Behavioral/Cognitive

$D_{2/3}$ Agonist during Learning Potentiates Cued Risky Choice

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Impulse control and/or gambling disorders can be triggered by dopamine agonist therapies used to treat Parkinson's disease, but the cognitive and neurobiological mechanisms underlying these adverse effects are unknown. Recent data show that adding win-paired sound and light cues to the rat gambling task (rGT) potentiates risky decision-making and impulsivity via the dopamine system, and that changing dopaminergic tone has a greater influence on behavior while subjects are learning task contingencies. Dopamine agonist therapy may therefore be potentiating risk-taking by amplifying the behavioral impact of gambling-related cues on novel behavior. Here, we show that ropinirole treatment in male rats transiently increased motor impulsivity but robustly and progressively increased choice of the high-risk/high-reward options when administered during acquisition of the cued but not uncued rGT. Early in training, ropinirole increased win-stay behavior after large unlikely wins on the cued rGT, indicative of enhanced model-free learning, which mediated the drug's effect on later risk preference. *Ex vivo* cFos imaging showed that both chronic ropinirole and the addition of win-paired cues suppressed the activity of dopaminergic midbrain neurons. The ratio of midbrain:prefrontal $cFos^+$ neurons was lower in animals with suboptimal choice patterns and tended to predict risk preference across all rats. Network analyses further suggested that ropinirole induced decoupling of the dopaminergic cells of the VTA and nucleus accumbens but only when win-paired cues were present. Frontostriatal activity uninformed by the endogenous dopaminergic teaching signal therefore appeared to perpetuate risky choice, and ropinirole exaggerated this disconnect in synergy with reward-paired cues.

Key words: $D_{2/3}$; dopamine; gambling; risky decision making; ropinirole; sensory cues

Significance Statement

$D_{2/3}$ receptor agonists, used to treat Parkinson's disease, can cause gambling disorder through an unknown mechanism. Ropinirole increased risky decision-making in rats, but only when wins were paired with casino-inspired sounds and lights. This was mediated by increased win-stay behavior after large unlikely wins early in learning, indicating enhanced model-free learning. cFos imaging showed that ropinirole suppressed activity of midbrain dopamine neurons, an effect that was mimicked by the addition of win-paired cues. The degree of risky choice rats exhibited was uniquely predicted by the ratio of midbrain dopamine:PFC activity. Depriving the PFC of the endogenous dopaminergic teaching signal may therefore drive risky decision-making on-task, and ropinirole acts synergistically with win-paired cues to amplify this.

Introduction

Flashing light and sound cues have long been used in electronic gaming and gambling products to signal rewarding outcomes. Although they may superficially seem harmless to the lay person, these sensory cues increase risky choices in both rats and humans playing laboratory-based gambling games (Barrus and Winstanley, 2016; Cherkasova et al., 2018; Spetch et al., 2020). Risky decision-making in turn facilitates the development and maintenance of a range of addiction disorders (Bechara et al., 2001; Goldstein and Volkow, 2002; Verdejo-Garcia et al., 2007). Together, these observations raise concerns about the role sensory cues may play not only in promoting problematic engagement with electronic games but also in developing and maintaining other addictive behaviors. Yet, the question remains: how do sensory cues induce these effects on risky choice?

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Numerous studies indicate that the neurochemical regulation of the decision-making process is significantly altered by the addition of win-paired cues (Adams et al., 2017; Betts et al., 2021; Chernoff et al., 2021). However, the neural circuitry responsible for the risk-promoting effects of win-paired cues remains opaque. Data from rats suggest that repeated engagement with heavily cued probabilistic schedules of reinforcement may actually sensitize the mesolimbic dopamine system to the reinforcing effects of psychostimulants (Singer et al., 2012; Zack et al., 2014; Zeeb et al., 2017; Ferland et al., 2019; Mascia et al., 2019). Medications that potentiate dopamine signaling may therefore potentiate the impact of win-paired cues on behavior. Indeed, dopamine $D_{2/3}$ receptor agonists ropinirole and pramipexole, used in the treatment of movement disorders, such as Parkinson's disease and restless leg syndrome, induce impulse control and addiction disorders, including gambling disorder (GD), in a significant minority of patients (Weintraub et al., 2006, 2010; Grall-Bronnec et al., 2018). Multiple animal studies report that chronic administration of these drugs promotes impulsive, risky, or compulsive behaviors in both pseudo-Parkinsonian and otherwise healthy animals (Rokosik and Napier, 2012; Cocker et al., 2016; Tremblay et al., 2017; Jiménez-Urbieta et al., 2019). However, the cognitive and neurobiological mechanisms driving these psychiatric side effects are still largely ambiguous, and it is unclear what role reward-paired cues play in driving or modulating this effect.

Theoretically, a bias toward the risky options could result from either hyperlearning from rewards or diminished learning from punishments. Computational modeling based on reinforcement learning algorithms on the rat gambling task (rGT) strongly indicate reward-concurrent cues potentiate risky choice by reducing animals' ability to learn from negative outcomes of an unsuccessful gamble (i.e., penalty time-outs) (Langdon et al., 2019). Additionally, choice patterns on the rGT appear to become less sensitive to changes in reinforcer value in the presence of win-paired cues (Hathaway et al., 2021). However, numerous studies demonstrate that dopamine is released following the unexpected delivery of rewards or the cues which predict their occurrence (Schultz et al., 1997). Dopamine is also essential for reward-predictive cues to acquire motivational drive ("incentive salience") (Robinson and Berridge, 1993; Flagel et al., 2011). Based on such studies, we hypothesized that dopamine agonists should boost risky choice through potentiating learning from rewards.

We previously found that chronic administration of ropinirole failed to alter risky choice in the cued rGT when administered after the acquisition of the task (Tremblay et al., 2019). Since computational analyses suggest that these win-paired cues have their biggest effect early in acquisition of the rGT (Langdon et al., 2019), in the current study we hypothesized that ropinirole would amplify risky choice in the cued version of the task if administered when animals are learning the reinforcement contingencies and developing a choice strategy. We therefore administered ropinirole chronically during acquisition of either the cued or uncued version of the rGT. We also conducted cFos imaging and network analyses *ex vivo* to determine whether performance of the cued versus uncued rGT, in ropinirole-treated versus control rats, was associated with patterns of brain activation across mesocorticostriatal circuits indicative of lower model-based executive control.

Materials and Methods

Subjects

Subjects were 112 male Long-Evans rats from Charles River laboratories ($n = 64$ in the high-dose cohort, i.e., 32 saline-treated and 32 ropinirole-treated; $n = 48$ in the low-dose cohort, i.e., 16 saline-treated and 32 ropinirole-treated) weighing 300-480 g at the beginning of the experiment. They were fed 14-16 g standard rat chow per day to maintain 85% of their free-feeding weight. Water was available *ad libitum* in the home cages. Animals were group-housed (2 or 3 animals per cage) and kept in a climate-controlled colony room at 21°C with reversed dark-light cycle (lights off at 8:00 A.M.). All training and testing took place between 7:00 A.M. and 10:00 A.M. by the same female experimenter. Housing and testing conditions were in accordance with the Canadian Council on Animal Care, and all experimental protocols were approved by the University of British Columbia Animal Care Committee.

Behavioral apparatus

A total of 32 standard five-hole operant chambers were used for both training and testing across two testing rooms. All chambers were placed in a ventilated and sound-attenuating cabinet (Med Associates) and were controlled by a Med-PC software written by CAW run on IBM-compatible computers. An array of five equidistant stimulus holes was mounted within one wall of each operant chamber. A light could be illuminated within each stimulus hole. A food tray located on the opposite wall of the chamber dispensed sucrose pellets (45 mg; BioservJ). All five stimulus holes as well as the food tray were enabled with vertical infrared beams to detect nose-poke responses. Each box was additionally equipped with a sound output apparatus producing high-pitched tones at six different frequencies.

rGT training and testing

Animals were first habituated to the operant chambers over 30-minute sessions, during which sucrose pellets were placed in stimulus holes and the food tray. They were able to freely explore the chamber. Habituation continued until animals consumed all pellets during the allotted time.

Subsequently, animals were trained on an rGT variant of the 5-Choice Serial Reaction Time task (5-CSRT) (Carli et al., 1983). In this task, animals nose-poke in a pseudorandomly illuminated stimulus hole to obtain a sugar pellet. Training continued until rats detected a 10 s stimulus light with $>$ 80% accuracy and $<$ 20% omission (i.e., failure to nose-poke in any hole during the 10 s light period) during a 30 min session consisting of 100 trials. In the original 5-CSRT task, the stimulus light varies across all five stimulus holes. However, in the rGT variant, the middle-most hole is eliminated for consistency with the four options available in the rGT. All 112 subjects achieved the training criteria within 10 training sessions. The experimental timeline after the 5-CSRT training is depicted in Figure 1*B*.

