), such that rats chose the punished lever more frequently as delay increased (Fig. 6a). There was an effect of quinpirole exposure ($F(3, 24.080) = 17.723$, $p < .001$), revealing that acute D2 receptor activation reduced punished choice (Fig. 6a). Additionally, there was a significant drug × delay interaction ($F(15, 180) = 5.803$, $p < .001$), such that D2 activation did not affect choice of the punished reward when punishment was immediate but reduced punishment lever choice when punishment was delayed (Fig. 6a). There was no significant sex difference ($F(1, 12) = .259$, $p = .620$), drug x sex interaction ($F(3, 36) = 1.460$, $p = .242$), or sex × delay × drug interaction ($F(15, 180) = 1.554$, $p = .091$). However, there was a significant delay × sex interaction ($F(15, 180) = 5.803$, $p < .001$), such that males and females showed comparable choice of the large reward with immediate punishment, but females chose this option less frequently with delayed punishment (Fig. 6b-c). In summary, D2 receptor activation caused a dose-dependent reduction in choice of delayed but not immediate punishment.

There was no effect of drug or sex on locomotion (*p*s > .301; Fig. 6d-f). However, there was a near significant drug

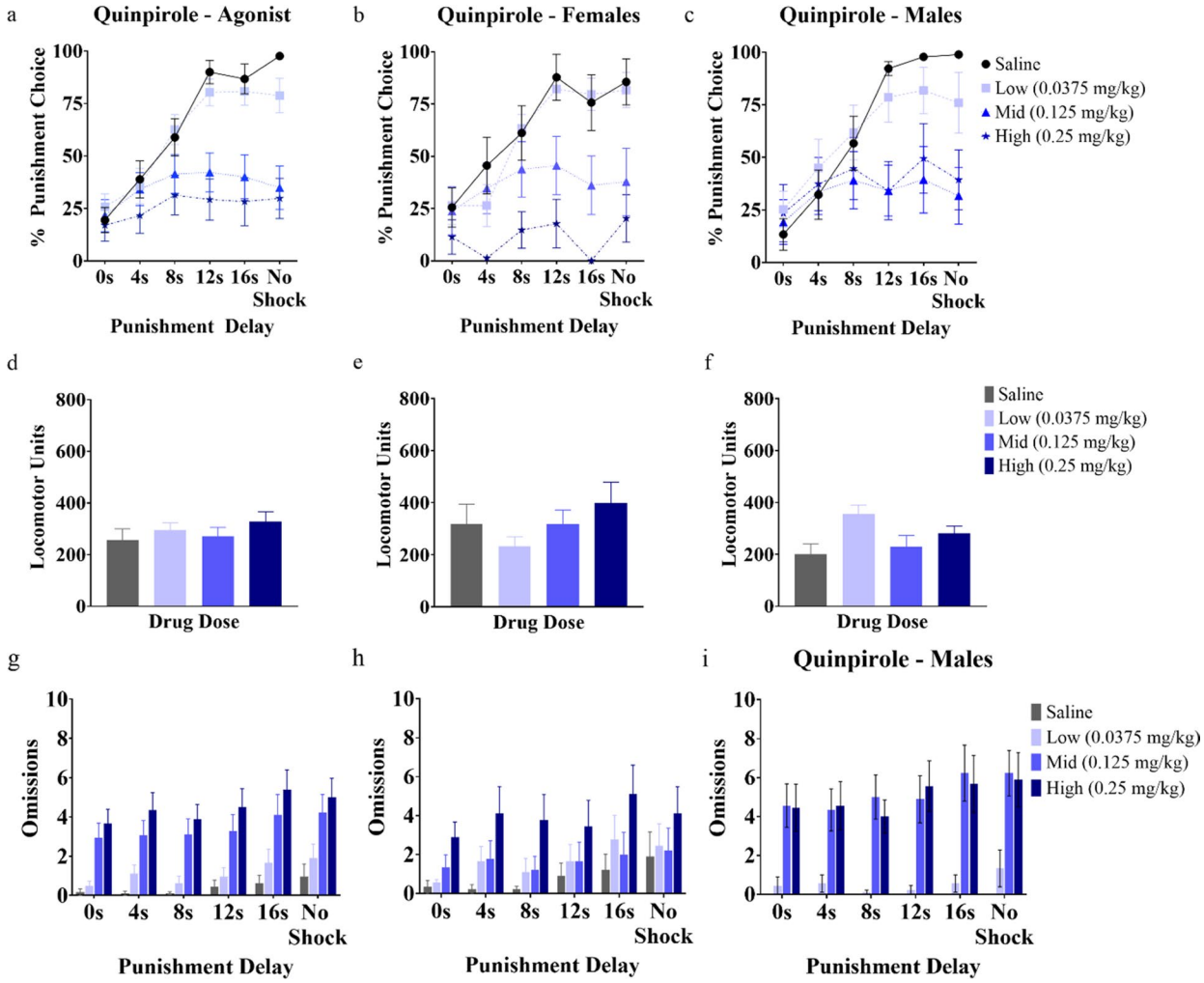

Fig. 6 Effects of quinpirole on decision-making, locomotion, and omissions during DPDT. Quinpirole caused a dose-dependent reduction in choice of the delayed (but not immediate) punishment (**a**). This pattern was observed in both female (**b**) and male (**c**) rats. Quinpirole caused a drug-dependent increase in total locomotor units; all doses of quinpirole caused an increase in locomotion compared to saline (**d**). This effect was not pronounced in females (**e**) but was seen in males (**f**). Quinpirole increased omissions across the session (**g**). This effect was more prominent in males (**h**) than females (**i**). Data are depicted as mean ± standard error of the mean

x sex interaction ($F(3, 36) = 2.644$, $p = .064$), such that low dose quinpirole reduced movement in females and increased it in males. There was a significant drug effect on omissions ($F(2.300, 36.803) = 16.502$, $p < .001$), indicating that quinpirole reduced completed trials (Fig. 6g). There also was a significant effect of delay on omissions ($F(2.977, 47.630) = 6.253$, $p = .001$), such that omissions occurred more frequently as delay increased (Fig. 6g). There was no drug × delay interaction ($F(4.709, 75.351) = .326$, $p = .887$). There also was no significant sex difference ($F(1, 16) = .704$, $p = .414$), but there was a significant drug × sex interaction ($F(3, 36.803) = 5.146$, $p = .004$), such that quinpirole caused a greater increase in omissions in males compared with saline (Fig. 6h-i). There was no significant sex × delay × drug interaction ($F(15, 75.351) = 1.000$, $p = .455$).

During administration of the D2 antagonist eticlopride, there was a significant effect of delay ($F(1.950, 27.302) = 43.875$, $p < .001$). Rats again chose the punished option more frequently as punishment delay increased (Fig. 7a). There was no effect of drug ($F(3, 27.302) = 1.824$, $p = .157$) or drug × delay interaction ($F(15, 210) = 1.150$, $p = .314$). There was no significant sex difference ($F(1, 14) = .493$, $p = .494$), drug × sex interaction ($F(3, 42) = .550$, $p = .651$), or sex × delay × drug interaction ($F(15, 210) = .562$, $p = .901$) (Fig. 7b-c). In summary, unlike D2-receptor activation, D2 receptor blockade did not affect choice of delayed punishment.

