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Dopamine activity in the nigrostriatal pathway alters cue-induced risky choice patterns in female rats

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Abstract

Deficits in cost/benefit decision making is a critical risk factor for gambling disorder. Reward-paired cues may play an important role, as these stimuli can enhance risk preference in rats. Despite extensive research implicating the dorsal striatum in the compulsive aspects of addiction, the role of nigrostriatal dopaminergic activity in cue-induced risk preference remains unclear, particularly in females. Accordingly, we examined the effects of manipulating the dopaminergic nigrostriatal pathway on cue-induced risky choice in female rats. TH:Cre rats were trained on the cued version of the rat Gambling Task. This task was designed such that maximal reward is attained by avoiding the high-risk, high-reward options and instead favouring the options associated with lower per-trial gains, as they feature less frequent and shorter time-out penalties. Adding reward-paired audiovisual cues to the task leads to greater risky choice on average. To assess the role of the nigrostriatal pathway, a viral vector carrying either Cre-dependent inhibitory or excitatory DREADD was infused into the substantia nigra. Rats then received clozapine-N-oxide either during task acquisition or after a stable performance baseline was reached. Inhibition of this pathway accelerated the development of risk preference in early sessions and increased risky choice during performance, but long-term inhibition actually improved decision making. Activation of this pathway had minimal effects. These results provide evidence for the involvement of the dopaminergic nigrostriatal pathway in cue-induced risk preference in females, therefore shedding light on its role in cost/benefit decision-making deficits and expanding our knowledge of the female dopaminergic system.

Abbreviations: CNO, clozapine N-oxide; DA, dopamine; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DREADD, designer receptors exclusively activated by designer drugs; DV, dependent variable; ITI, inter-trial interval; PD, Parkinson's disease; rGT, rat Gambling Task; RPE, reward prediction error; SNc, substantia nigra pars compacta; Tg−, transgene-negative; Tg+, transgene-positive; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

1 | INTRODUCTION

Gambling disorder is associated with significant health, social and financial consequences (Ladouceur et al., 1994). Previous literature suggests that deficits in risky decision making are a significant factor in the development and maintenance of problem gambling (Goudriaan et al., 2005). Extensive research has addressed the critical role of dopaminergic activity originating from the ventral tegmental area (VTA) in modulating risky choice (Bissonette & Roesch, 2016; Stopper et al., 2014), which greatly differs between sexes (Hynes et al., 2020, 2021). Substantially less work has examined the role of nigrostriatal dopamine activity in risky decision making and whether sex differences impact its function. In individuals with gambling disorder, dopamine response to amphetamine in the dorsal striatum is correlated with gambling severity (Boileau et al., 2014). Thus, understanding the contribution of the nigrostriatal pathway to risky choice and the role of biological factors such as sex could advance our understanding of gambling disorder.

Risk preference in rats can be assessed with the rat Gambling Task (rGT), loosely analogous to the Iowa Gambling Task used clinically (Bechara et al., 1994; Zeeb et al., 2009). In both tasks, maximal reward is attained by avoiding the high-risk, high-reward options and instead favouring the options associated with lower per-trial gains. On the rGT, these low-risk, low-reward options result in less frequent and shorter time-out penalties, and therefore, more sugar pellets are earned overall. Adding salient audiovisual cues concurrent with reward delivery to this task results in a higher proportion of rats preferring the disadvantageous risky options and renders decision making more sensitive to dopamine manipulations (Barrus & Winstanley, 2016; Mortazavi et al., 2023). The impact of reward-paired cues on risky choice has also been observed in humans (Cherkasova et al., 2018).

In males, chemogenetic inhibition of dopaminergic neurons in the VTA reduces risky choice on the cued rGT (Hynes et al., 2020, 2021). Surprisingly, the same manipulation has the opposite effect in females, increasing risky choice (Hynes et al., 2021). Chemogenetic activation of the VTA also results in diametrically opposing effects across sexes, with males developing a preference for the risky options more rapidly and females more slowly (Hynes et al., 2024). Dopamine activity in the dorsal striatum, innervated by the substantia nigra pars compacta (SNc), has not yet been investigated on this task and may contribute to the development of choice patterns. Given that oestradiol impacts the excitability of dopamine terminals and D2 receptor binding in the dorsal striatum, dopamine activity in this region may play a particularly important role in decision making in females versus males (Becker, 2016; Yoest et al., 2018).

The nigrostriatal pathway has long been implicated in action initiation and the acquisition of motor skills (Jin & Costa, 2010; Thorn et al., 2010; Yin et al., 2009). This pathway also contributes to higher-order cognitive functions such as decision making. There is now an established role of the dorsal striatum in reinforcement learning, particularly in learning the value of actions and their associated outcomes (Hollon et al., 2021; Ramayya et al., 2014). Dynamic changes in SNc activity causally contribute to action selection (Howard et al., 2017). Additionally, patients with early Parkinson's disease, prior to receiving dopamine replacement therapy, exhibit deficits in performance on tasks assessing risky decision making and cognitive flexibility (Dirnberger & Jahanshahi, 2013).

Reward prediction errors (RPEs) are classically thought to be carried by the VTA, in which unexpected reward induces burst firing, and omission of expected reward causes neurons to pause firing (Schultz et al., 1997). Dopaminergic neurons in the medial SNc also show characteristics of prediction errors and have distinct intrinsic properties and inputs from neurons located more laterally (Lerner et al., 2015; Zhang et al., 2017). The lateral portion of the SNc instead tracks salience, as it is equally excited by appetitive and aversive events (Zhang et al., 2017). This medial-to-lateral gradient in function is preserved in the striatum, with the medial SNc innervating the dorsomedial striatum (DMS) and the lateral portion innervating the dorsolateral striatum (DLS; Lerner et al., 2015). Accordingly, these regions of the striatum are thought to perform different functions. The DMS contributes to flexible, goal-directed behaviour, and the DLS is involved in habit formation (Balleine & Dickinson, 1998; Burton et al., 2015; Everitt & Robbins, 2013). At least in males, reward-paired cues reduce flexibility in choice patterns on the rGT following reinforcer devaluation, indicative of lower goal-directed control of decision making (Hathaway et al., 2021). As such, nigrostriatal dopamine activity may be involved in the establishment of choice patterns on the cued rGT.

Given the surprising effect in females following manipulation of the VTA on the cued rGT and the impact of oestradiol on dopaminergic signalling in the dorsal striatum, examining the role the nigrostriatal pathway in females is of particular interest. Thus, the current set of studies employed the DREADDs technique to manipulate activity in SNc dopamine neurons during both the acquisition and performance phases of the cued rGT in female rats, with the goal of determining whether the nigrostriatal pathway contributes to the development of risky choice patterns when reward-paired cues are present.