Animals then received osmotic pump implantation and started training on the rGT. They performed a forced-choice variant of either the cued or uncued rGT, with half of the animals in each cohort randomly assigned to each group. The forced-choice phase of the rGT training is designed to ensure animals respond roughly an equal number of times to each of the four stimulus holes used in the rGT. Each hole is associated with a certain probability and magnitude of sugar pellet reward and time-out penalty. For each animal, the contingencies associated with each hole as well as the presence or absence of win-paired cues remained the same across forced-choice and free-choice sessions. All rats performed seven sessions of the forced-choice rGT before beginning the free-choice version.

Each session began by illumination of the food tray light. Upon a nose-poke in the food tray, a 5-second intertrial interval (ITI) began, during which all lights were off and the animal had to refrain from making a nose-poke response. After the ITI, one of the four stimulus holes was illuminated in the forced-choice rGT, whereas in the free-choice rGT, all four stimulus holes were illuminated simultaneously to allow the animal to designate its choice with a nose-poke. Each choice would then yield either a reward or a time-out punishment according to the unique reinforcement schedule associated with that hole. A schematic of the task is presented in Figure 1*A*. This schematic was inspired by the

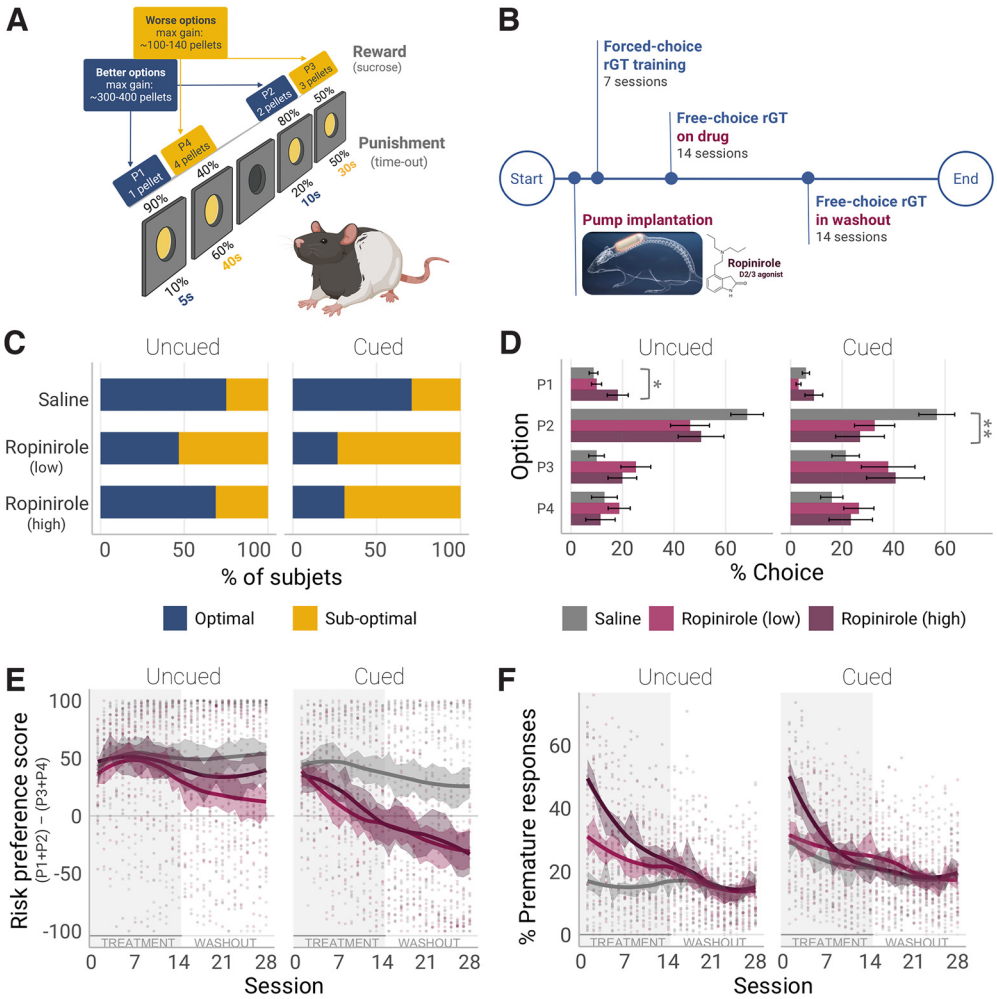

Figure 1. Ropinirole increases impulsivity only transiently but has long-term and progressive effects on risk preference in the presence of reward-paired cues. ***A***, Schematic of the rGT illustrates the contingencies associated with each stimulus aperture, which are counterbalanced across animals but remain consistent within-individual across the whole experiment. ***B***, Experimental timeline of the experiment. Animals received chronic treatment of ropinirole via an osmotic pump for 28 d as they acquired the rGT (including 7 sessions of forced-choice training followed by 14 sessions of free-choice). Testing continued for 14 sessions in washout. ***C***, Optimal versus suboptimal choosers on the cued and uncued rGT. *x* axis indicates percentage of animals preferring the low-risk/low-reward options (Optimal; blue) versus those preferring the high-risk/high-reward options (Suboptimal; yellow) at baseline (i.e., during the last five sessions, when animals were statistically stable in their choice). The majority of control animals in both uncued and cued versions of the task had already adopted an optimal choice strategy. In the cued rGT, however, the majority of ropinirole-treated animals had a suboptimal choice pattern. ***D***, Percent choice of each option at baseline. In the uncued task, ropinirole significantly increased choice of P1. In the cued task, ropinirole significantly decreases choice of P2, which is the most lucrative option. ***E***, The risk preference score during treatment and washout sessions. The score was calculated as the percentage of low-risk/low-reward choices (P1 and P2) minus the percentage of high-risk/high-reward choices (P3 and P4). Relative preference for the high-risk/high-reward options increased only in the ropinirole-treated animals performing the cued rGT and become progressively worse even after prolonged washout. ***F***, Motor impulsivity as measured by premature responses increased in the ropinirole-treated animals during the treatment but returned to normal in washout. All panels: Each point represents 1 animal. Lines/bars indicate mean ± SEM. $*p < 0.05$. $**p < 0.01$. $***p < 0.001$.

Table 1. Win-paired cues in the cued rGT[a]

Option	Cue duration (s)	Auditory cues	Visual cues	Variable
P1	2	1 tone	Flash H1, 2.5 Hz, 2 s	No
P2	2	2 tones, in sequence, 1 s each	Flash H4, 2.5 Hz, 2 s	No
P3	2	3 tones, in sequence, 0.2 s each	Flash H5, 5 Hz, 1 s; Flash H2, H3, H4, 5 Hz, 1 s	Yes; 2 patterns
P4	2	6 tones, in sequence, 0.2 s each	Flash H2, 5 Hz, 1 s; Flash H1, H2, H3, H4, H5, 5 Hz, 1 s	Yes; 4 patterns

[a]Audiovisual cues associated with wins increase in complexity as the magnitude of reward increases (Barrus and Winstanley, 2016).

cartoon in Winstanley and Floresco (2016) and created with www.BioRender.com.

On a rewarded trial, the light in the stimulus aperture would be extinguished, and the corresponding number of sucrose pellets would be delivered in the now-illuminated food tray. In the cued rGT, the delivery of the reward is paired with audiovisual cues that increase in complexity as the magnitude of reward increases (Table 1). A nose-poke in the food tray would then initiate a new trial.

If the response was punished, a corresponding time-out period would begin, during which the selected aperture flashed at a rate of

0.5 Hz, and no response was registered. After the time-out period, the food tray would be illuminated, and the animal was able to initiate a new trial by nose-poking in the food tray. If the rat nose-poked in any of the stimulus holes during the ITI, a premature response was registered, and a 5 s time-out period began marked by illumination of the house light. The animal was similarly unable to register a response during this time-out and can afterward initiate a new trial by nose-poking in the illuminated food tray.

The reinforcement schedule on the four options is summarized in Figure 1*A*. The position of each option was counterbalanced across subjects to mitigate any thigmotaxic biases toward the more medial or lateral holes. In Version A, the options were arranged P1, P4, P2, and P3 from left to right, whereas in Version B, the arrangement was P4, P1, P3, and P2.

Behavioral measures

To measure the relative choice of the low-risk/low-reward to high-risk/high-reward options, as a proxy of optimal risk-based decision-making, a variable called score was developed to reflect the extent to which each animal's choice was optimal. The risk preference score variable was calculated as [(percent choice of the P1 option) + (percent choice of the P2 option)] – [(percent choice of the P3 option) + (percent choice of the P4 option)] (Zeeb and Winstanley, 2011). Since choice of either P1 or P2 yields a higher number of sugar pellets as well as fewer and shorter time-out periods across a session, any positive value of the score variable reflects a rationally advantageous choice preference. In contrast, negative values indicate a preference for suboptimal high-risk/high-reward options. This variable is similarly often used to reflect choice preference on the Iowa Gambling Task (Bechara et al., 1994).