There were no significant effects of sex or drug on locomotor activity ($ps > .160$; Fig. 7d-f). There was no drug effect on omissions ($F(1.634, 26.149) = .136$, $p = .832$) nor a

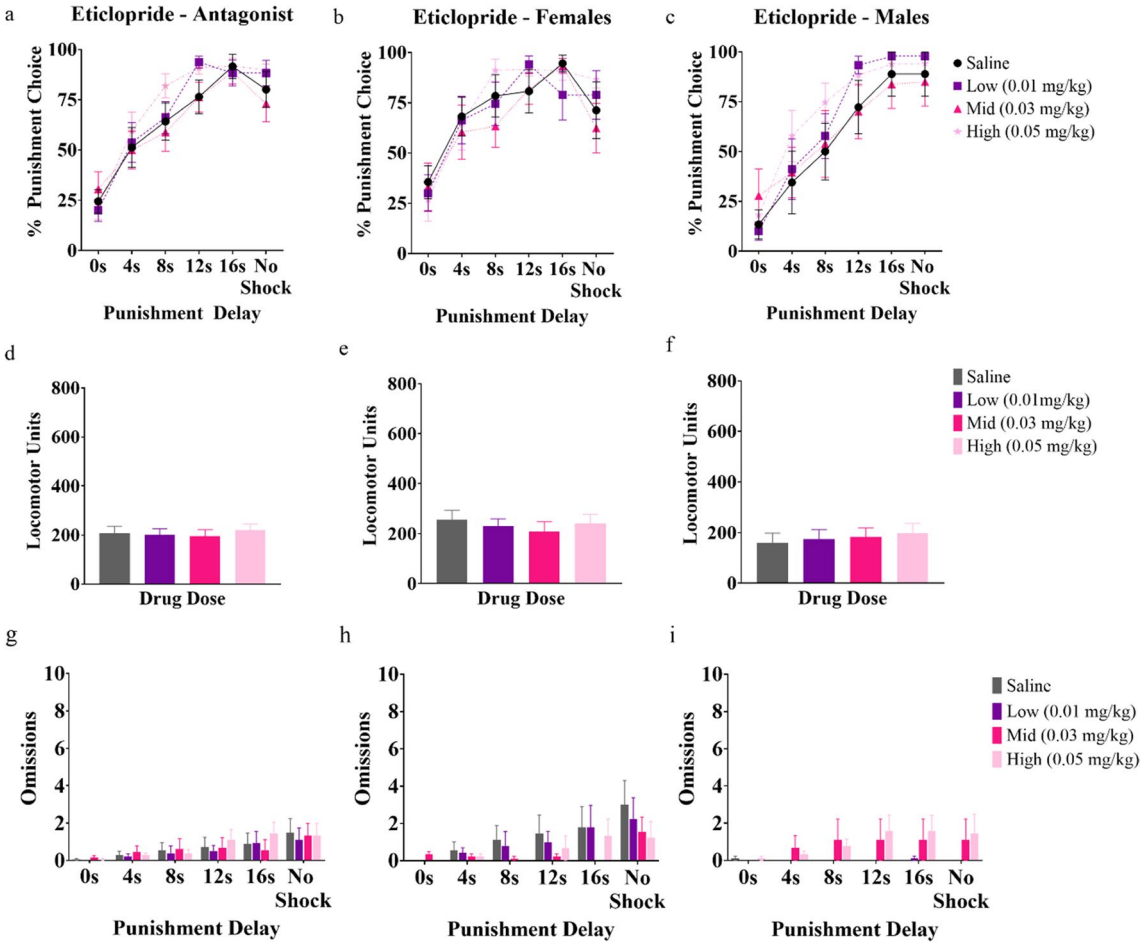

Fig. 7 Effects of eticlopride on decision-making, locomotion, and omissions during DPDT. Eticlopride did not lead to changes in choice of the delayed (or immediate) punishment (**a**). This was observed in both female (**b**) and male (**c**) rats. Eticlopride did not lead to a difference in locomotor units (**d**). There was no difference in locomotion between females (**e**) or males (**f**). Eticlopride increased omissions across the session (**g**). This effect was more prominent in males (**h**) than females (**i**). Data are depicted as mean ± standard error of the mean

drug x delay interaction ($F(15, 240) = .388$, $p = .981$). There was a significant effect of delay on omissions ($F(1.952, 31.233) = 5.091$, $p = .013$), such that omissions occurred more frequently as delay increased (Fig. 7g). There was no main effect of sex ($F(1, 16) = .663$, $p = .427$), but there was a significant drug × sex interaction ($F(3, 26.149) = 2.895$, $p = .045$), such that males showed a greater increase in omissions after D2 blockade compared with saline (Fig. 7h-i).

Effect of cocaine with D2 receptor blockade on sensitivity to delayed punishment during DPDT

Cocaine and D2 activation exerted comparable effects on delayed punishment decision-making, reducing choice of delayed punishment without affecting choice of immediate punishment (Figs. 3a and 6a). Accordingly, we hypothesized that cocaine's effects on decision-making were dependent on D2 receptor activation. We tested this by blocking D2 receptors with the D2 antagonist eticlopride (0.05 mg/kg) and then administering systemic cocaine (15 mg/kg). This was compared to saline/cocaine, eticlopride/saline, and saline/saline injections. Each rat (n = 18; 9 females/9 males) received all four doses across an 8-day counterbalanced schedule. Two females were not included in final analyses due to excessive omissions. If D2 receptor activation was indeed necessary for cocaine's effects on decision-making, the D2 antagonist was predicted to attenuate cocaine's effects on choice of delayed punishment.

Across all treatments, we again observed that rats chose the punished lever more frequently as delay increased ($F(2.301, 32.214) = 48.214$, $p < .001$; Fig. 8a). There also was a significant drug effect ($F(3, 42) = 3.793$, $p = .017$) and drug x delay interaction ($F(15, 210) = 6.126$, $p < .001$), revealing that cocaine again reduced choice of delayed (but not immediate) punishment (Fig. 8a). Interestingly, the effects of cocaine were unaffected by D2 receptor blockade