2 | MATERIALS AND METHODS

2.1 | Subjects

Four cohorts of 32–48 female transgenic rats (total $N = 160$) were bred in-house against a Long–Evans background. Transgene-positive (Tg+) rats express Cre recombinase (Cre) in neurons containing tyrosine hydroxylase (TH:Cre rats from Long–Evans-Tg (TH-Cre) 3.1Deis, RRRC #00659; Rat Resource and Research Centre; WT rats obtained from Charles River Laboratories). Transgene-negative (Tg−) litter mates were used as the control group for all experiments, with the exception of four Tg+ rats who received saline injections in the inhibition during acquisition experiment. Rats bred in-house were weaned at postnatal day 21, housed in groups of three or four animals per cage and had access to ad libitum standard rat chow until an average weight of ∼250 g was reached. Rats were then transferred to the main vivarium. Animals were food-restricted and maintained at least 85% body weight of an age- and sex-matched control (training days: ∼75–200 sucrose pellets during task, 9-g standard rat chow provided after task training; non-training days: 11 g standard rat chow). Water was available ad libitum. Rats were housed under a reverse 12 h light/dark cycle in a temperature-controlled colony room maintained at 21°C. Huts and paper towel were provided as environmental enrichment. Behavioural testing took place 5 days per week. Experimental protocols were approved by the Animal Care Committee of the University of British Columbia and were conducted in accordance with the Canadian Council of Animal Care.

2.2 | Behavioural apparatus

Testing took place in 32 standard five-hole operant chambers, each of which was enclosed in a ventilated, sound-attenuating chamber (Med Associates Inc, Vermont). Chambers were fitted with an array composed of five equidistantly spaced response holes. A stimulus light was located at the back of each hole, and nose-poke responses into these apertures were detected by vertical infrared beams. On the opposite wall, sucrose pellets (45 mg; Bioserv, New Jersey) were delivered to the magazine via an external pellet dispenser. The food magazine was also fitted with a tray light and infrared sensors to detect sucrose pellet collection. A house light could illuminate the chamber. The operant chambers were operated by software written in Med-PC by CAW, running on an IBM-compatible computer.

2.3 | Behavioural testing

Rats were first habituated to the operant chambers in two daily 30-min sessions, during which sucrose pellets were present in the nose-poke apertures and food magazine. Rats were then trained on a variant of the five-choice serial reaction time task and the forced-choice variant of the rGT, as described in previous reports (Barrus & Winstanley, 2016; Zeeb et al., 2009).

A task schematic of the rGT is provided in Figure 1. During the 30-min session, trials were initiated by making a nose-poke response within the illuminated food magazine. This response extinguished the light and was followed by a 5-s inter-trial interval (ITI) in which rats were required to inhibit their responses to proceed with the

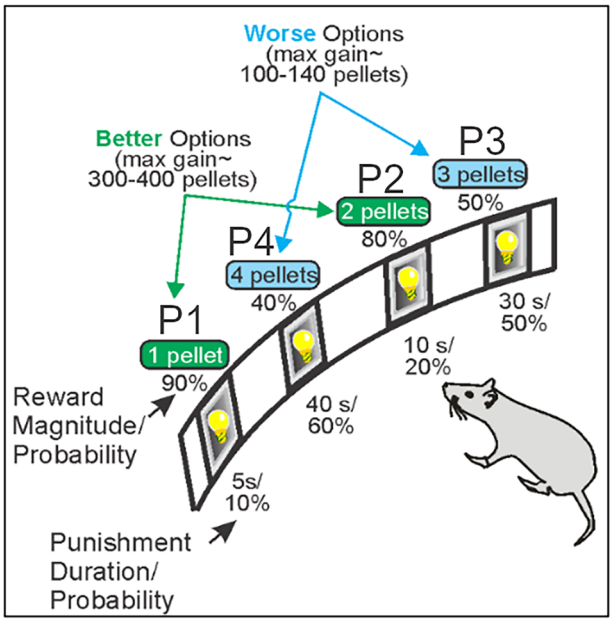

FIGURE 1 The rat Gambling Task. A nose poke response in the food tray extinguished the tray light and initiated a new trial. After an inter-trial interval (ITI) of 5 s, four stimulus lights were turned on in holes 1, 2, 4 and 5, each of which was associated with a different number of sugar pellets. The order of the options from left to right was counter-balanced within each cohort to avoid development of a simple side bias (version A (shown): P1, P4, P2, P3; version B: P4, P1, P3, P2). The animal was required to respond at a hole within 10 s. This response was then rewarded or punished depending on the reinforcement schedule for that option. If the animal lost, the stimulus light in the chosen hole flashed at a frequency of 0.5 Hz for the duration of the time-out penalty, and all other lights were extinguished. The maximum number of pellets available per 30-min session shows that P1 and P2 are more optimal than P3 and P4. The percent choice of the different options is one of the primary dependent variables. A score variable is also calculated, as for the IGT, to determine the overall level of risky choice as follows: [(P1 + P2) − (P3 + P4)]. The figure is modified from Winstanley and Floresco (2016).

trial. Any response in the five-hole array during the ITI was recorded as a premature response and punished by a 5-s time-out period, during which the house light was illuminated and no response could be made. Following the ITI, apertures 1, 2, 4 and 5 in the five-hole array were illuminated for 10 s. Aperture 3 was not used for this task. A lack of response after 10 s was recorded as an omission, at which point the food magazine was re-illuminated and rats could initiate a new trial. A nose-poke response within one of the illuminated apertures was either rewarded or punished according to that aperture's reinforcement schedule. Probability of reward varied among options (0.9–0.4, P1–P4), as did reward size (1–4 sucrose pellets). Punishments were signalled by a light flashing at 0.5 Hz within the chosen aperture for the duration of a time-out penalty that lasted for 5–40 s depending on the aperture selected. The task was designed such that the optimal strategy to earn the highest number of sucrose pellets during the 30-min session would be to exclusively select the P2 option, due to the relatively high probability of reward (0.8) and short, infrequent time-out penalties (10 s, 0.2 probability). Although options P3 and P4 provide higher per-trial gains of three or four sucrose pellets, the longer and more frequent time-out penalties associated with these options greatly reduce the occurrence of rewarded trials. Consistently selecting these options results in fewer sucrose pellets earned across the session and is therefore considered disadvantageous. The position of each option was counterbalanced across rats to mitigate potential side bias. Half the animals in each study were trained on version A (left to right arrangement: P1, P4, P2, P3) and the other half on version B (left to right arrangement: P4, P1, P3, P2).