Choice of each individual option was calculated as follows: (total number of choice of a given option)/(total number of trials completed) × 100. Percentage of choice, rather than raw number of choices, was used as a measure of choice preference to control for the variability in total number of trials completed in each session.

Any responses made during the ITI would count as a premature response and has been previously shown to be a well-validated and reliable measure of motor impulsivity in the 5-CSRT task and derivatives of it using a similar paradigm, including the rGT (Voon et al., 2014). Premature responses were calculated as a percentage variable: (total number of premature responses)/(total number of trials initiated) × 100.

Other behavioral measures were sum of omitted responses, sum of completed trials, and average latencies to choose an option and to collect rewards.

Surgery to implant osmotic pumps

After the 5-CSRT training and before the beginning of the forced-choice rGT training, animals were implanted with a Model 2ML4 osmotic pump (Alzet, Durect) delivering either 5 mg/kg/d of ropinirole hydrochloride (Tocris Biosciences, R&D Systems; $n = 32$), 2.5 mg/kg/d of ropinirole ($n = 32$), or 0.9% saline solution ($n = 48$). Osmotic pumps allowed for steady delivery of the drug, which is consistent with prolonged-release pills in human patients (Nashatizadeh et al., 2009). The low dose used here was derived from dose conversion from human to rat (Shin et al., 2010; Nair and Jacob, 2016) based on the 24 mg/d used in prolonged-release pills in PD patients (Nashatizadeh et al., 2009; Zhu and Chen, 2021). The high dose was chosen based on previous publications (Cocker et al., 2016; Tremblay et al., 2017, 2019; Russell et al., 2021). The pumps delivered ropinirole over 28 d and were left in place for the maximum duration allowed (i.e., 42 d). Based on the capacity of the pumps, the rate of drug delivery, and the manufacturer's specifications, no drug should have been delivered during the 14 washout days. Animals performed 7 sessions of forced-choice and 14 sessions of free-choice rGT with the drug on board (during the first 28 d after pump implantation). They then performed 14 additional washout sessions (see Fig. 1*B*).

The osmotic pumps were sterilely filled with concentration of solution based on each rat's weight a day before implantation. They were then kept overnight in a sterile 50 ml falcon tube filled with 0.09% saline solution to allow for immediate release of the solution following implantation. Calculations for formulating the solution were performed using the Alzet guide. Animals were anesthetized with isoflurane (4% induction) and monitored continuously during surgery. Levels of the inhalant were adjusted (to ~2.5%) to maintain a surgical plane of anesthesia throughout the surgery. Ketoprofen and bupivacaine (both from AVP Supplies) were administered subcutaneously as systemic and local analgesic, respectively. Pumps were implanted subcutaneously on the back of the animal posterior to the scapulae.

Animals remained group-housed in their home cages. To control for the dynamic of cages, each cage consisted of rats receiving ropinirole and saline. Testing resumed after 2 d following surgery. All animals were closely monitored and treated every day with consultation of the veterinarian staff. Ropinirole seemed to cause some skin irritations around the modulator of the implanted pumps (not the incision site) starting ~10 d after surgery. Therefore, on regular consultation with the veterinary staff, tropical treatments were provided to mitigate irritations. Despite this, a few animals kept scratching at the pump, and this led to breakage of the skin and exposing the pump. For this reason, 3 animals in the high-dose and two in the low-dose cohort had to be excluded from analyses involving repeated-measures ANOVA.

cFos immunohistochemistry

Following the last session, 60-90 minutes after performing the task, rats were transcardially perfused with 1% PBS followed by 4% PFA. Brains were then cryoprotected in 30% sucrose and sliced into 40 µm coronal sections containing the medial and lateral orbitofrontal cortex (mOFC and lOFC), basolateral amygdala (BLA), infralimbic cortex (IL), prelimbic cortex (PrL), NAcc, dorsal striatum (DStr), substantia nigra pars compacta (SNc), and ventral tegmental area (VTA). All brain regions were identified using the Rat Brain Atlas (Paxinos and Watson, 1998). Tissue was processed for immunohistochemistry in a subset of individuals (minimum of $n = 7$ per cued/uncued and saline/high-dose ropinirole condition). The sections were then blocked in 3% NGS and incubated in monoclonal rabbit anti-cFos (Millipore, RM374, 1:1000) for 24 h at 4°C in PBST. Sections containing the VTA and SNc were also incubated with monoclonal chicken anti-TH (Millipore, SAB5700892, 1:200) at the same time as anti-cFos. Sections were then washed and incubated in AlexaFluor-488 goat anti-rabbit (Invitrogen, Fisher Scientific, A11034; 1:400), and AlexaFluor-568 goat anti-mouse (Invitrogen, Fisher Scientific, A11004; 1:500) for midbrain VTA/SNc sections, for 2 h at room temperature followed by DAPI (Millipore, 1:1000). Sections were then cover-slipped under Krystalon mounting medium (Millipore-Sigma). Slides were then imaged in a single *z* plane on an SP8 confocal microscope (Leica). Two randomly selected Regions of Interest (ROIs) of 69,000-112,000 μm^2 were captured for each brain ROI, specified above, for each subject. Two researchers blind to the experimental conditions manually counted cells within the ROI boundary that were $cFos^+$. For midbrain VTA/SNc sections, the number of $cFos^+$ cells that coexpressed TH were also counted. Subjects were pseudorandomly counterbalanced between the two researchers. Cell count data were recorded as density of cFos or $cFos/TH^+$ cells (i.e., number of cells counted/area of ROI).

Data analyses

Behavioral data. All analyses were conducted using R (version 4.1.2). Repeated-measures ANOVA was performed to test the effect of drug dose (3 levels: 0, 2.5, and 5 mg/kg/d; between-subject), cue (2 levels: cued and uncued rGT; between-subject), and session (14 levels; within-subject) on behavioral measures. The main dependent variables were the score variable, percent choice of each option (four levels: P1, P2, P3, and P4; within-subject), and premature responses. Additional dependent variables included omissions, trials completed, choice, and collect latency. Analyses were conducted separately for the sessions during drug administration (14 sessions) and in washout (14 sessions). To prevent ceiling effects, percent variables were arcsine transformed for statistical tests but were plotted as percentage values. Here, we define baseline as the last five sessions, during which animals were stable in their choice patterns, as identified by a nonsignificant main effect of session on choice and a nonsignificant session by option interaction (all t values < 1.2; p values > 0.23).

Trial-by-trial analyses. In order to assess how outcome of a chosen option affects the decision to switch to a different choice category (i.e.,

high-risk/high-reward vs low-risk/low-reward), a logistic mixed-effects model was fitted to the trial-by-trial data from the first five acquisition sessions with subject as random effect. All trials, on which animals either failed to respond (i.e., omitted trials) or had a premature response, were excluded for the purpose of trial-by-trial analyses.

cFos data quantification. The number of $cFos^+$ cells were first averaged across samples within each region for any single animal. Effects of cue, drug, and cue by drug on the number of $cFos^+$ cells were then tested for seven ROIs.

cFos network analysis. To gain insight into the functional relationship between ROIs, we calculated the correlation between any two regions using a Spearman rank correlation within each group (drug treatment vs saline and cued vs uncued task). We then report edges with $p < 0.05$ weighted by the strength of the association in Figure 6. To compare these paired associations between experimental groups, we used a Fisher's Z transformation to convert correlation coefficients to Z scores. R code to perform these analyses was adapted from the published code by Ruiz et al. (2021).

Results

As expected from previous studies, rats performing the cued rGT generally had lower scores, indicative of greater risky choice, than those performing the uncued version of the task during both drug treatment and washout (main effect of cue: treatment: $F_{(1,101)} = 6.44$, $p = 0.013$; washout: $F_{(1,101)} = 9.57$, $p = 0.003$). We perform further analyses separately for cued and uncued tasks because we had specific predictions that disadvantageous risky choice would be exacerbated in presence of win-paired cues as this effect has been repeatedly replicated in previous reports (Barrus and Winstanley, 2016; Adams et al., 2017; Ferland et al., 2019; Tremblay et al., 2019).

Animals chronically treated with ropinirole decreased their preference for the optimal choices only when rewards were paired with audiovisual cues. Control animals typically adopt an optimal pattern of choice when they reach choice stability, which is preferring the low-risk/low-reward options. We observe here that the majority of control animals had already adopted an optimal choice pattern by the last five sessions in both uncued (75%) and cued (72%) versions of the task. In the cued rGT, however, this pattern was flipped such that only a minority of ropinirole-treated animals had an optimal choice pattern (27% in low dose; 31% in high dose; Fig. 1*C*). Ropinirole-treated animals did not differ from controls in the uncued task (χ^2 (2, $N = 55$) = 3.39, $p = 0.184$) but had a significantly higher number of suboptimal choosers in the cued rGT (χ^2 (2, $N = 52$) = 9.29, $p = 0.0096$).