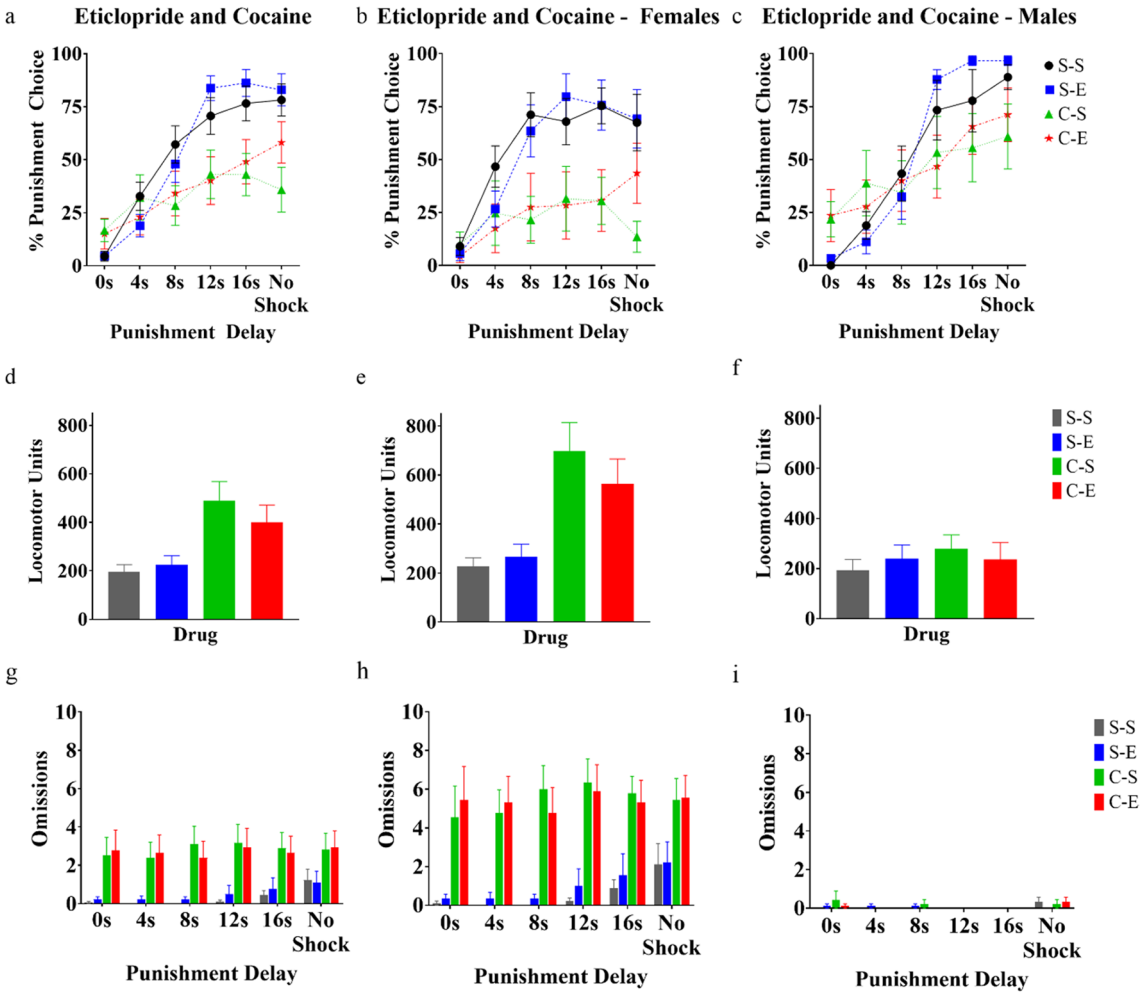

Fig. 8 Effect of cocaine with D2 receptor blockade on sensitivity to delayed punishment during DPDT. Cocaine reduced choice of delayed (but not immediate) punishment and the effects of cocaine were unaffected by D2 receptor blockade (**a**). This was observed in both females (**b**) and males (**c**). There was a cocaine-induced increase in locomotion (**d**). This effect was more pronounced in females (**e**) than males (**f**). Cocaine increased omissions across the session (**g**). This effect was more prominent in females (**h**) than males (**i**). Data are depicted as mean ± standard error of the mean

(individual comparisons: saline-saline vs. cocaine-saline: $p = .019$; saline-eticlopride vs. saline-saline: $p = .911$; cocaine-eticlopride vs. cocaine-saline: $p = .874$; eticlopride-cocaine vs. saline-saline: $p = .022$). As previously, D2 blockade had no effect on decision-making (eticlopride-saline vs. saline-saline: $p = .911$). There was no significant sex difference ($F(1, 14) = 3.401$, $p = .086$), drug x sex interaction ($F(3, 42) = 1.793$, $p = .163$), or drug x delay x sex interaction ($F(15, 210) = 1.320$, $p = .192$; Fig. 8b-c). In summary, because D2 blockade did not attenuate systemic cocaine effects on delayed punishment decision-making, cocaine's effects are likely not dependent on D2 receptor activation.

Next, we assessed drug effects on locomotion. There was a significant drug effect ($F(1.946, 31.138) = 12.685$, $p < .001$), supporting that cocaine leads to an increase in locomotion (Fig. 8d). There also was a significant drug x sex interaction ($F(3, 31.138) = 7.082$, $p < .001$; Fig. 8e-f); individual comparisons revealed that cocaine increased locomotion in females ($p < .001$) but not males ($p = .416$). Additionally, there was a significant sex difference ($F(1, 15) = 6.969$, $p = .018$), indicating that females had an increase in cocaine-induced locomotion compared with their male counterparts (Fig. 8e-f).

Finally, we assessed drug effects on omissions. There was no significant effect of delay ($F(1.979, 31.657) = 1.297$, $p = .287$) as well as no drug x delay interaction ($F(4.700, 75.196) = .542$, $p = .733$). There was, however, a significant drug effect ($F(1.643, 26.294) = 15.636$, $p < .001$), such that cocaine led to an increase in omissions (Fig. 8g). Additionally, there was a significant sex difference ($F(1, 16) = 59.739$, $p < .001$; Fig. 8h-i), which shows that overall,

females omitted more frequently than their male counterparts, as well as a significant drug x sex interaction ($F(3, 26.294) = 14.904$, $p < .001$), individual comparisons revealed that cocaine increased omissions in females ($p < .001$) but not males ($p = .488$).

Effects of increased synaptic norepinephrine on sensitivity to Delayed punishment during DPDT

In addition to elevating synaptic dopamine, cocaine enhancement of synaptic norepinephrine. However, the role of norepinephrine in regulating decision-making with delayed punishment remains a mystery. For this final experiment, we investigated the effects of increased synaptic norepinephrine on DPDT using the norepinephrine transporter inhibitor atomoxetine. Each rat (n = 18; 9 females/9 males) received all four doses across an 8-day counterbalanced schedule. Six females and one male were not included in final analyses because of excessive omissions. As with all other experiments, rats chose the punished lever more frequently as punishment delay increased ($F(2.349, 21.145) = 14.513$, $p < .001$; Fig. 9a). There was a significant drug effect ($F(3, 27) = 9.064$, $p < .001$), showing that increasing norepinephrine transmission reduced punished choice (Fig. 9a). There was no significant drug x delay interaction ($F(15, 135) = .925$, $p = .539$), such that atomoxetine reduced choice of the punished option when punishment was both immediate and delayed. However, individual comparisons revealed that there was only an effect of drug in the blocks with a delay ($ps < .024$), whereas there was no difference with immediate punishment ($p = .143$).