2.4 | Viral vector injections

Rats were anaesthetized with isoflurane (5% induction, 2%–3% maintenance) prior to surgery. Ketoprofren (5 mg/kg) was given as an analgesic and bupivicaine was administered at the surgical site for local anaesthesia. For the inhibitory DREADD experiment, 1 μL of 1.0 × 10^9 genomic copies/μL of AAV5-hSyn-DIO-hM4D(Gi)-mCherry (UNC Vector Core, USA) was injected bilaterally in the SNc (coordinates relative to bregma: AP: −5.3, ML: ±2.1, DV: −7.5 from skull), at a rate of 0.1 μL/min. This low rate resulted in frequent blockages within the injectors used for infusions, and therefore, multiple rats exhibited unilateral or no viral expression. Accordingly, the rate was increased to 0.2 μL/min for the remaining experiments. The volume of viral vector (excitatory: 1.0 × 10^9 genomic copies/μL of AAV5-hSyn-DIO-hM3D (Gq)-mCherry; UNC Vector Core, USA) was reduced to 0.8 μL to reduce the spread of viral particles to the VTA. Clozapine N-oxide (CNO) administration started at least 6 weeks after surgeries, allowing sufficient time for viral expression to stabilize (Smith et al., 2016).

2.5 | CNO preparation and administration

CNO (Toronto Research Chemicals) was dissolved in 100% DMSO and diluted with saline (0.9% NaCl) to a final DMSO concentration of 3%. CNO was injected at 1 mL/kg via the intraperitoneal route 30 min prior to the start of the behavioural task. For acquisition experiments, all animals (Tg+ experimental group, Tg− controls) received 1 mg/kg of CNO prior to each rGT session for 30 sessions, at which point performance was statistically stable across all measures (see Section 2.7). For performance experiments, CNO was not administered until animals reached a statistically stable baseline. Following task training, animals received CNO (0, 0.1, 1, 3 mg/mL; 0.1 mg/mL omitted for inhibition experiment due to pandemic-related logistic constraints) in a diagram-balanced Latin square drug design (for doses A–D: ABCD, BDAC, CADB, DCBA). Drug injections were given on a 3-day cycle, starting initially with a baseline session. The following day, rats received a drug or vehicle injection prior to testing. On the third day, animals were not tested and remained in their home cages to ensure no carry-over effects were observed.

2.6 | Histology

Rats were deeply anaesthetized and perfused transcardially with ice-cold phosphate-buffered saline followed by 4% paraformaldehyde in PBS. Brains were removed, stored in 4% paraformaldehyde solution, cryoprotected in a 30% sucrose solution for at least 24 h and sectioned (40 μm) with a cryostat. Free-floating sections were then immunostained with a chicken mCherry primary (1:1000; Abcam ab205402) and goat anti-chicken secondary stain (Alexa 633, 1:500; Invitrogen A21103) and mounted on slides. A subset of three rats per DREADD type was immunostained with rabbit tyrosine hydroxylase (TH) primary (1:500, Sigma-Aldrich AB152) and goat anti-rabbit secondary stain (Alexa 488; 1:500; Invitrogen A-11008) to confirm the specificity of DREADD transfection to dopaminergic neurons. DREADD virus transfection and spread were verified using a confocal microscope (Leica TCS SP8) by a researcher blind to condition. Confocal images were acquired with a 10× objective and a 63× oil immersion objective.

2.7 | Data analysis

All statistical analyses were completed using SPSS Statistics 27.0 software (IBM) and RStudio. As per previous reports, the following rGT variables were analysed: percentage choice of each option ([number of times option chosen/total number of choices] × 100), risk score (calculated as choice of [(P1 + P2) − (P3 + P4)]), percentage of premature responses ([number of premature responses/total number of trials initiated] × 100), sum of omitted responses, sum of trials completed and average latencies to choose an option and collect reward. Variables that were expressed as a percentage were subjected to an arcsine transformation to limit the effect of an artificially imposed ceiling (i.e., 100%) (McDonald, 2009). For performance experiments, a statistically stable baseline was determined by a repeated-measures ANOVA across data from three consecutive sessions, in which both the session factor and session × choice interaction were not significant, prior to CNO administration.

For performance experiments, animals with a mean positive baseline risk score were designated as 'optimal decision-makers', whereas rats with negative risk scores were classified as 'risk-preferring'. For the Latin Square dosing regimen, choice data were analysed with a two-way repeated measures ANOVA with dose (three or four levels: vehicle, 0.1, 1 and 3 mg/kg) and choice (four levels: P1, P2, P3 and P4) as within-subject factors. For all other variables, dose was the only within-subjects factor. Transgene status (Tg^+, Tg^-) and risk status (optimal, risk-preferring) were included as between-subjects factors for all statistical analyses. If sphericity was violated as determined by Mauchley's test, a Huynh–Feldt correction was applied, and corrected p values' degrees of freedom were rounded to the nearest integer. Any main effects or interactions of significance were further analysed via post hoc one-way ANOVA or paired samples t-tests with a Bonferroni correction applied for the number of comparisons made.

For the analysis of data from the acquisition experiments, we used a multilevel model with two levels: sessions nested within subjects. All 30 sessions for which rats were dosed with CNO were included in analyses. P1–P4 choice was analysed using a binomial mixed effects logistic regression model, predicting choice of a given option out of all outcomes. Other dependent variables (DV; risk score, premature responses, omissions, trials completed and latencies to choose an option and collect reward) were analysed using linear mixed effects models. For each DV, the full model included session as the level 1 predictor, and experimental group and final risk score at the end of the dosing period (final score in equations below) as level 2 predictors. We accounted for subject-level differences in the intercepts by including a random intercept component that varied across subjects and modelled individual differences in the evolution of the dependent variable across sessions by including a random slope component for session. This resulted in the following model:

Level 1 model:

$$\mathrm{DV}_{ij} = \beta_{0j} + \beta_{1j}\mathrm{session}_{ij} + e_{ij}$$

Level 2 model:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}\text{final score}_j \times \text{experimental group}_j + \gamma_{02}\text{final score}_j + \gamma_{03}\text{experimental group}_j + u_{0j}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11}\text{final score}_j \times \text{experimental group}_j + \gamma_{12}\text{final score}_j + \gamma_{13}\text{experimental group}_j + u_{1j}$$

To determine the best-fitting model for each DV, we compared the Akaike information criterion between the full model and reduced models in which each fixed effect, interaction term and random slope was removed respectively. Significant main effects and interactions involving experimental group for the best-fitting model were further analysed by examining simple slopes for the experimental versus control groups. For interaction terms with final score, post hoc analyses at the mean and ±1 SD were performed.