Regarding specific choice profiles, ropinirole influenced the choice of the low-risk/low-reward options. Specifically, in the cued task, ropinirole decreased the choice of P2, which is the most lucrative option (treatment: $F_{(2,49)} = 5.41$, $p = 0.008$; washout: $F_{(2,49)} = 5.93$, $p = 0.005$; Fig. 1*D*). In the uncued task, ropinirole-treated animals significantly increased their choice of P1 (treatment: $F_{(2,52)} = 5.17$, $p = 0.009$; washout: $F_{(2,52)} = 6.50$, $p = 0.003$). Follow-up analyses comparing the two doses of ropinirole showed that there was no significant difference between the two doses on the choice of P2 in the cued rGT (in treatment and washout: both F values < 0.53, both p values > 0.48) and a trending difference in choice of P1 in the uncued task (treatment: $F_{(1,26)} = 3.77$, $p = 0.063$; washout: $F_{(1,26)} = 3.03$, $p = 0.094$). With regards to the high-risk/high-reward options, the drug had no significant effect on the percent choice of either P3 or P4 options (P3: cued treatment: $F_{(2,49)} = 2.50$, $p = 0.093$; –washout: $F_{(2,49)} = 1.93$, $p = 0.156$; P3: uncued treatment: $F_{(2,52)} = 0.37$, $p = 0.690$; –washout: $F_{(2,52)} = 2.32$, $p = 0.108$; P4 cued and uncued: all F values < 1.2; all p values > 0.34). During the last five sessions (i.e., at baseline), when animals were statistically stable in their choice patterns (for all four options: all t values < 1.2; p values > 0.23), these differences were preserved (uncued-P1: $F_{(2,52)} = 3.75$, $p = 0.03$; cued-P2: $F_{(2,49)} = -4.34$, $p = 0.018$; Fig. 1*D*). Overall, these choice profiles (increased P1 in uncued and decreased P2 in the cued task) are consistent with an increase in suboptimal decision-making observed only in the cued task.

The ropinirole-induced reduction in advantageous decision-making in the cued task continued progressively after termination of drug administration (Fig. 1*E*). This effect was revealed by a significant main effect of drug on the risk preference score in the cued, but not uncued group when controlling for the effect of session during both treatment and washout periods (cued treatment: $F_{(2,49)} = 3.30$, $p = 0.045$; –washout: $F_{(2,49)} = 3.98$, $p = 0.025$; uncued treatment: $F_{(2,52)} = 0.17$, $p = 0.84$; –washout: $F_{(2,52)} = 1.72$, $p = 0.188$). On the cued rGT, the risk-preference score did not differ between the two doses of ropinirole in either treatment or washout (both F values < 0.25, both p values > 0.62). During the last five sessions, ropinirole-treated animals in the uncued task similarly did not significantly differ from controls ($F_{(2,52)} = 2.50$, $p = 0.092$), whereas in the cued task, they were significantly more risk preferring ($F_{(2,49)} = 4.20$, $p = 0.021$). *Post hoc* pairwise t tests in the cued group revealed that there was a significant difference between saline and low-dose with a large effect size ($t_{(37)} = 2.45$, $p = 0.019$, Cohen's $d = 0.81$) as well as saline versus high-dose group ($t_{(35)} = 2.27$, $p = 0.030$, Cohen's $d = 0.78$), but no significant difference between the two doses ($t_{(26)} = -0.025$, $p = 0.98$).

In contrast to the long-lasting effects ropinirole had on risk preference in the cued rGT, premature responses were only marginally elevated as a result of drug condition during ropinirole treatment itself ($F_{(2,49)} = 2.60$, $p = 0.084$) and reverted to control levels before washout ($F_{(2,49)} = 0.83$, $p = 0.443$; Fig. 1*F*). Follow-up pairwise comparisons during treatment suggest that premature responses were significantly increased only in the high-dose group compared with the saline group in the cued task ($F_{(1,35)} = 4.48$, $p = 0.041$). In the uncued rGT, despite a null effect of ropinirole on risk preference, premature responses were elevated by ropinirole treatment ($F_{(2,52)} = 22.44$, $p < 0.0001$), but this effect similarly dissipated once the drug was no longer on board ($F_{(2,52)} = 1.88$, $p = 0.163$). The effect of ropinirole on premature responses was dose-dependent in the uncued task, with the higher dose producing a more pronounced increase in premature responses than the lower dose (low-dose vs saline: $F_{(1,37)} = 4.17$, $p = 0.048$; low-dose vs high-dose: $F_{(1,29)} = 18.55$, $p < 0.001$). In both versions of the task, ropinirole did not affect motor impulsivity beyond the drug administration period. Together, these results show that the effects of ropinirole on choice were dissociable from motor impulsivity.

Across both task variants, we also found a significant main effect of condition on omissions (treatment: $F_{(2,101)} = 7.01$, $p = 0.001$; washout: $F_{(2,101)} = 13.07$, $p < 0.001$), trials completed (treatment: $F_{(2,101)} = 5.37$, $p = 0.006$; washout: $F_{(2,101)} = 8.81$, $p < 0.001$), choice latency (treatment: $F_{(2,101)} = 30.26$, $p < 0.001$; washout: $F_{(2,101)} = 15.07$, $p < 0.001$), and collect latency (treatment: $F_{(2,101)} = 4.34$, $p = 0.016$), though the latter effect was marginal during washout ($F_{(2,101)} = 2.50$, $p = 0.087$). *Post hoc* pairwise analyses revealed that animals receiving the low dose of ropinirole did not differ from controls in their latency to choose and collect reward (all F values < 0.9, all p values > 0.3), but animals receiving the high dose were faster than controls to make a choice (treatment: $F_{(1,73)} = 51.35$, $p < 0.001$; washout: $F_{(1,73)} = 20.76$, $p < 0.001$; Fig. 2*A*) and to collect rewards (treatment: $F_{(1,73)} = 7.26$, $p = 0.009$; washout: $F_{(1,73)} = 7.37$, $p = 0.008$; Fig. 2*B*).

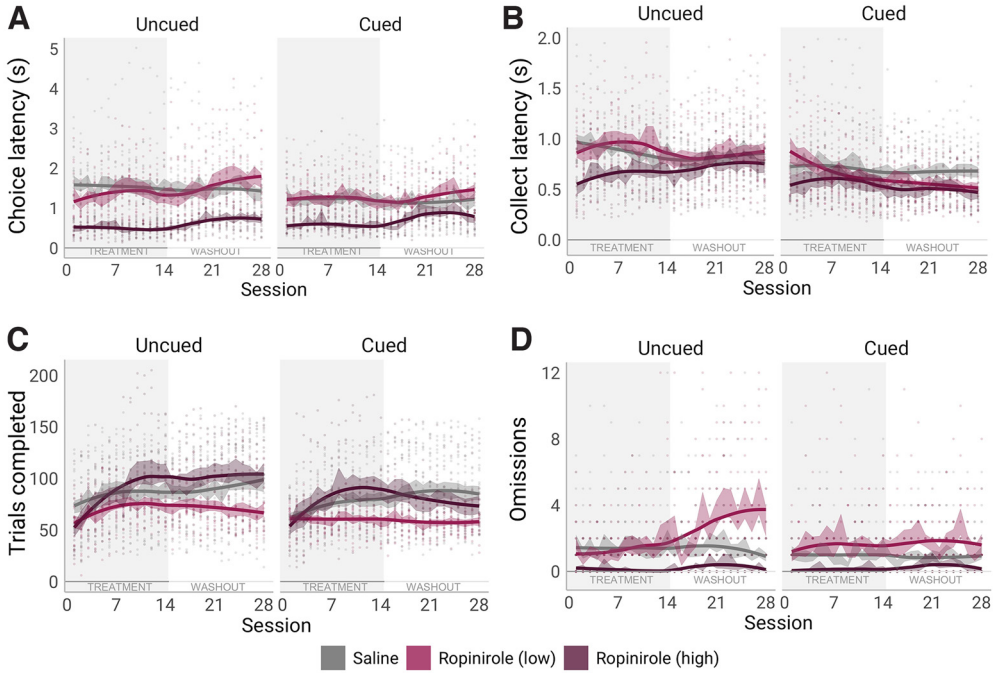

Figure 2. Other behavioral variables over sessions. ***A***, ***B***, Animals receiving the high dose, but not the low dose, were faster than controls to make a choice and to collect rewards. ***C***, The low-dose group, but not the high-dose group, completed fewer trials relative to controls throughout testing. ***D***, The high-dose group had significantly fewer omitted trials across sessions, but the low-dose group omitted more trials during washout. All panels: Each point represents 1 animal. Lines/bars indicate mean ± SEM. $*p < 0.05$. $**p < 0.01$. $***p < 0.001$.