There also was a significant sex difference ($F(1, 9) = .003$, $p < .001$); females chose the punished lever choice less compared with their male counterparts (Fig. 9b-c). There was no drug x sex interaction ($F(3, 27) = .665$, $p = .581$) or sex x delay x drug interaction ($F(15, 135) = 1.135$,

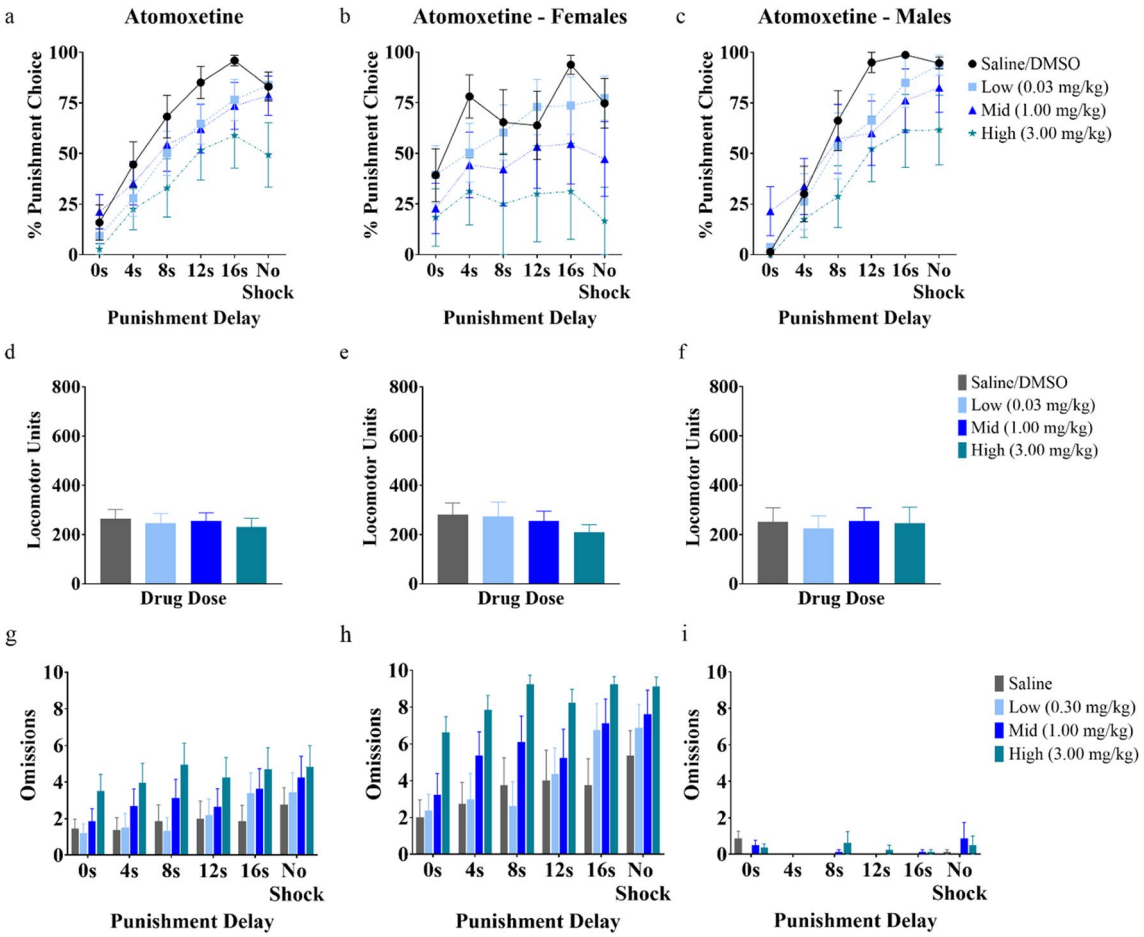

Fig. 9 Effects of atomoxetine on decision-making, locomotion, and omissions during DPDT. Atomoxetine caused a dose-dependent reduction in choice of the delayed (but not immediate) punishment (**a**). This pattern was observed in both female (**b**) and male (**c**) rats. Atomoxetine did not lead to a difference in locomotor units (**d**). There was no difference in locomotion between females (**e**) or males (**f**). Atomoxetine increased omissions across the session (**g**). This effect was more prominent in females (**h**) than males (**i**). Data are depicted as mean ± standard error of the mean

$p = .332$). In summary, elevating synaptic norepinephrine caused a dose-dependent reduction in choice of delayed but not immediate punishment.

Atomoxetine had no effects on locomotion (*p*s > .137) (Fig. 9d-f). There was a significant effect of delay for omissions ($F(2.740, 38.365) = 9.673$, $p < .001$), indicating that omissions increased as the delay increased (Fig. 9g). A significant drug effect was shown ($F(3, 42) = 14.802$, $p = .000$), indicating that Atomoxetine led to an increase in omissions (Fig. 9g). Additionally, there was a significant drug x delay interaction ($F(15, 210) = 2.211$, $p = .007$), such that omissions with saline or low/mid doses of atomoxetine increased as the session progressed, but high dose of atomoxetine (3.00 mg/kg) increased omissions comparably across the session (Fig. 9g). There also was a significant sex difference in omissions ($F(1, 14) = 31.158$, $p < .001$), such that females had overall more omissions than their male counterparts (Fig. 9h-i). There were significant drug x sex ($F(3, 42) = 12.218$, $p < .001$) and sex x delay x drug interactions ($F(15, 210) = 1.955$, $p = .020$), indicating that atomoxetine increased omissions in females but not males (Fig. 9h-i).

Testing drug effects on DPDT with descending delays (RevDPDT)

Cocaine, atomoxetine, and quinpirole each reduced choice of the large reward with delayed punishment in DPDT. It is possible that these shifts were driven by drug-evoked inflexibility, which would reduce ability to shift choice with increasing punishment delays (leading to a "flat" curve). To assess this, a group of drug naïve rats (n = 10, 6 females/4 males) were tested in a modified version of DPDT with descending instead of ascending delays (RevDPDT) and then retested with the effective doses of these drugs. If these treatments induced inflexibility, this would manifest as persistent choice of the large reward even after the addition of punishment.

First, the highest dose (15 mg/kg) of cocaine was tested on RevDPDT, because this dose evoked a shift in responding in DPDT that may be attributable to inflexibility (Fig. 3a-c). One male rat was removed from analyses due to failure to complete any trials. As previously, high-dose cocaine increased omissions ($t(8) = 4.39$, $p = .001$; saline mean = 12.5/session, cocaine mean: 38.4/session). Surprisingly, cocaine had no effect on choice in RevDPDT (effect of drug: $F(1,8) = .219$, $p = .652$; drug × block interaction $F(5,40) = 1.996$, $p = .100$). This suggests that cocaine only alters choice of delayed punishment when punishment is initially immediate, then preceded by ascending delays (as in DPDT). Furthermore, the lack of effect on RevDPDT suggests that the effects of cocaine on DPDT (Fig. 3a) were not solely caused by cocaine-induced deficits in behavioral flexibility. There was a significant effect of sex, with females selecting the large reward more frequently more frequently than their male counterparts ($F(1,7) = 15.169$, $p = .006$, although this should be interpreted with caution because of imbalanced and small sample size (6 females/3 males) and titration of individual shock values for each rat to produce a baseline discounting curve. There were no significant interactions involving sex (*p*s > 098).