For all models, the level 1 residual variances/covariances followed a first-order autoregressive structure; level 2 residual variances/covariances were unstructured:

$$e_{ij} \sim N\left(0, \sigma^2\right)$$

$$\Sigma = \sigma^2 \begin{bmatrix} 1 & & & & \\ \rho & 1 & & & \\ \rho^2 & \rho & 1 & & \\ \vdots & \vdots & \ddots & \ddots & \\ \rho^{29} & \rho^{28} & \ldots & \rho & 1 \end{bmatrix}$$

$$\begin{pmatrix} u_{0j} \\ u_{1j} \end{pmatrix} \sim \mathrm{MVN}\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_{00} & \\ \tau_{01} & \tau_{11} \end{bmatrix} \right)$$

For all analyses except trials completed, data were excluded from any animal that performed <20 trials. Results were deemed to be significant if p-values were less than or equal to an α of 0.05. Analyses yielding a p-value between 0.05 and 0.09 were reported as a trend.

3 | RESULTS

3.1 | DREADDs expression

Viral vectors were highly expressed by cell bodies within the SNc (Figure 2). Rats with no or unilateral expression were excluded from analyses ($n = 15$ of 80 Tg+ rats). Expression was well-contained within the dopaminergic midbrain, largely centred on the SNc. Some light expression in lateral VTA was noted in a small subset of rats. Nine rats across all four cohorts died as a result of complications from surgery or from ill health not obviously related to the experimental manipulations. Data were also excluded from rats that failed to complete at least 20 trials per session (inhibition during acquisition: $n = 5$; inhibition during performance: $n = 4$) resulting in the following sample sizes for each experimental cohort: SNc dopamine (DA) inhibition/activation during acquisition: 17 Tg+ (3 saline controls), 12 Tg−/12 Tg+, 15 Tg−; SNc DA inhibition/activation during performance: 12 Tg+, 20 Tg−/18 Tg+, 23 Tg−.

3.2 | Inhibition/activation of nigrostriatal dopamine neurons during task acquisition

3.2.1 | Choice

To assess the impact of modulating SNc activity on risky choice across training, we analysed the evolution of score across the 30 dosing sessions using a multilevel logistic regression model, comparing the effects of CNO in Tg+ experimental animals versus the control group (inhibition: Tg− rats and Tg+ rats injected with saline; activation: Tg− rats; referred to as control group hereafter). Regression analyses were used as a parsimonious way to evaluate group differences in initial values at the start of training (intercept) and the progression of choice preferences over time (slope). Raw data for risk scores, separated by risk-preferring and optimal rats, are shown in Figures 3b and 4b, to provide a comparison with predicted scores from regression analyses.

Following chemogenetic inhibition of the nigrostriatal dopamine neurons, we observed a significant

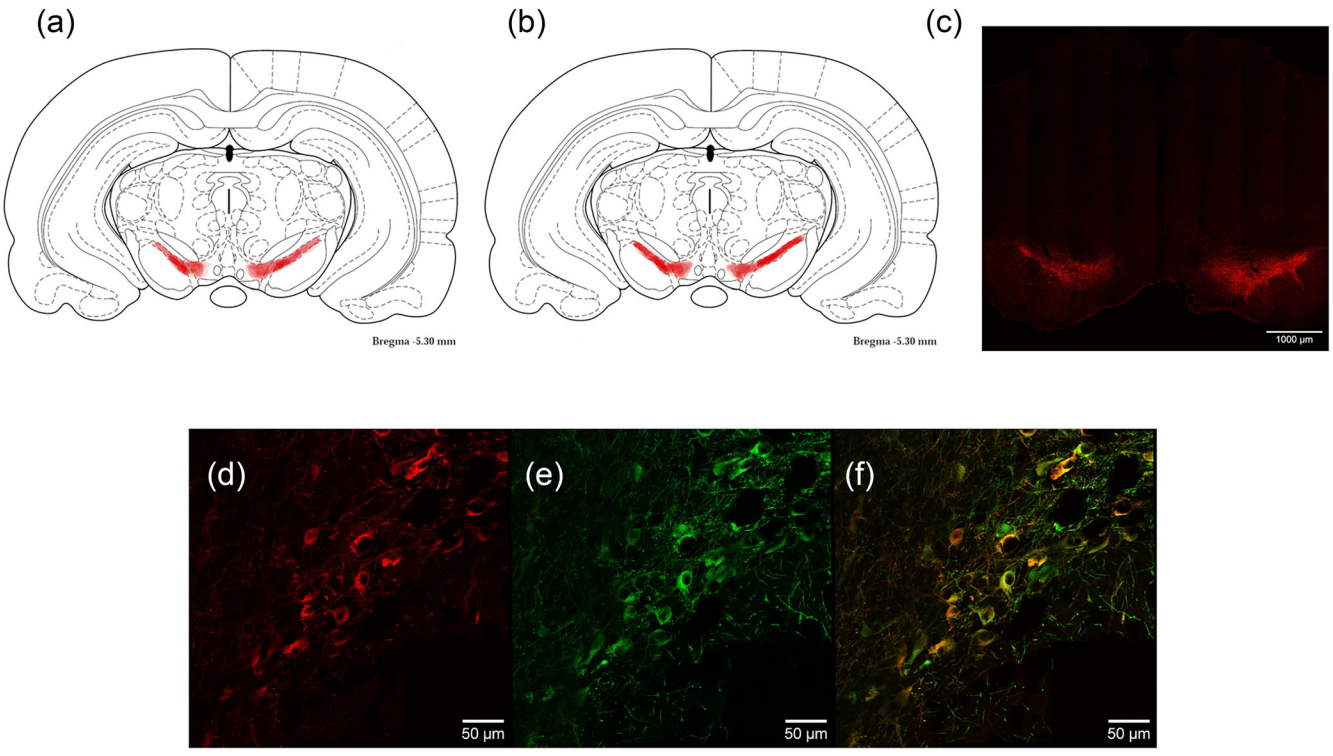

FIGURE 2 Location of all acceptable SNc expression patterns. Diagrams depicting the extent of viral expression within the SNc for inhibitory DREADD (a) and excitatory DREADD (b) are shown; viral spread was largely contained within the SNc, with a small subset of rats exhibiting light expression in the lateral VTA. Panel C shows the greatest medial-lateral spread within the midbrain at 10× magnification. (d–f) Representative images of DREADD expression within the SNc at 63× magnification: mCherry expression (d), TH expression (e) and a merged image showing specificity of DREADDs transfection to TH-containing neurons (f) are shown. Coordinates are relative to bregma. Plates modified from Paxinos and Watson (1998).