Comparing the number of omissions and trials completed suggested a rather nonlinear effect of dose. Compared with controls, the low-dose group omitted more trials during washout ($F_{(1,74)} = 11.33$, $p = 0.001$) but not during treatment ($F_{(1,74)} = 0.71$, $p = 0.40$), whereas the high-dose group had significantly fewer omitted trials during both treatment ($F_{(1,73)} = 13.02$, $p < 0.001$) and washout ($F_{(1,73)} = 10.17$, $p = 0.002$; Fig. 2*D*). Additionally, the low-dose group completed fewer trials relative to controls throughout testing (treatment: $F_{(1,74)} = 8.97$, $p = 0.004$; washout: $F_{(1,74)} = 14.38$, $p < 0.001$; Fig. 2*C*), whereas this effect was not evident in rats treated with the high dose (both F values < 0.5, both p values > 0.50). Biphasic responses to the locomotor effects of D_2 receptor agonism, such that lower doses produce more pronounced inhibition than higher doses which ultimately stimulate activity, have been reported for decades (Eilam and Szechtman, 1989). However, other measures on task were either affected uniformly by both doses or showed linear dose sensitivity. The exact reasons for these behavior-specific effects of ropinirole are not easy to discern from the current dataset but may reflect differences in receptor occupancy and activation of different subpopulations of D_2-receptor expressing cells across distinct regions (e.g., Hartesveldt et al., 1992; Peczely et al., 2022).

Trial-by-trial analyses

Following up on the choice effects in the cued task, we investigated the trial-by-trial choice during the initial acquisition sessions. We asked whether differences in win-stay/loss-shift patterns could account for the robust differences in risk preference that were firmly established in the last five sessions when behavior was statistically stable. Since there was no difference between the choice profiles of animals receiving low and high doses of ropinirole in the cued task, the two doses were pooled for the following analyses.

In the last five sessions (s24-s28), ropinirole-treated animals preferred the high-risk/high-reward options, whereas control animals preferred the more lucrative low-risk/low-reward options ($t_{(50)} = 2.93$, $p = 0.005$; Fig. 3*A*). In the initial five sessions, control animals rapidly learned to more often avoid repeating a rewarded rare win, but we found that ropinirole-treated animals were more likely to keep choosing a high-risk/high-reward option after experiencing an unlikely large win ($t_{(50)} = 3.77$, $p < 0.001$; Fig. 3*B*). Drug and saline-treated animals were not significantly different in their subsequent choice following any of the other possible outcomes (all t values < 1.88, all p values > 0.05). Probabilities of switching or staying after each outcome were estimated using a logistic regression model fitted to the trial-by-trial choice.

When controlling for the probability of switching choice categories after a large unlikely win, the effect of drug on choice preference was no longer significant (Fig. 3*C*). Using a bootstrapped causal mediation model (with 3000 simulations; *mediation* R package 4.5.0) (Tingley et al., 2014), we found that lower probability of switching to a low-risk/low-reward choice after experiencing a large unlikely win mediated the effect of drug on later risk preference score (average causal mediation effect = −0.26, [−0.51, −0.06] 95% CI). In other words, animals that had a higher probability of switching back to a safe option after experiencing a large unlikely win were more likely to have an optimal choice pattern later on at baseline.

cFos immunohistochemistry

To gain insight into the neural correlates of the observed behavior in animals, we looked at expression of cFos *ex vivo*. Compared with rats trained in the uncued rGT, those trained in the cued rGT showed significantly lower cFos expression in the dopaminergic neurons of the VTA and SNc (main effect of cue in VTA; $F_{(3,45)} = -7.26$, $p < 0.0001$; SNc: $F_{(3,45)} = -5.50$, $p < 0.0001$) but marginally higher cFos expression in the striatum (main effect of cue in NAcc; $F_{(3,33)} = 2.07$, $p = 0.047$; DStr:

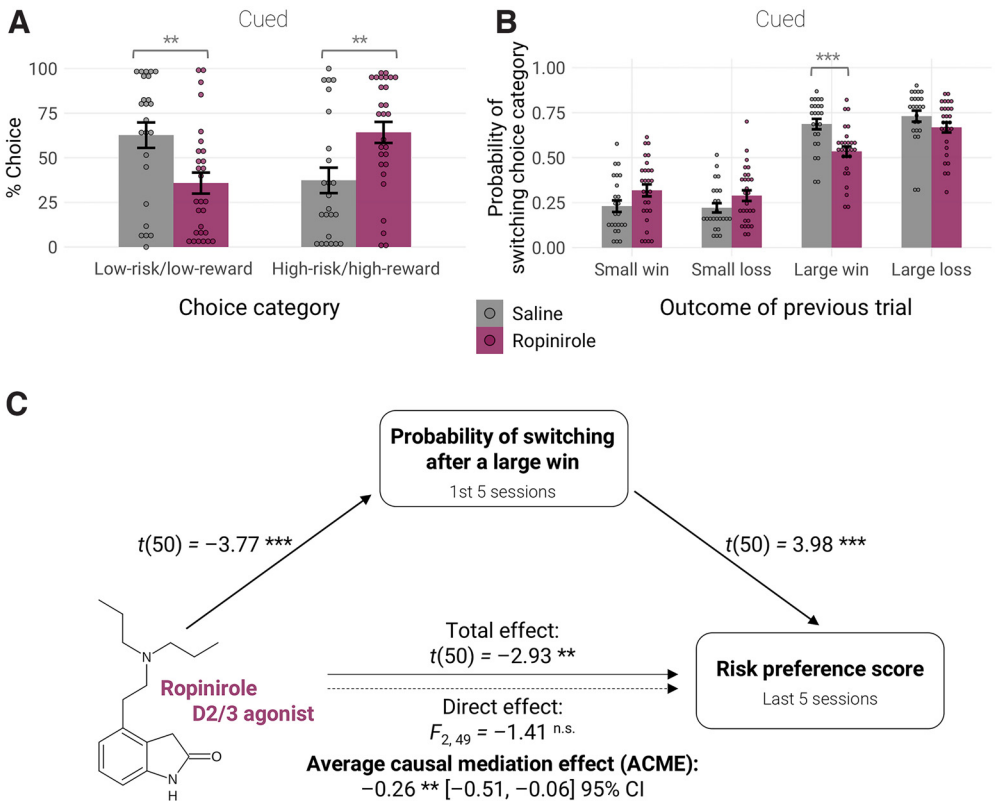

Figure 3. Choice following a highly cued large unlikely win during acquisition mediates the effect of ropinirole on later preference for high-risk/high-reward options. ***A***, In the last five sessions (s24-s28), ropinirole-treated animals preferred the high-risk/high-reward options, whereas control animals preferred the more lucrative low-risk/low-reward options. ***B***, In the initial five sessions, control animals rapidly learned to more often avoid repeating a rewarded rare win, but ropinirole-treated animals were more likely to keep choosing a high-risk option after experiencing an unlikely large win. Drug and saline-treated animals were not significantly different in their subsequent choice following any of the other possible outcomes. ***C***, When controlling for the probability of switching choice categories after a large unlikely win, the effect of drug on choice preference was no longer significant. Using a bootstrapped causal mediation model (with 3000 simulations), we found that the probability of switching after a large unlikely win mediated the effect of drug on later risk preference score. All panels: Each point represents 1 animal. Lines/bars indicate mean ± SEM. $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

$F_{(3,33)}$ = 1.85, $p = 0.07$; all other regions: F values < 1.14, p values > 0.49).

Compared with saline controls, ropinirole treatment in the uncued task enhanced cFos activity in the prefrontal and striatal regions but diminished it in the midbrain regions (Table 2; Fig. 4). Since there were similar patterns among our experimental groups across midbrain regions (VTA and SNc), striatal regions (DStr and NAcc), and all four prefrontal regions (lOFC, mOFC, IL, and PrL), we averaged across those regions (Fig. 5*B*).

cFos expression in the BLA had a trending negative association with score on the corresponding last session (spearman *Rho* = −0.28, $p = 0.076$), but none of the other tested regions were significantly correlated with the main rGT behavioral measures (all R values < 0.33, p values > 0.10).

We were initially surprised to see stronger differences in cFos activity in the uncued group, in which we do not see changes in risk preference and wondered whether some of these changes may reflect compensatory mechanisms. Therefore, we asked whether relative activity of these regions is important for regulation of choice in the task. To do this, we derived a ratio metric, calculated as the within-subject ratio of $cFos^{+}$ cells in each region group. We further log-transformed the ratios to avoid artificial asymmetry between the effects of the numerator and denominator (Hedges et al., 1999). Relating this measure to behavior on the last session, midbrain:prefrontal activity was significantly lower in the animals with a suboptimal choice pattern who favored the high-risk/high-reward options compared with those preferring the low-risk/low-reward options across all groups ($t_{(22)} = -2.41$; $p = 0.025$; Fig. 5*C*). Treating risk preference as a continuous variable, midbrain:prefrontal activity was also marginally associated with risky decision-making across all rats ($t_{(21)} = 1.97$; $p = 0.06$; Fig. 5*D*).