High-dose atomoxetine (3 mg/kg), a norepinephrine reuptake inhibitor, had no effect on omissions in RevDPDT ($t(9) = 4.17$, $p = .343$; data not shown). Atomoxetine also had no effect on choice of the punished option (effect of drug: $F(1,9) = .471$, $p = .510$; drug × block interaction $F(5,45) = 1.413$, $p = .238$), suggesting that, as with cocaine, atomoxetine only alters decision-making with delayed punishment when delays occur in ascending fashion. There were no significant effects of sex or sex-based interactions ($p > .32$).

D2 receptor activation with quinpirole at multiple doses caused avoidance of the delayed punishment-associated reward in DPDT across both sexes that persisted even after the shock was removed in the final block (Fig. 6a). Due to this enduring punishment avoidance, as well as D2 activation evoked punishment avoidance in other decision-making tasks (Simon et al., 2011), we tested if quinpirole increased sensitivity to aversive foot shocks. Neither mid (0.125 mg/kg) nor high (0.25 mg/kg) dose of quinpirole altered shock threshold compared to saline ($F(2,16) = 1.779$, $p = .201$; Fig. 10c) or drug × sex interaction ($F(2,16) = .409$, $p = .671$), suggesting that D2 activation did not impact shock sensitivity. There was a significant difference between males and females, such that males required a higher shock amplitude to elicit a response ($F(1,8) = 16.547$, $p = .004$; male mean = .338 mA, female mean = .164 mA).

We next tested the effects of mid- and high-dose quinpirole on Rev DPDT. Quinpirole increased trial omissions at both doses compared with saline ($F(2,16) = 39.654$, $p < .001$; saline = 12.2/session, 0.125 mg/kg quinpirole = 43.9/session, 0.25 mg/kg quinpirole = 47.7/session). As with cocaine and atomoxetine, there were no effects of quinpirole on choice (effect of drug: $F(2,8) = .985$, $p = .395$; drug × block interaction: $F(10,80) = .526$, $p = .867$), suggesting that D2 receptor activation only alters choice of delayed punishment when delays are ascending. There were no effects of sex or sex-based interactions (*p*s > .513). Based on the planned hypothesis that quinpirole impairs flexibility in this task as well as visual inspection of the data revealing a flattened curve after treatment (Fig. 10c), we performed additional analyses to determine whether quinpirole reduced ability to shift decision-making preference across blocks. With saline exposure, there was an effect of delay, such that rats shifted choice when shock was introduced ($F(4,45) = 3.407$, $p = .011$). However, quinpirole abolished the effect of delay at both doses (0.125 mg/kg: $F(5,45) = .925$, $p = .475$; 0.25 mg/kg: $F(5,45) = .350$, $p = .938$). Therefore,

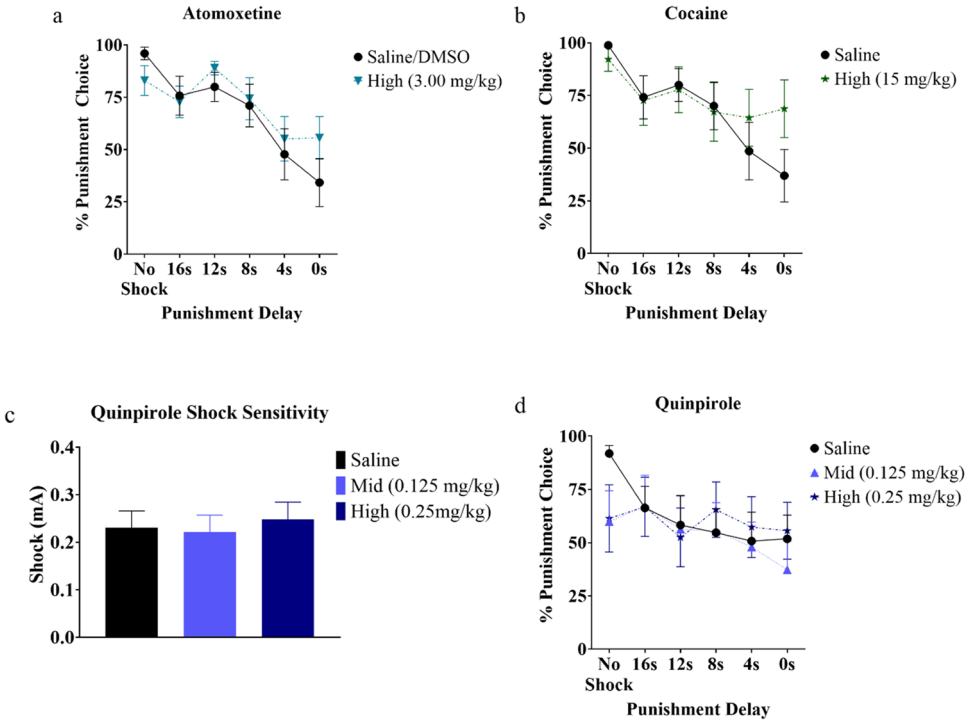

Fig. 10 Effects of drugs on DPDT with descending delays (RevDPDT). High-dose atomoxetine had no effect on decision-making in RevDPDT (**a**). High-dose cocaine did not alter decision-making (**b**). The D2 agonist quinpirole did not influence shock sensitivity (**c**) and evoked inflexible decision-making in RevDPDT. Data are depicted as mean ± standard error of the mean

D2 receptor activation causes inflexible responding during DPDT with both ascending and descending delays, although the RevDPDT results should be interpreted with caution due to the high number of omitted trials.

Discussion

We determined that sensitivity to delayed punishment during decision-making is sensitive to acute systemic dopaminergic and noradrenergic manipulation, although these effects are task-dependent. Cocaine caused a dose-dependent reduction in choice of rewards associated with delayed but not immediate punishment in both male and female rats, although this effect was limited to DPDT with ascending delays. While neither activation nor blockade of D1-like receptors affected decision-making, D2-like receptor activation reduced choice of rewards associated with delayed but not immediate punishment in both males and females, although this effect may be driven by reduced behavioral flexibility. D2 blockade did not affect DPDT performance in either sex. Additionally, D2 receptor blockade did not affect cocaine's effects on decision-making, indicating that cocaine modulation of delayed punishment choice is not dependent on D2 receptor activation. Finally, increasing synaptic norepinephrine caused a dose-dependent reduction in choice of rewards associated with delayed punishment in DPDT with ascending but not descending delays.

Effects of acute cocaine on sensitivity to delayed punishment during DPDT

Acute cocaine had no effect on choice of rewards accompanied by immediate punishment but reduced choice of delayed punishment. This selectivity suggests that cocaine's neurochemical effects mitigate the effects of delay on punishment salience, causing rats to avoid delayed punishment in a manner comparable to immediate punishment. Accordingly, we speculate that cocaine is reducing delay discounting of punishment. We also observed that females chose the punished lever less frequently than males, which has been observed in previous punished decision-making tasks (Liley et al., 2019; Orsini et al., 2022).