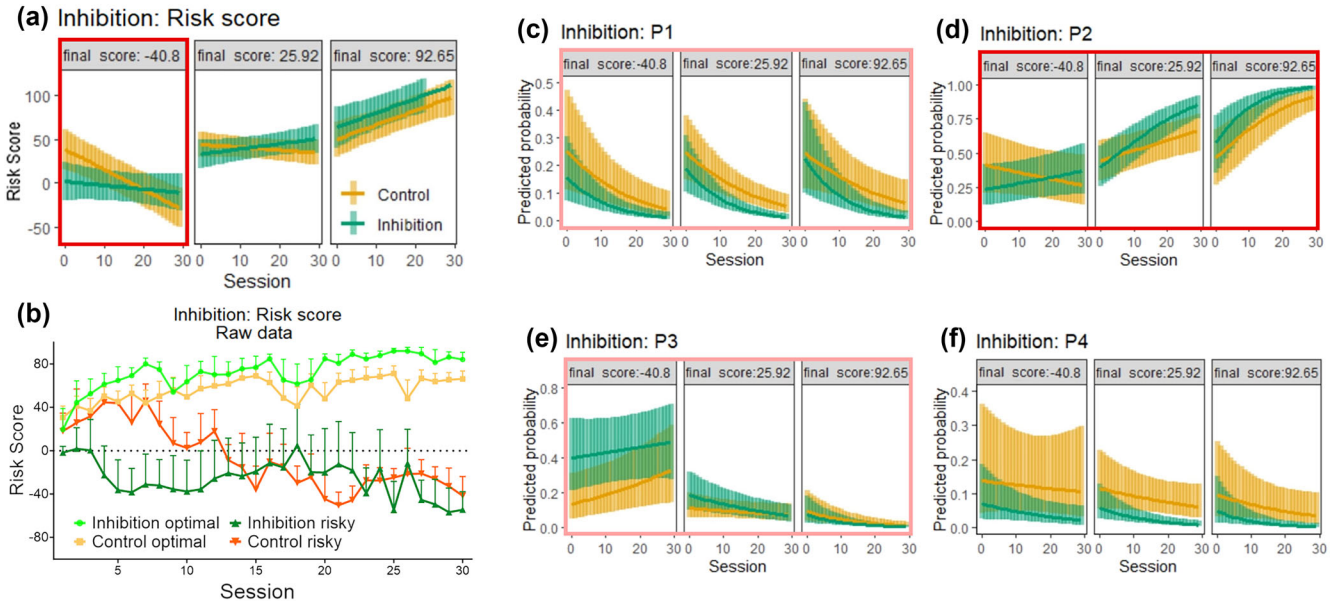

FIGURE 3 Nigrostriatal inhibition impacts choice patterns during task learning. Panel a: simple slopes analysis of the experimental group × final score × session interaction, comparing SNc inhibition to controls. Experimental risk-preferring rats were significantly riskier early in training compared with control risky rats (a, left panel); no differences were found between groups for rats at the mean (a, middle panel) or optimal risk level (a, right panel). Raw risk scores for each group (split by optimal and risk-preferring) are provided in panel b. (c–f) Predicted probability of P1–P4 choice across the 30 dosing sessions, split by the mean and ±1 SD final score. Experimental rats show a significantly greater choice of P2 across training (d), and a tendency to show an initial stronger preference for P3 (e) combined with a weak trend for lower choice of P1 (c). Red boxes indicate a significant difference in slopes and/or intercepts between groups, $p < .05$. Pink boxes indicate a trend (p between .05 and .09).

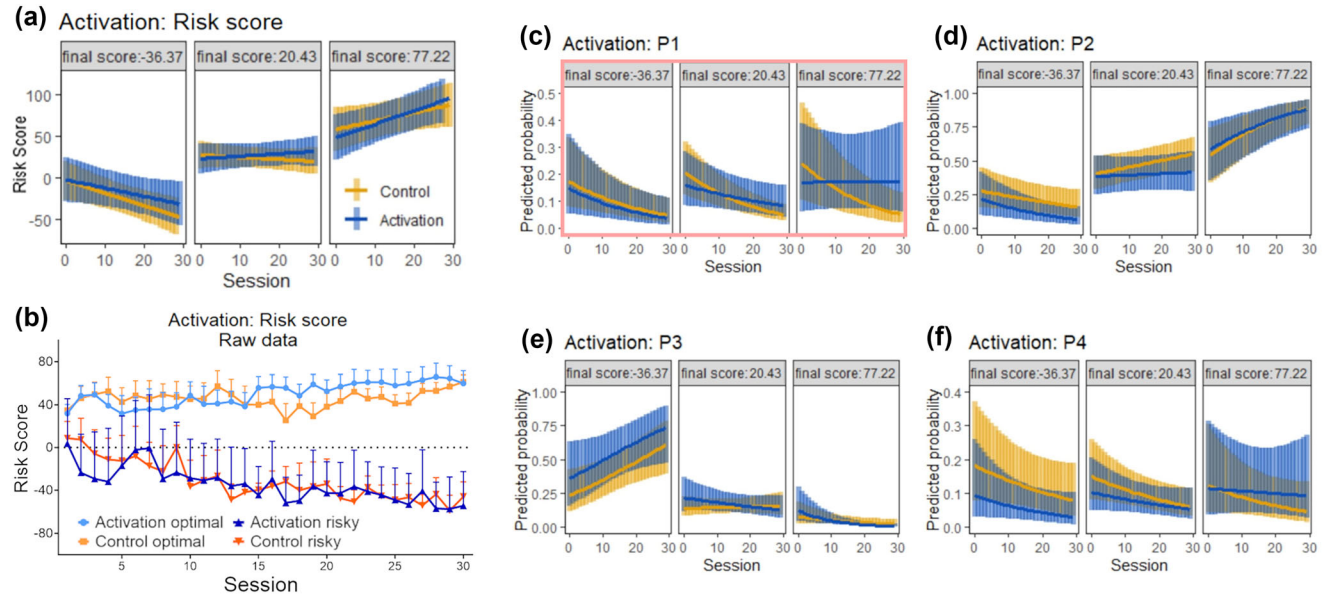

FIGURE 4 Nigrostriatal activation does not impact choice patterns during task learning. Simple slopes analysis of the experimental group × final score × session interaction, activation versus controls, split by the mean and ±1 SD final score. No significant differences were found on score (a) or P1–P4 choice (c–f), with the exception of a weakly trending increase in P1 selection across sessions (c). Pink box indicates a trend (p between .05 and .09).