Table 2. Regional expression of $cFos^{+}$ neurons

		Cue		Drug		Cue × drug	
	df	*F*	*p*	*F*	*p*	*F*	*p*
mOFC	3, 27	0.14	0.89	3.89***	0.0006	−2.19*	0.038
lOFC	3, 27	0.16	0.87	3.06**	0.005	−1.89†	0.069
PrL	3, 27	0.91	0.37	3.42**	0.002	−2.58*	0.016
IL	3, 27	0.10	0.92	2.75*	0.011	−1.53	0.137
DStr	3, 33	1.85†	0.074	2.92**	0.006	−3.26**	0.003
NAcc	3, 33	2.07*	0.047	2.68*	0.011	−3.69***	0.0008
BLA	3, 36	0.31	0.76	2.93**	0.006	0.35	0.726
VTA	3, 45	−7.26***	<0.0001	−4.50***	<0.0001	3.13**	0.003
SNc	3, 45	−5.50***	<0.0001	−4.06***	0.0002	2.85**	0.007

$^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$. †$p < 0.1$.

Network analyses

During the uncued task, control animals showed significant functional correlation among the three prefrontal regions (IL, PrL, and OFC), between dorsal and ventral striatum, as well as between the midbrain regions (SNc and VTA; Fig. 6*A*). Presence of cues, however, was characterized by significant functional

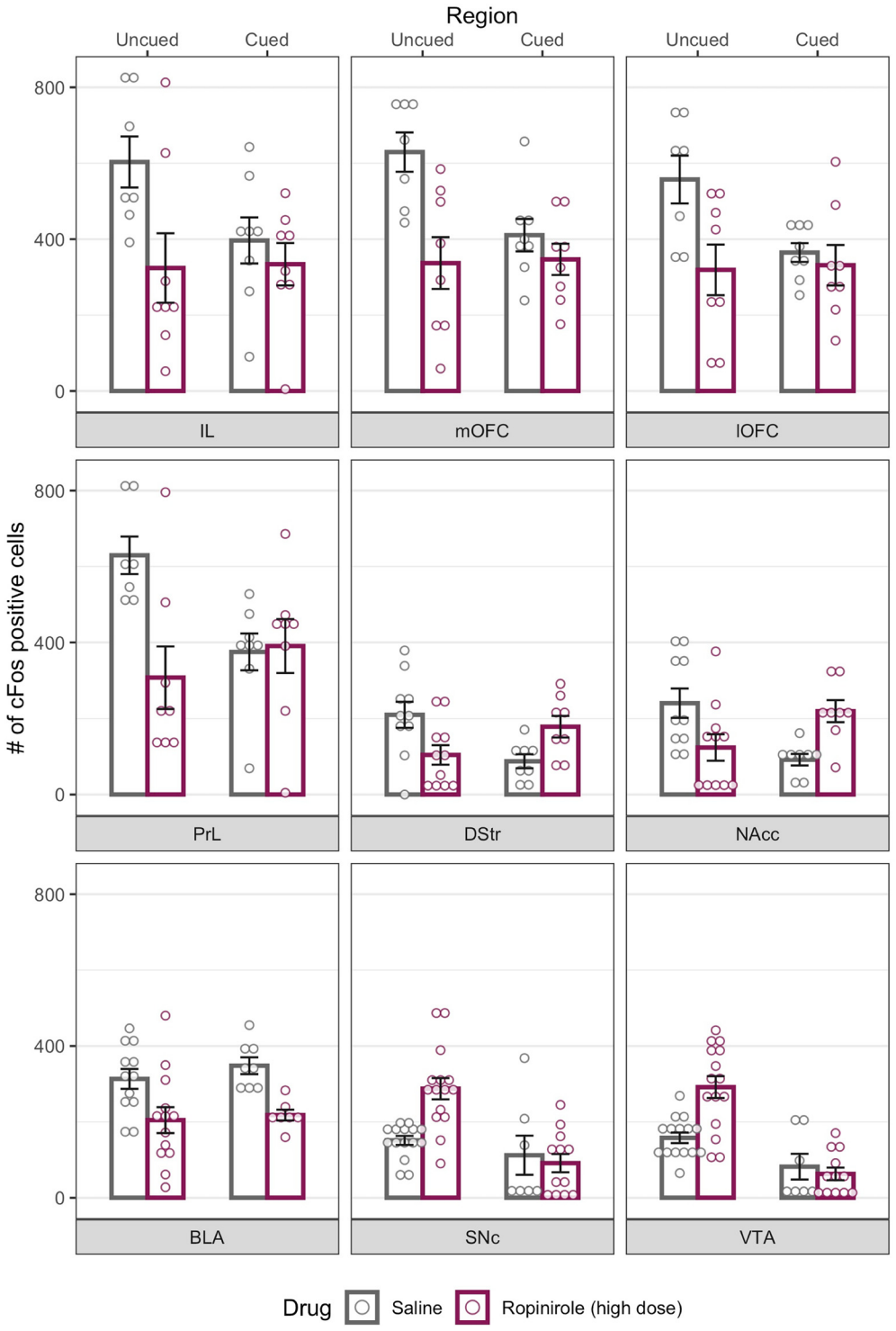

Figure 4. Regional expression of cFos. Each dot is the average count for each region for each subject. Bar columns and error bars indicate mean ± SEM (for statistics, see Table 1).

coupling between the VTA and the NAcc, NAcc and OFC, but decoupling between the OFC and the BLA (Fig. 6*B*).

Treatment with ropinirole qualitatively reduced the correlated edges in both cued and uncued task (Fig. 6*C*,*D*). In the uncued task, ropinirole increased the strength of a negative correlation between the PrL and the BLA (saline: *Rho* = –0.37, $p = 0.47$; ropinirole: *Rho* = –1.0, $p < .001$; Fisher $Z = -17.98$; Fig. 6*A*,*C*,*E*) as well as switching the negative correlation between the OFC and SNc (saline: *Rho* = –0.60, $p = 0.21$; Fig. 6*A*) to a positive association (ropinirole: *Rho* = 0.90, $p = 0.037$; Fisher $Z = 2.17$; Fig. 6*C*,*E*). In the cued task, ropinirole switched from a significant positive correlation between NAcc and VTA (*Rho* = 0.90, $p = 0.037$; Fig. 6*B*) to an anticorrelation (*Rho* = –0.75, $p = 0.05$; Fig. 6*D*), a change that was also significant (Fisher $Z = -2.45$; Fig. 6*F*).

Discussion

Here we show that the effect of reward-paired cues on risky choice can be potentiated with $D_{2/3}$ agonist administration while