Although the effects of cocaine on discounting of delayed punishment have not been studied previously, it has been observed that acute cocaine increases impulsive choice, indicative of increased delay discounting (Evenden & Ryan, 1996; Smethells & Carroll, 2015). The current results contrast with these data, as increased delay discounting would be expected to increase choice of delayed punishment (with delay reducing the influence of punishment on choice). Therefore, acute cocaine may have dissociative effects

on delay discounting based on outcome valence, causing increased discounting of delayed rewards and reduced discounting of delayed punishment. Along with the lack of correlation between decision-making tasks driven by reward vs punishment (Liley et al., 2019) and differential sensitivity to inactivation of brain regions (Liley et al., 2022), this provides further evidence that delayed rewards and punishment are mediated by disparate neural mechanisms.

One possible explanation for the cocaine-induced reduction in choice of delayed (but not immediate) punishment is cocaine-evoked inflexibility, leading to inability to adapt choice strategy with increasing punishment delays. Acute cocaine causes perseverative responding in humans (Ersche et al., 2008), and cocaine exposure caused inflexibility in response to changes in punishment risk during a rat risky decision-making task (Simon et al., 2009). To test this possibility, rats were trained in RevDPDT, a version of DPDT with descending instead of ascending delays. Cocaine exposure did not affect the slope of the RevDPDT curve, suggesting that the effects in DPDT are not driven by broad flexibility deficits. However, this experiment revealed that acute cocaine does not reduce choice of delayed punishment when delays are presented in descending (instead of ascending) order, suggesting that cocaine exposure alters decision-making in a task-specific manner. Similar task-specific results were obtained with basolateral amygdala inactivation, which selectively reduced choice of delayed punishment with ascending but not descending delays (Liley et al., 2022). We propose that punishment-based decision-making beginning with a highly salient choice including multiple variables (high vs. low reward magnitude and punishment vs no punishment) is more sensitive to neurobiological manipulations than decision-making beginning with a punishment-free condition (as in RevDPDT).

It also is possible that cocaine-driven reduced choice of delayed punishment in DPDT was not caused by changes in sensitivity to delayed punishment but instead was reflective of an overall increase in punishment sensitivity regardless of delay. However, this seems unlikely, because cocaine has antinociceptive properties (Pertovaara et al., 1991), which would be predicted to increase choice of foot shock, the opposite of what was observed. Moreover, cocaine exposure did not increase avoidance of rewards associated with immediate punishment in DPDT and increased choice of immediate punishment in the final block of RevDPDT.

Effects of D1-like receptor manipulation on sensitivity to delayed punishment during DPDT

Neither blockade (SCH23390) nor activation (SKF81297) of the D1 receptor altered choice of immediate or delayed punishment. This lack of effect on delayed punishment suggests that the D1 receptor is not involved in punishment-drive economic decision-making regardless of delay. This is corroborated by previous studies showing that neither D1 activation (Oinio et al., 2017; Simon et al., 2011, Zeeb et al., 2009) nor D1 blockade (Oinio et al., 2017; Simon et al., 2011, Zeeb et al., 2009) affected on choice of punished rewards during risky decision-making.

Notably, this experiment used systemic D1 receptor manipulation. When D1 activation and blockade were performed in specific brain regions, studies did show D1 modulation of risky decision-making. Larkin and colleagues (2016) looked at activation and blockade of D1 receptors in the basolateral amygdala (BLA) during probabilistic risky decision-making. They found that activation of the intra-BLA with SKF81297 increased risky choice in high probability conditions and decreased risk-taking in low probability conditions (Larkin et al., 2016). A similar pattern was observed after D1 activation in the nucleus accumbens (NAcc) (Stopper et al., 2013). D1 receptor blockade in the NAcc reduced risky choice decisions (Stopper et al., 2013), which parallels D1 blockade in the BLA (Larkin et al., 2016). Additionally, a decrease in risky choice decisions was found after the blockade of D1 receptors in the prefrontal cortex (PFC; St Onge et al., 2011). However, activation of the D1 receptors in the PFC led to no significant change in risky choice decisions (St Onge et al., 2011). Therefore, despite the lack of effects of systemic D1 receptor manipulation, it is possible that D1 receptors localized to specific regions mediate decision-making with delayed punishment.

Effects of D2-receptor manipulation on sensitivity to delayed punishment during DPDT

Blockade of the D2 receptor with eticlopride had no effect on choice of immediate or delayed punishment. However, activation of the D2 receptor with quinpirole reduced choice of delayed but not immediate punishment in DPDT. Notably, this punished reward avoidance persisted to the final, punishment-free block of trials. Therefore, it is possible that D2 activation did not directly affect sensitivity to delayed punishment but instead altered other cognitive/perceptual processes involved with decision-making. We observed that quinpirole had no impact on a shock threshold test, suggesting that the enduring punishment avoidance was not a result of increased pain sensitivity. Next, we tested if D2 activation was impairing ability to flexibly alter responding with changes in outcome. Rats were trained in the DPDT with descending instead of ascending punishment delays, and quinpirole did not reduce choice of delayed punishment. Instead, quinpirole "flattened" the response curve, indicative of either inflexible responding or a general inability to comprehend the parameters of the task. Therefore, the reduced choice of delayed punishment in DPDT following D2

receptor activation was likely a result of cognitive impairment rather than altered sensitivity to delayed punishment.

D2 manipulation in specific brain regions has been demonstrated to influence other forms of decision-making. As observed here, D2 activation in medial PFC impaired ability to adapt to changes in task contingencies (St Onge et al., 2011). Activation of D2 receptors in the BLA reduced risky choice (Larkin et al., 2016). Blockade of D2 receptors in the orbitofrontal cortex also decreased risky choice (Morgado et al., 2015), whereas specific blockade of D2 in the intra-BLA resulted in no overall effect on risky choice decisions (Larkin et al., 2016). Interestingly, neither blockade nor activation of D2 receptors in the NAcc affected risky decision-making (Stopper et al., 2013). Additionally, neither systemic blockade of the D2 receptor or D2 blockade in NAcc had any effects on delay discounting of reward (Li et al., 2015; Yates & Bardo, 2017). A similar lack of effect was seen with systemic D2 receptor activation on reward delay discounting (Castrellon et al., 2021; Koffarnus et al., 2011). Interestingly, both D2 activation and blockade in the medial PFC increased impulsive choice during delay discounting of rewards, while neither activation nor blockade in OFC affected delay discounting (Yates et al., 2014).

Notably, D2 receptor activation produced a reduction in delayed punished choice in DPDT that was qualitatively comparable to acute cocaine, which acts as an indirect D2 receptor agonist by reducing dopamine reuptake. Thus, it is possible that cocaine's effects on DPDT were driven by D2 receptor activation. This was tested by combining cocaine administration with a D2 antagonist. Interestingly, effects of cocaine were unaffected by blockade of the D2 receptor, suggesting that D2 receptor activation is not necessary for cocaine's modulation of sensitivity to delayed punishment. It is possible that cocaine is affecting choice via D1 receptor activation; however, selective D1 receptor manipulation did not affect delayed punishment decision-making. Therefore, it seems more likely that cocaine's norepinephrine enhancing effects may account for the reduction in punished reward choice, with D2 activation instead causing cognitive impairment/inflexible decision-making.