group × session × final score interaction ($\beta = -0.014$, $p = .03$) for the risk score variable, suggesting an alteration in the rate at which rats developed a preference for the optimal or risky options. Post hoc analyses revealed that experimental risk-preferring rats (mean − 1 SD final score) had a lower intercept compared to control risk-preferring rats (session 1: $\beta = -36.10$, $t[25] = -2.42$, $p = .02$) and a flatter slope (inhibition group: $\beta = -0.42$,

control: $\beta = -2.28$, $t = 3.03$, $p = .003$; Figure 3a, left panel). As such, experimental rats that ultimately preferred the risky options at the end of training developed this preference significantly earlier than the control group, even though levels of risk preference were comparable by the final session. No significant differences were found between experimental groups at the mean or mean + 1 SD level of risk preference (all $t < 1.12$, all $p > .27$; Figure 3a, middle/right panel). Raw risk scores separated by risk-preferring and optimal rats are provided in Figure 3b.

Somewhat in keeping with the effect of inhibiting SNc DA neurons on risk score reported above, we also observed a trend-level difference between groups in the intercept for P3, indicating that experimental rats tended to choose P3 more at the start of training (group: $\beta = 0.93$, $p = .08$; Figure 3e). This was not dependent on final score (group × final score: $\beta = .00005$, $p = .8$). More surprisingly, we also observed a significant group × session effect for P2 ($\beta = 0.04$, $p = .01$; Figure 3d). Simple slope analyses revealed a significant difference in slopes between groups across sessions (inhibition: $\beta = 0.03$, control: $\beta = 0.07$, $p = .03$), indicating that experimental rats selected P2 more as training progressed. Again, this change was not dependent on final score, indicating this effect was also present in those that ultimately preferred the risky options (group × session × final score: $\beta = -6 \times 10^{-6}$, $p = .98$). There was also a very weak trend for selection of P1 to be lower in experimental rats as training progressed (session × group: $\beta = -.04$, $p = .09$; Figure 3c), whereas no group-dependent effects for P4 were observed (all $p > .15$; Figure 3f). This set of results indicates that rats in the inhibition group initially tended to show a greater preference for P3 but developed a stronger preference for P2 by the end of the 30 sessions.

Activation of the dopaminergic projections from the SNc did not result in any significant change in score (session × group: $\beta = 0.54$, $p = .3$; Figure 4a; see Figure 4b for raw risk scores). When analysing the choice of each option, we also did not observe any significant or trending terms involving experimental group, with the exception of a very weak trend for increased selection of P1 over time (session × group: $\beta = 0.029$, $p = .09$, Figure 4c; all other $p > .2$, Figure 4d–f).

3.2.2 | Other variables

Daily chemogenetic inhibition of SNc DA neurons resulted in a significantly lower rate of premature responding across training (session × group: $\beta = 0.005$, $p = .005$; group, session 1: $t(27) = 6.54$, $p < .0001$; session 30: $t(27) = 5.50$, $p < .0001$; Figure 5a). The rate of premature responding declined progressively in control rats with training ($\beta = -0.006$, $p < .0001$) yet stayed consistently low in the experimental group ($\beta = -.0005$, $p = .69$). In contrast, there was a very weak trend for premature responding to be higher in experimental rats at the first session following activation of SNc DA neurons; no difference in the slopes across sessions was found (group: $\beta = .08$, $p = .09$; group × session: $\beta = -.0002$, $p = .95$; Figure 5b).

Following inhibition of SNc DA neurons, experimental rats were slower to make a choice and became slower over time, whereas controls sped up as training progressed (group × session: $\beta = .023$, $p = .001$; control: $\beta = -.009$, inhibition: $\beta = .014$; control vs. inhibition: $t[827] = -3.28$, $p = .001$; Figure 5c). By contrast, following SNc activation, there was a trending difference between groups at the first session that was dependent on final score (group × final score: $\beta = .005$, $p = .07$). Simple slope analyses revealed that risk-preferring activation rats were significantly quicker to choose than risky control rats at Session 1 (final score mean − 1 SD = −347; $t[23] = 2.78$, $p = .01$; Figure 5d, left panel). Reward collection latency also tended to be slower in experimental rats following inhibition but significantly decreased across sessions, whereas control rats remained constant (group × session: $\beta = -.02$, $p = .08$; inhibition: $\beta = -.03$, $p = .002$; control: $\beta = -.008$, $p = .16$; Figure 5e). However, high initial values in the experimental group were likely driven by an outlier that was very slow to collect reward in early sessions (e.g., latency of 15 s on session 5). Residuals were not normally distributed for this model, further suggesting that the effect was skewed by an outlier.

In keeping with this pattern of generally lower and slower activity caused by inhibition of SNc DA neurons, although control rats that ultimately developed the most optimal choice profile completed progressively more trials as training progressed, this tended to be less apparent in their experimental counterparts (session × group × final score: $\beta = -.008$, $t = .06$; mean + 1 SD final score, inhibition: $\beta = 0.68$, control: $\beta = 1.39$; inhibition versus control: $t[837] = -1.75$, $p = .08$; Figure 6a, right panel). Activation of SNc DA caused the opposite effect, in that experimental rats completed progressively more trials per session as training progressed, whereas control rats did not (group × session: $\beta = 0.64$, $p = .08$; activation: $\beta = 0.71$, $p = .01$; control: $\beta = .07$, $p = .76$; Figure 6b). In the inhibition condition, experimental rats also made progressively more omissions, whereas control rats reliably omitted low numbers of trials (session × group: $\beta = 0.24$, $p = .0001$; inhibition: $\beta = 0.26$, $p < .0001$; control: $\beta = 0.03$, $p = .47$; Figure 6c). Activation of SNc DA neurons did not alter omission rates (Figure 6d).