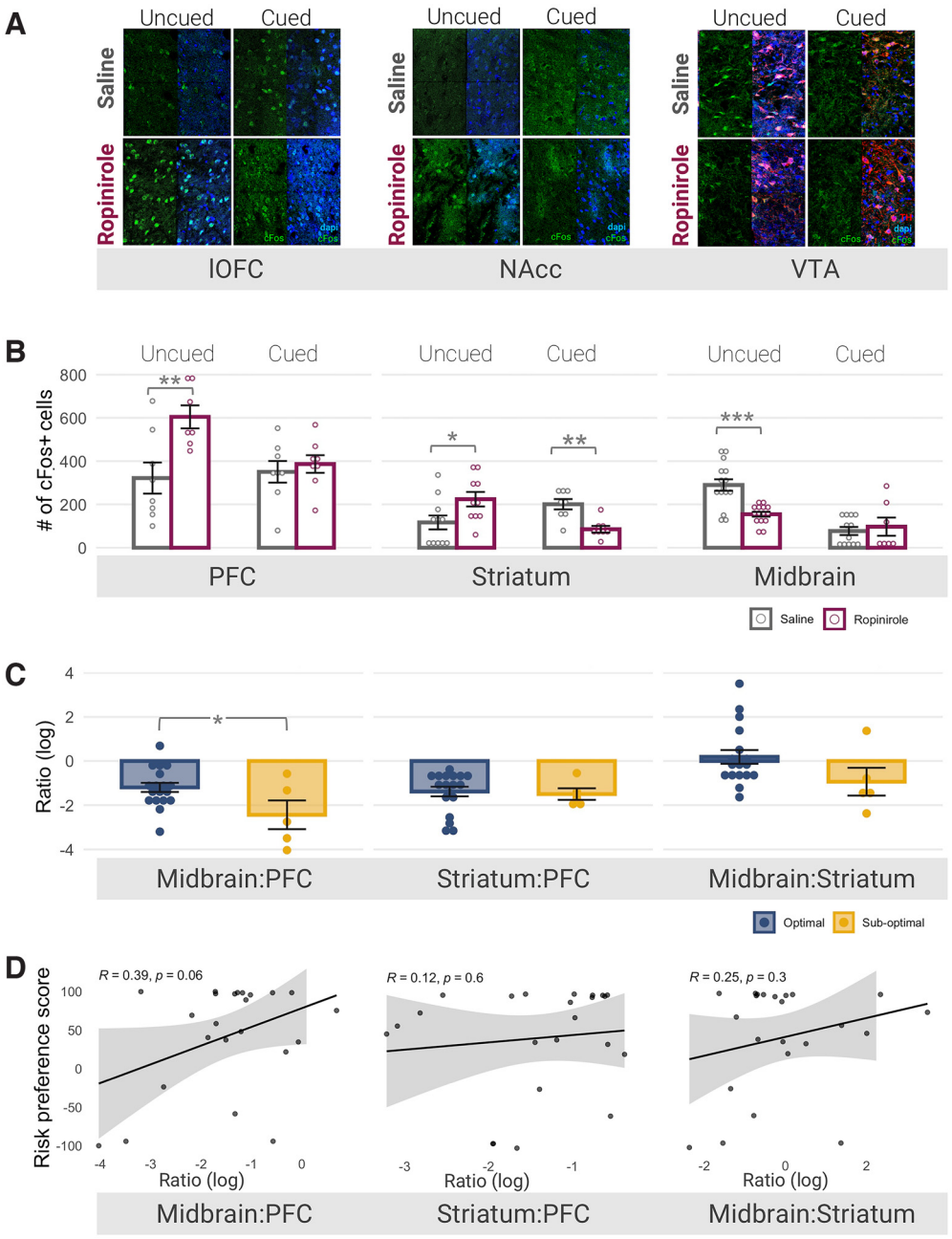

Figure 5. Regional expression of cFos across treatment group and task variant. ***A***, Representative slices from IOFC, NAcc, and VTA. Blue represents DAPI. Green represents cFos. Red represents TH. In each image, rectangles on the left represent cFos only, whereas rectangles on the right represent colocalization of cFos and/or DAPI and TH on the same slice section. ***B***, Number of $cFos^+$ cells averaged over prefrontal regions (including lOFC, mOFC, IL, and PrL), striatal regions (NAcc and dorsal striatum), and midbrain regions (SNc and VTA dopaminergic neurons). ***C***, Ratio of $cFos^+$ cells (log-transformed) in each of the three regions calculated within individuals and plotted for optimal versus suboptimal choosers. Midbrain:prefrontal (PFC) ratio was significantly lower in suboptimal animals. ***D***, Across all animals, the ratio of midbrain:PFC tended to correlate with individual differences in risk preference score. All panels: Each point represents 1 animal. Bars indicate mean ± SEM. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

healthy rats learned a gambling-like task. This bias was mediated by an increase in "win-stay" behavior on risky options after a large win, although the chances of such a win re-occurring were low. *Ex vivo* immunohistochemistry analyses revealed lower levels of cFos expression in midbrain dopaminergic neurons in rats trained on the cued rGT, an effect that was also mirrored in rats treated chronically with ropinirole. The ratio of midbrain:prefrontal $cFos^+$ neurons was lower in animals with a suboptimal choice pattern, and the degree of risky decision-making tended to correlate with the ratio of midbrain:prefrontal $cFos^+$ neurons across all rats. Loss of midbrain regulation of prefrontal regions could therefore be driving risky choice.

Ropinirole seems to influence risky decision-making when administered specifically during task acquisition, as we previously found no effect when treatment began after rats' choice had stabilized (Tremblay et al., 2019). Although this conclusion is weakened by the cross-study nature of the comparison, critical factors were consistent in both studies (e.g., identical surgical procedure, identical equipment, similar time of day for behavioral testing, etc.) which increases our confidence when contrasting the results. In PD patients, the majority who

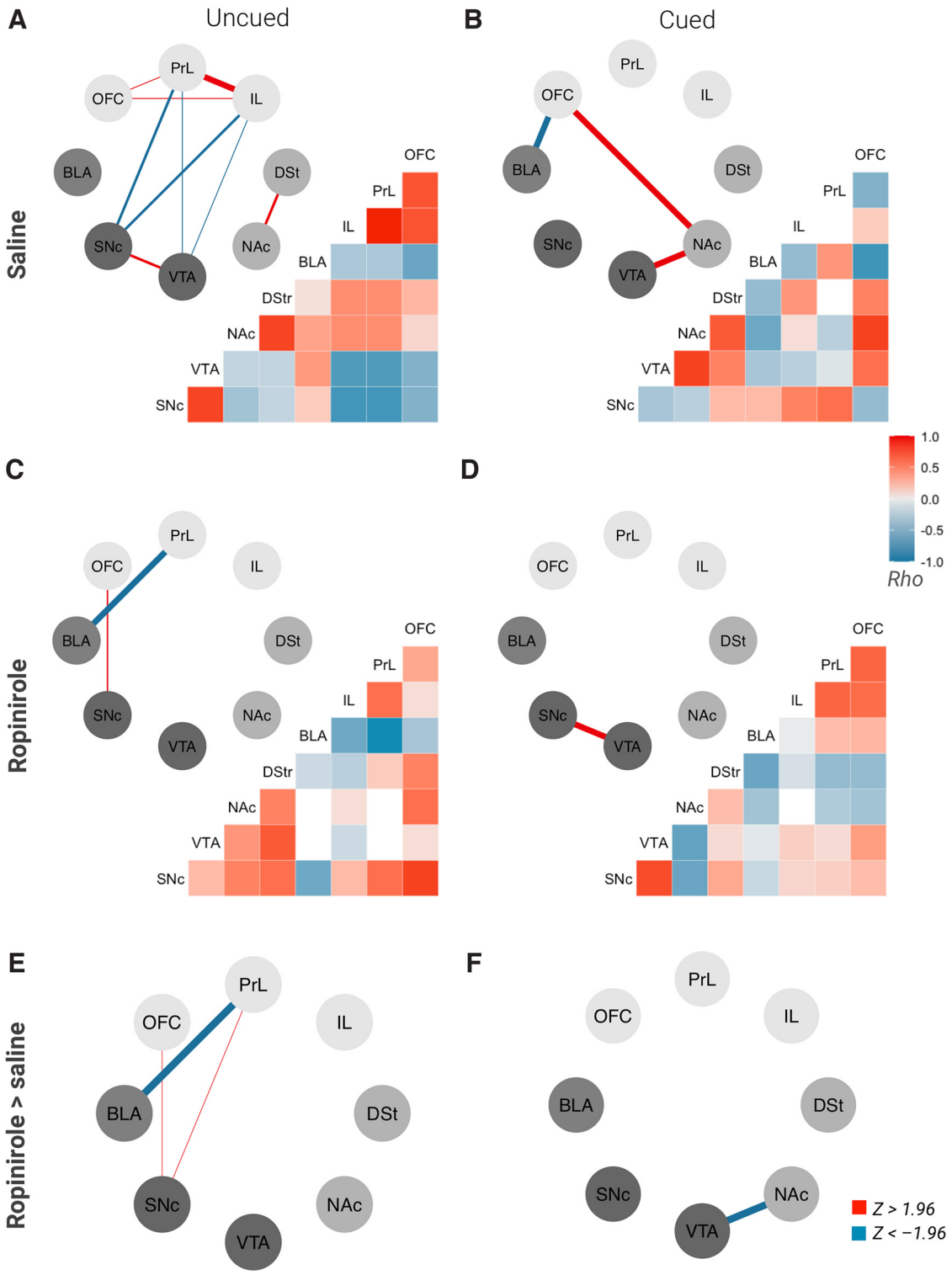

Figure 6. Network analysis based on regional cFos expression. The width of the edges on the circle diagrams is indicative of the weight of the edge; that is, the absolute value of the Spearman *Rho*, and is thresholded to only show significant correlations at α of $p < 0.05$. The correlation matrices represent the Spearman *Rho* coefficients for all pairs of regions, regardless of significance. ***A***, Functional correlations in the saline-treated group performing the uncued rGT showed significant positive correlations among the prefrontal regions, as well as within striatal and midbrain subregions, but negative correlations between midbrain and IL/PrL regions. ***B***, Saline-treated animals in the cued rGT showed strong positive association between VTA and the NAcc, NAcc and OFC, but an anticorrelation between the OFC and the BLA. ***C***, Ropinirole-treated rats in the uncued rGT did not show the within-region connectivity observed in controls but had an anticorrelation between the BLA and PrL and a positive correlation between SNc and OFC. ***D***, Ropinirole-treated rats in the cued rGT only had a significant functional coupling within the midbrain. ***E***, ***F***, Fisher's *Z* test conducted on the contrast between ropinirole > saline for uncued (***E***) and cued rGT (***F***). Only the significant edges are shown (i.e., $Z > 1.96$ and $Z < -1.96$).

develop GD after starting dopamine agonist therapy had never gambled before, and this poses a major risk factor for iatrogenic GD (Djamshidian et al., 2011). In another multicenter study of 388 patients taking anti-Parkinson's medication, most patients who had already regular but minor gambling expenditure did not show a change in their gambling engagement after treatment (Grosset et al., 2007). Our findings are therefore consistent with clinical observations that dopamine agonists mainly pose a risk for development of GD in patients who do not have a history of recreational gambling.