Increased synaptic norepinephrine increases sensitivity to delayed punishment during DPDT

In addition to elevating synaptic dopamine, cocaine also functions as a norepinephrine transporter inhibitor (Sofuoglu & Sewell., 2009). Therefore, cocaine-induced reduction in choice of delayed punishment may have been driven by norepinephrine rather than dopamine transmission. We tested this by administering the norepinephrine reuptake inhibitor atomoxetine before DPDT and revDPDT. Atomoxetine had no effect on choice of immediate punishment but reduced choice of delayed punishment. Interestingly, these effects were not evident in revDPDT with descending punishment delays. Collectively, effects of atomoxetine resembled cocaine exposure, which also disrupts norepinephrine reuptake. Therefore, it is possible that cocaine's effects on decision-making are regulated by norepinephrine receptor activation. Further research is necessary to determine if increased synaptic norepinephrine is necessary for cocaine's effects and which noradrenergic circuits specifically regulate sensitivity to delayed punishment.

Overall summary

This study was the first to investigate the pharmacological mechanisms underlying sensitivity to delayed vs immediate punishment during reward-seeking. A deeper understanding of discounting of delayed punishment is a crucial step toward understanding psychiatric disorders characterized by insensitivity to punishment (Kräplin et al., 2020; Nestadt et al., 2016; Pushkarskaya et al., 2015; Starcke et al., 2010). Critically, because consequences of substance use (such as withdrawal or financial problems) often occur after a delay, understanding discounting of delayed punishment is a key aspect in understanding and treating maladaptive decision making in SUD. The current study highlights task-dependent contributions of dopamine and norepinephrine to evaluation of delayed punishment, suggesting that manipulation of these circuits may have potential to improve suboptimal decision-making in psychiatric disorders.

Acknowledgments The authors thank Dr. Daniel Gabriel, Tiya Qualls, Boula Baskhairoun, Zachary Mikkelson, Sharoderick Lowe, Jason Leonidas Martinez, and Kayrine Cortes for technical assistance.

References

Beaulieu, J. M., Espinoza, S., & Gainetdinov, R. R. (2015). Dopamine receptors – IUPHAR Review 13. *British Journal of Pharmacology, 172*(1), 1–23.

Blaes S. L., Orsini C. A., Mitchell M. R., Spurrell M. S., Betzhold S. M., Vera K., Bizon J. L., & Setlow B. (2018). Monoaminergic modulation of decision-making under risk of punishment in a rat model. *Behavioural Pharmacology, 29*(8), 745–761.

Cardinal R. N., Robbins T. W., & Everitt B. J. (2000). The effects of d-amphetamine, chlordiazepoxide, alpha-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. *Psychopharmacology, 152*(4), 362–375.

Cardinal R. N. (2006). Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks, 19*(8), 1277–1301.

Castrellon J. J., Meade J., Greenwald L., Hurst K., & Samanez-Larkin G. R. (2021). Dopaminergic modulation of reward discounting in healthy rats: A systematic review and meta-analysis. *Psychopharmacology, 238*(3), 711–723.

dela Peña, I., Gevorkiana, R., & Shi, W. X. (2015). Psychostimulants affect dopamine transmission through both dopamine transporter-dependent and independent mechanisms. *European Journal of Pharmacology, 764*, 562–570.

De Wit H., Enggasser J., & Richards J. (2002). Acute administration of d-Amphetamine decreases impulsivity in healthy volunteers. *Neuropsychopharmacology, 27*, 813–825.

Ersche K. D., Roiser J. P., Robbins T. W., Sahakian B. J. (2008). Chronic cocaine but not chronic amphetamine use is associated with perseverative responding in humans. *Psychopharmacology, 197*(3), 421–431.

Evenden, J. L., & Ryan, C. N. (2016). The pharmacology of impulsive behavior in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology, 128*(2), 161–70.

Faraone, S. V. (2018). The pharmacology of amphetamine and methylphenidate: Relevance to the neurobiology of attention-deficit/hyperactivity disorder and other psychiatric comorbidities. *Neuroscience & Behavioral Reviews, 87*, 255–270.

Fischhoff B., Broomell S. B. (2020). Judgment and decision making. *Annual Review of Psychology, 71*, 331–355.

Floresco, S. B., & Magyar, O. (2006). Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology, 188*(4), 567–585.

González-Barriga F., & Orduña V. (2022) Spontaneously hypertensive rats show higher impulsive action, but equal impulsive choice with both positive and aversive consequences. *Behavioural Brain Research, 427*, 113858.

Hori Y., Nagai Y., Mimura K., Suhara T., Higuchi M., Bouret S., & Minamimoto T. (2021) D1- and D2-like receptors differentially mediate the effects of dopaminergic transmission on cost-benefit evaluation and motivation in monkeys. *PLoS Biology, 19*(7), e3001055.

Jacobs D. S., & Moghaddam B. (2020). Prefrontal cortex representation of learning of punishment probability during reward-motivated actions. *The Journal of Neuroscience, 40*(26), 5063–5077.

Jean-Richard-Dit-Bressel P., Killcross S., & McNally G. P. (2018). Behavioral and neurobiological mechanisms of punishment: Implications for psychiatric disorders. *Neuropsychopharmacology, 43*(8), 1639–1650.

Kräplin A., Höfler M., Pooseh S., Wolff M., Krönke K. M., Goschke T., Bühringer G., & Smolka M. N. (2020). Impulsive decision-making predicts the course of substance-related and addictive disorders. *Psychopharmacology, 237*(9), 2709–2724.

Koffarnus M. N., Newman A. H., Grundt P., Rice K. C., & Woods J. H. (2011). Effects of selective dopaminergic compounds on a delay-discounting task. *Behavioural Pharmacology, 22*(4), 300–311.

Larkin, J. D., Jenni, N. L., & Floresco, S. B. (2016). Modulation of risk/reward decision making by dopaminergic transmission within the basolateral amygdala. *Psychopharmacology, 233*(1), 121–136.

Li, Y., Zuo, Y., Yu, P., Ping, X., & Cui, C. (2015). Role of basolateral amygdala dopamine D2 receptors in impulsive choice in acute cocaine-treated rats. *Behavioral Brain Research, 287*, 187–95.

Liley A. E., Gabriel D. B. K., Sable H. J., & Simon N. W. (2019). Sex differences and effects of predictive cues on delayed punishment discounting. *eNeuro*, *6*(4).

Liley, A. E., Gabriel, D. B. K., & Simon, N. W. (2022). Lateral orbitofrontal cortex and basolateral amygdala regulate sensitivity to delayed punishment during decision-making. *eNeuro, 9*(5).

Mishra, A., Singh, S., & Shukla, S. (2018). Physiological and functional basis of dopamine receptors and their role in neurogenesis: Possible implication for Parkinson's disease. *Journal of Experimental Neuroscience, 12*, 1179069518779829.

Mitchell, M. R., Weiss, V. G., Beas, B. S., Morgan, D., Bizon, J. L., & Setlow, B. (2014). Adolescent risk taking, cocaine self-administration, and striatal dopamine signaling. *Neuropsychopharmacology, 39*(4), 955–962.