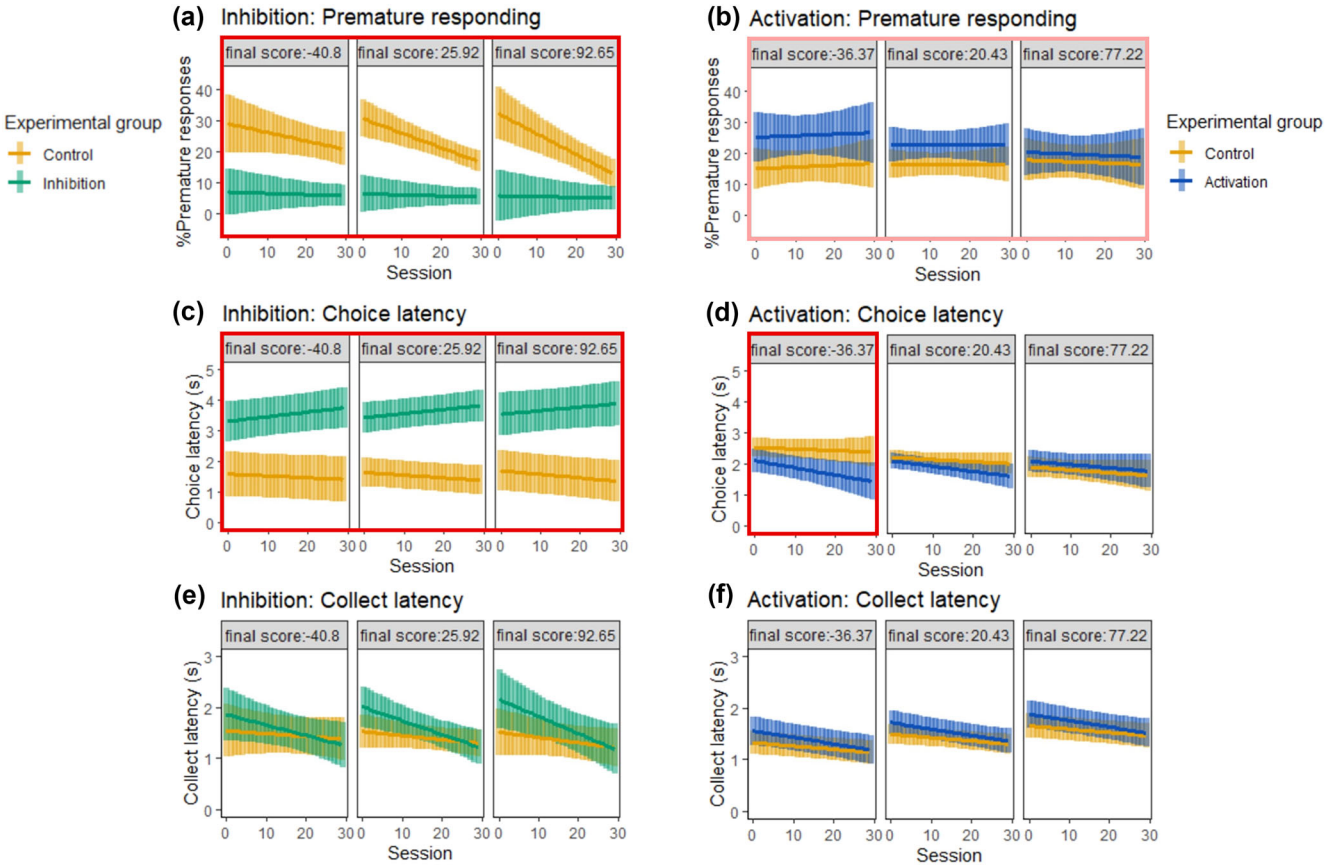

FIGURE 5 Nigrostriatal manipulation during task learning: Premature responding and latencies. Inhibition of the nigrostriatal dopamine pathway resulted in decreased premature responding (a), whereas there was a trending increase with nigrostriatal activation (b). Inhibition also increased choice latencies (c) whereas activation resulted in a decrease in this measure only in risk-preferring rats (d). Inhibition may have also increased latency to collect reward, but this was likely driven by an outlier (e); no effects were found in the activation condition (f). Red boxes indicate a significant difference in slopes and/or intercepts between groups, $p < .05$. Pink boxes indicate a trend (p between .05 and .09).

To explore whether the impacts of nigrostriatal inhibition on motor slowing versus decision making were related, correlation tests were conducted. The size of change was quantified by calculating a difference score between the predicted value for the first session for each experimental rat and the mean of the predicted values for control rats, for the variables for which a significant effect was observed at the start of training: risk score, premature responding and choice latency. Risk score difference scores were not correlated either with premature responding difference scores ($R^2 = -0.02$, $p = .94$), or choice latency difference scores ($R^2 = 0.05$, $p = .86$). This indicates that rats showing the largest deficits in decision making did not also exhibit the highest degree of motor slowing. This was also the case when risk-preferring and optimal rats were tested separately (risk-preferring: premature responding, $R^2 = -0.48$, $p = .41$; choice latency, $R^2 = 0.65$, $p = .24$; optimal: premature responding, $R^2 = 0.24$, $p = .52$; choice latency, $R^2 = -0.35$, $p = .35$). Furthermore, choice latency and premature responding difference scores were highly correlated ($R^2 = -0.83$, $p = .0002$), suggesting that reductions in premature responding were associated with motor slowing rather than changes to cognitive processes (i.e., increased response inhibition).

To test for off-target effects of CNO, Tg+ rats who received saline injections in the inhibition experiment were compared with rats who received CNO. Results indicated that although rats receiving saline showed a trending decrease in choice latencies across training, rats who were administered CNO did not (CNO × session: $\beta = 0.02$, $p = .07$; saline: $\beta = -0.02$, $p = .06$; CNO: $\beta = 0.003$, $p = .39$). This may indicate that chronic CNO administration resulted in some degree of motor slowing over time. However, no other variable was affected (all $p > .14$), suggesting that off-target effects of CNO were relatively minor.