Ropinirole's ability to perpetuate risky decision-making during learning of the cued rGT was mediated by greater win-stay behavior after a risky win during early learning. Given the probabilistic reinforcement schedules in place, a risky win is a greater predictor of the next trial being unrewarded than a risky loss, such that win-stay behavior is arguably maladaptive. Repeating a previously rewarded action when reward delivery is rare is the hallmark of model-free behavior in the classic "two-step" task, whereas shifting to a different course of action that optimizes subsequent reward is only possible when subjects are using model-based decision-making strategies (Daw et al., 2011). A lower reliance on model-based strategies is associated with greater insensitivity to reinforcer devaluation, one of the classic definitions of habitual control (Gillan et al., 2015). As such, constant low-level $D_{2/3}$ receptor activation could promote learning through systems that are more habitual and less model-based.

This observation fits with the increase in compulsive behaviors observed clinically following chronic $D_{2/3}$ agonist administration (Weintraub et al., 2006). Impulsive behaviors, such as kleptomania and hypersexuality, can also manifest. Here, ropinirole increased impulsive (premature) responding in both the cued and uncued task. A pronounced role for mesolimbic dopamine in regulating premature responding has been known for decades, and both amphetamine and dopamine reuptake inhibitors amplify premature responding in a $D_{2/3}$-dependent manner (van Gaalen et al., 2006). High impulsivity can also result from increased arousal (Sun et al., 2010; Swann et al., 2013), and this physiological state facilitates dominance of behavior by less flexible and more automatic control systems (Arnsten, 1998). Alternatively, subjects may not adequately consider the costs in pursuit of a reward (for discussion, see, e.g., Fineberg et al., 2014), and therefore act impulsively but purposively in an effort to obtain a goal. Such behavior can reflect enhanced incentive motivation, and is thought to underpin aspects of drug-seeking (Robinson and Berridge, 1993). It is increasingly recognized that addictive drugs can result in aberrant yet highly effective goal-directed behavior and habit formation concurrently, rather than a simple "habit dominant" state (Daw, 2015). Ropinirole may likewise be acting through both behavioral control systems.

Alternatively, ropinirole may be enhancing sensitivity to reward, thereby promoting risk-taking after risky wins and disinhibiting actions associated with reward delivery leading to premature responding. In humans, reward sensitivity, as measured by pupil dilation to potential rewards, is enhanced in PD patients following dopaminergic medications (Manohar and Husain, 2015; Muhammed et al., 2016). However, following this account, it is not easy to see why reward-concurrent cues would be necessary for ropinirole's effects. Boosting accumbal dopamine signaling enhances conditioned reinforcement (Cador et al., 1991; Parkinson et al., 1999). Although we could find no evidence that basal levels of cue-driven risky choice are mediated by conditioned reinforcement, ropinirole administration could nevertheless enhance the ability of reward-paired cues to act as conditioned reinforcers, thereby potentiating learning from larger wins as documented here. Dopamine agonists also enhance reward anticipation. Using fMRI in humans, numerous studies have shown that activity increases in the NAcc during cues predictive of reward delivery, and activation scales with reward size (e.g., Knutson et al., 2001; Galvan et al., 2005; Srirangarajan et al., 2021). The magnitude of this signal correlates with striatal dopamine release (Schott et al., 2008), and pramipexole amplifies this effect (Ye et al., 2011). If reward-paired cues on the rGT elicit a similar gain anticipation signal in the NAcc, then ropinirole may promote choice of risky options by enhancing it.

The cFos imaging results may help to parse whether ropinirole's effects on choice are best explained by potentiated habitual control of learning, or enhanced dopaminergic modulation of anticipated reward. It is important to note that brain samples were taken 2 weeks after ropinirole administration should have ceased, based on amount of drug loaded and predicted rate of delivery. Failure to confirm drug was unequivocally absent at this time point is unfortunately a weakness in our methodology, but one that does not critically alter our conclusions. Risky choice remained elevated and was further exacerbated during this time period on the cued rGT, suggesting that drug-induced changes in neural regulation were still engaged. Although the number of $cFos^+$ neurons was higher in the NAcc of the cued rGT group, this was lower in ropinirole-treated animals, rather than higher as might be expected if ropinirole amplified an NAcc-dependent gain anticipation signal. Fewer $cFos^+$ neurons were observed in midbrain dopaminergic nuclei in animals performing the cued versus the uncued rGT, consistent with previous reports of reduced accumbal dopamine efflux following cued rGT training (Ferland et al., 2019). Network analyses suggested a decoupling of functional connectivity between the NAcc and the VTA in response to drug treatment in the cued task. Given that the anticipated reward signal in the accumbens correlates with activity in the SNc/VTA, this is hard to reconcile with a ropinirole-induced amplification of such an anticipatory signal.

$cFos^+$ counts in any brain region alone did not correlate with risky decision-making. However, activity in frontal, striatal, and midbrain regions all changed differentially across task variant in response to ropinirole administration, suggesting that the balance of relative activity might be important. In support of this hypothesis, the ratio of midbrain dopamine: prefrontal activity within each subject dissociated risk-preferring and optimal decision-makers, and also somewhat predicted rats' risk preference across all tasks and conditions. As such, it seems that prefrontal activity either uninformed by endogenous dopaminergic signaling, or responding to impoverished dopamine output, drove risky decision-making, and this was facilitated by prior administration of ropinirole. Indeed, recent data highlight the importance of midbrain dopamine to frontal coherence in optimizing cognitive performance in both humans and rodents (Parker et al., 2015; Kim et al., 2017; Q. Zhang et al., 2021).

Given its therapeutic efficacy in treating motor symptoms resulting from dopamine loss, it may seem surprising that a $D_{2/3}$ agonist would suppress the activity of dopamine neurons. Within the midbrain, D_2 receptors act as autoreceptors on dopaminergic cell bodies, such that an agonist would be expected to reduce their likelihood of firing (Aghajanian and Bunney, 1977; Courtney et al., 2012). Previous studies have also found decreases in dopamine levels following chronic treatment with $D_{2/3}$ agonists (Koeltzow et al., 2003; de Haas et al., 2011). Nevertheless,

the idea that decreasing dopaminergic activity could increase risk-taking and impulsivity, and thereby facilitate GD, seems contrary to considerable literature showing that boosting dopamine signaling is critical for drug addiction. However, other findings strongly suggest that addictive behaviors can arise from a hypodopaminergic state, leading to reward deficiency (George et al., 1995; Blum et al., 2000; Volkow et al., 2007; Diana, 2011; L. Zhang et al., 2012; Siciliano et al., 2015).

Thinking about addictive behaviors as being triggered by "too much" or "too little" dopamine is hard to reconcile with the astounding complexity of this neuromodulatory system. Functional heterogeneity exists within dopaminergic cells of both the VTA and SNc, and these distinctions are not obvious from neuroanatomy alone (Lammel et al., 2014; Lerner et al., 2015; Margolis et al., 2008). In the striatum, $D_{2/3}$ receptors are located on cholinergic interneurons, medium spiny neurons, and on dopaminergic terminals. Activation of these cell types can influence each other in myriad ways that can vary across striatal subregion (e.g., Cai and Ford, 2018), such that predicting the net effect of chronic ropinirole is challenging. Theoretically, activation of D_2 receptors on cholinergic interneurons should inhibit their activity (Kreitzer, 2009), potentially impacting their ability to coordinate striatal MSN firing and enable cognitive flexibility (Aoki et al., 2015). Alternatively, chronic ropinirole may mimic the effect of repeated cocaine injections and decrease the sensitivity of striatal D_2 receptors by reducing the relative expression and coupling of G-protein subunits (Gong et al., 2022). A significant population of D_2 receptors are also found in frontal cortex, not only on layer V pyramidal neurons and parvalbumin-containing interneurons (Gee et al., 2012; Seaman et al., 2017), but spanning multiple cortical layers and interneuron subtypes (Khlghatyan et al., 2019). This latter study also reported a novel cluster of D_2^+ cells in both auditory and visual cortex, which may be relevant for the more pronounced effect of ropinirole when audiovisual cues were present.

As electronic games, replete with "bells and whistles," continue to dominate as forms of entertainment, it is important to realize that such sensory stimulation can interact with drug treatment to produce surprisingly long-lasting and impactful effects on brain function and decision-making. It will also be important to evaluate ropinirole's effects in female rats given the notable sex differences reported in both dopaminergic regulation of behavior and the trajectory of addiction (Williams et al., 2021). Understanding the processes through which the brain learns under such conditions could reveal new prospects for therapeutic interventions to combat impulsive and compulsive disorders.

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