Morgado, P., Marques, F., Ribeiro, B., Leite-Almeida, H., Pêgo, J. M., Rodrigues, A. J., Dalla, C., Kokras, N., Sousa, N., & Cerqueira, J. J. (2015). Stress induced risk-aversion is reverted by D2/D3 agonist in the rat. *European Neuropsychopharmacology, 25*(10), 1744–1752.

Murphy J., Vuchinich R., & Simpson C. (2001). Delayed reward and cost discounting. *Psychological Record, 51*(4), 571-588.

Nestadt G., Kamath V., Maher B. S., Krasnow J., Nestadt P., Wang Y., Bakker A., & Samuels J. (2016). Doubt and the decision-making process in obsessive-compulsive disorder. *Medical Hypotheses,* 96, 1–4.

Oinio, V., Bäckström, P., Uhari-Väänänen, J., Raasmaja, A., Piepponen, P., & Kiianmaa, K. (2017). Dopaminergic modulation of reward-guided decision making in alcohol-preferring AA rats. *Behavioural Brain Research, 326*, 87–95.

Orsini C. A., & Simon N. W. (2020). Reward/punishment-based decision making in rodents. *Current Protocol Neuroscience*, 93, e100.

Orsini C. A., Truckenbrod L. M., & Wheeler A. R. (2022). Regulation of sex differences in risk-based decision making by gonadal hormones: Insights from rodent models. *Behavioural Processes, 200*, 104663.

Owens M. M., Syan S. K., Amlung M., Beach S., Sweet L. H., & MacKillop J. (2019). Functional and structural neuroimaging studies of delayed reward discounting in addiction: A systematic review. *Psychological Bulletin, 145*(2), 141–164.

Pattij T., & Vanderschuren L. J. (2008). The neuropharmacology of impulsive behaviour. *Trends in Pharmacological Sciences, 29*(4), 192–199.

Pertovaara A., Mecke E., & Carlson S. (1991). Attempted reversal of cocaine-induced antinociceptive effects with naloxone, an opioid antagonist. *European Journal of Pharmacology, 192*(3), 349–353.

Phillips, P. E., Walton, M. E., & Jhou, T. C. (2007). Calculating utility: preclinical evidence for cost-benefit analysis by mesolimbic dopamine. *Psychopharmacology, 191*(3), 483–495.

Pushkarskaya H., Tolin D., Ruderman L., Kirshenbaum A., Kelly J. M., Pittenger C., & Levy I. (2015). Decision-making under uncertainty in obsessive-compulsive disorder. *Journal of Psychiatric Research, 69*, 166–173.

Robbins, T. W. (2003). Dopamine and cognition. *Current Opinion in Neurology, 16*(Suppl 2), S1–S2.

Rodríguez W., Bouzas A., & Orduña V. (2018). Temporal discounting of aversive consequences in rats. *Learn Behavior, 46*:38–48.

Shead N. W., & Hodgins D. C. (2009). Probability discounting of gains and losses: implications for risk attitudes and impulsivity. *Journal of the Experimental Analysis of Behavior, 92*(1), 1–16.

Smethells J. R., & Carroll M. E. (2015). Discrepant effects of acute cocaine on impulsive choice (delay discounting) in female rats during an increasing- and adjusting-delay procedure. *Psychopharmacology, 232*(14), 2455–2462.

Simon N. W., Gilbert R. J., Mayse J. D., Bizon J. L., & Setlow B. (2009). Balancing risk and reward: a rat model of risky decision making. *Neuropsychopharmacology, 34*(10), 2208–2217.

Simon NW, Montgomery KS, Beas BS, Mitchell MR, LaSarge CL, Mendez IA, Bañuelos C, Vokes C. M., Taylor A. B., Haberman R. P., Bizon J. L., & Setlow B. (2011). Dopaminergic modulation of risky decision-making. *The Journal of Neuroscience, 31*(48), 17460–17470.

Sofuoglu, M., & Sewell, R. A. (2009). Norepinephrine and stimulant addiction. *Addiction Biology, 14*(2), 119–129.

Soutschek, A., Gvozdanovic, G., Kozak, R., Duvvuri, S., de Martinis, N., Harel, B., Gray, D. L., Fehr, E., Jetter, A., & Tobler, P. N. (2020). Dopaminergic D_1 receptor stimulation affects effort and risk preferences. *Biological Psychiatry, 87*(7), 678–685.

Starcke K., Tuschen-Caffier B., Markowitsch H. J., & Brand M. (2010). Dissociation of decisions in ambiguous and risky situations in obsessive-compulsive disorder. *Psychiatry Research, 175*(1-2), 114–120.

St Onge J. R., & Floresco S. B. (2009). Dopaminergic modulation of risk-based decision making. *Neuropsychopharmacology, 34*(3), 681–697.

St. Onge, J. R., Abhari, H., & Floresco, S. B. (2011). Dissociable contributions by prefrontal D1 and D2 receptors to risk-based decision making. *The Journal of Neuroscience, 31*(23), 8625–33.

Stopper C. M., Khayambashi S., & Floresco S. B. (2013). Receptor-specific modulation of risk-based decision making by nucleus accumbens dopamine. *Neuropsychopharmacology, 38*(5), 715–728.

Wade, T., de Wit, H., & Richards, J. (2000). Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology, 150*, 90–101.

Westbrook A., van den Bosch R., Määttä J.I. , Hofmans L., Papadopetraki D., Cools R., & Frank M. J. (2020). Dopamine promotes cognitive effort by biasing the benefits versus costs of cognitive work. *Science (New York, N.Y.), 367*(6484), 1362–1366.

Woolverton, W. L., Freeman, K. B., Myerson, J., & Green, L. (2012). Suppression of cocaine self administration in monkeys: effects of delayed punishment. *Psychopharmacology, 220*(3), 509–517.

Winstanley, C. A., Theobald, D. E., Cardinal, R. N., & Robbins, T. W. (2004). Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *The Journal of Neuroscience, 24*(20), 4718–4722.

Yates, J. R., & Bardo, M. T. (2017). Effects of intra-accumbal administration of dopamine and ionotropic glutamate receptor drugs on delay discounting performance in rats. *Behavioral Neuroscience, 131*(5), 392–405.

Yates, J. R., Perry, J. L., Meyer, A. C., Gipson, C. D., Charnigo, R., & Bardo, M. T. (2014). Role of medial prefrontal and orbitofrontal monoamine transporters and receptors in performance in an adjusting delay discounting procedure. *Brain Research, 1574*, 26–36.

Zech M. P., Schäble S., & Kalenscher T. (2022). Discounting of future rewards and punishments in rats. *eNeuro 9*(6), ENEURO.0452-21.2022.

Zeeb F. D., Robbins T. W., & Winstanley C. A. (2009). Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. *American College of Neuropsychopharmacology, 34*(10), 2329–2343.

Open practices statement None of the experiments or analyses reported here were pre-registered. All raw data, behavioral protocols, and Matlab script are available upon request.