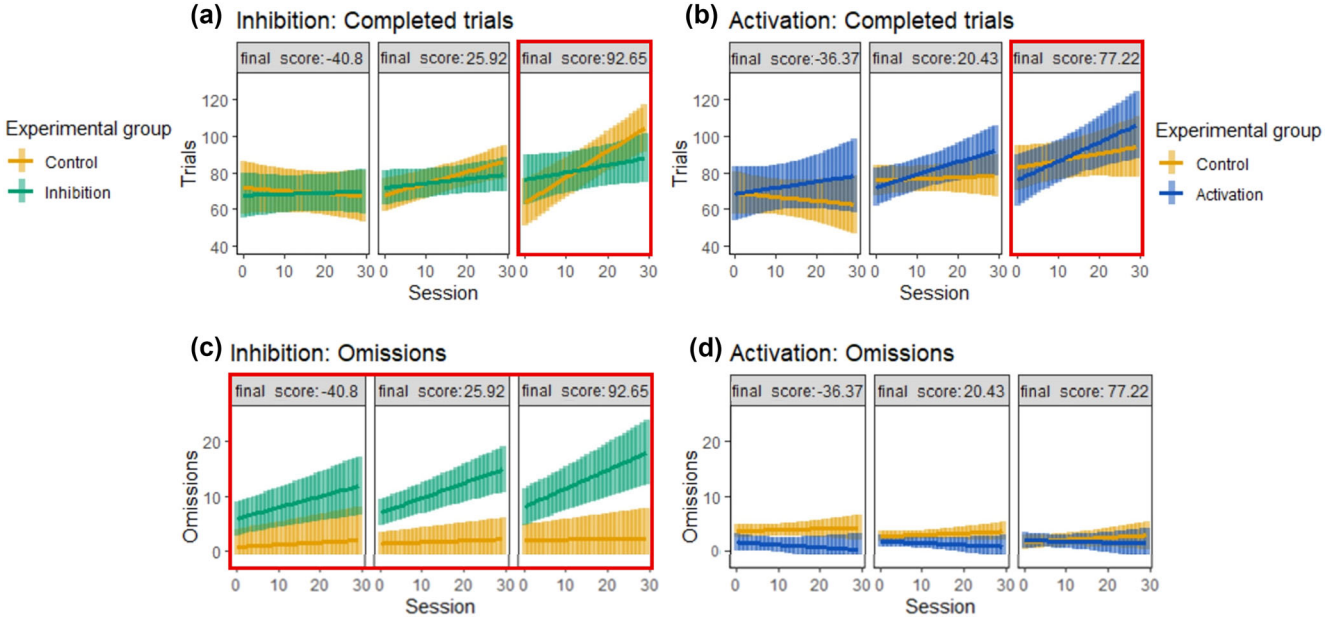

FIGURE 6 Nigrostriatal manipulation during task learning: Trials and omissions. Simple slope analyses of the trials and omissions variables. A bidirectional effect on trials completed was observed in optimal rats; in the inhibition condition, optimal rats completed fewer trials than controls as training progressed (a, right panel); conversely, in the activation condition, optimal rats completed more trials than controls as training progressed (b, right panel). Inhibition of SNc dopamine neurons also resulted in significantly more omitted trials (c); no differences were found with activation (d). Red boxes indicate a significant difference in slopes and/or intercepts between groups, $p < .05$.

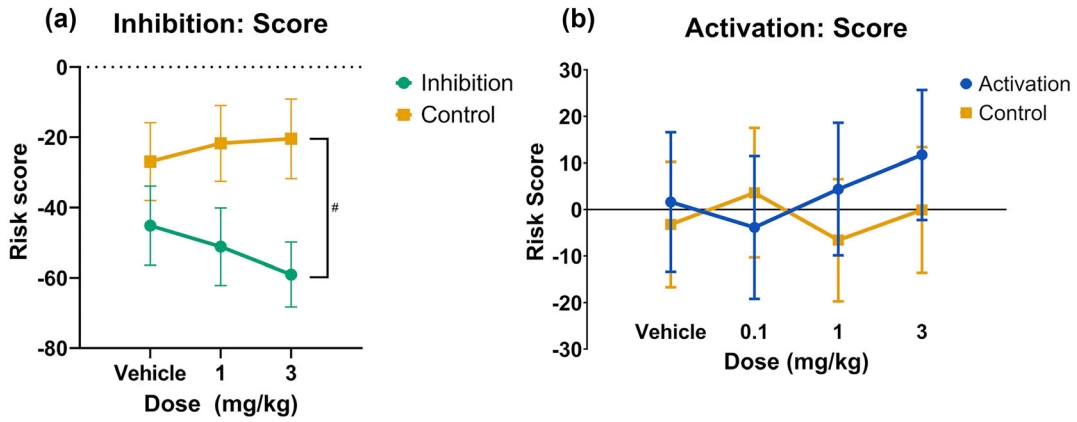

FIGURE 7 Nigrostriatal manipulation during task performance: Choice. Inhibition of SNc dopamine neurons (a) tended to decrease score (i.e., more risky choice) compared with controls; activation of SNc dopamine neurons (b) only weakly impacted risk scores in experimental animals and did not result in a significant difference from controls at any dose.

3.3 | Inhibition/activation of nigrostriatal dopamine neurons during task performance

3.3.1 | Choice

In the inhibition condition, score tended to be lower in experimental versus control rats at the highest dose, indicative of greater risky choice (dose × group: $F(2,48) = 2.82$, $p = .07$; inhibition vs. control at 3 mg/kg: $t(28) = 1.89$, $p = .07$, Figure 7a). No significant differences in choice of any individual option were observed (dose × choice × group: $F(5,138) = 1.33$, $p = .2$, data not shown). In the activation condition, a significant effect of dose on risk score was observed in experimental rats; however, these animals did not significantly differ from controls at any dose (dose × group: $F(3,111) = 3.25$, $p = .03$; dose, activation: $F(3,48) = 3.12$, $p = .04$; control: $F(3,63) = 1.68$, $p = 0.18$; activation vs. control at 3 mg/kg: $t(39) = -0.60$, $p = .0.55$; Figure 7b). When choice of each

option was analysed, the dose × choice × group interaction reached trend-level significance ($F(9,333) = 1.49$, $p = .06$). Although the choice patterns of experimental animals varied significantly following administration of CNO (activation, dose × choice: $F(9,144) = 1.96$, $p = .05$), comparison between the vehicle and highest dose did not reveal significant effects for any particular option (all $t < 1.71$, all $p > .11$, data not shown). Control rats were unaffected by CNO administration (dose × choice: $F(9,189) = 1.25$, $p = .27$). Taken together, these results indicate that inhibiting SNc dopamine neurons tended to decrease risk score (i.e., more risky choice); conversely, while activating these neurons did shift score in experimental animals, the effect was weak and did not result in a significant effect at any dose.

3.3.2 | Other variables

In the inhibition condition, experimental rats completed significantly fewer trials at the highest dose (dose × group: $F(2,54) = 44.71$, $p < .0001$; dose, inhibition: $F(2,20) = 32.52$, $p < .0001$; control: $F(2,32) = 0.86$, $p = .43$; inhibition, vehicle vs. 3 mg/kg: $t(15) = 2.64$, $p = .02$; Table 1). No other dose-dependent or group-dependent effects were found (all $F < 1.94$, all $p > .15$). In the activation condition, no group-dependent effects of CNO were observed for any variable analysed (all $F < 2.02$, all $p > .12$; Table 1). However, the highest dose tended to increase choice latency in all rats by an average of 0.19 s (dose: $F(3,111) = 2.47$, $p = .07$; vehicle vs. 3 mg/kg: $t(40) = -2.13$, $p = .04$).

4 | DISCUSSION

Here, we showed that in female rats, the nigrostriatal pathway contributes to choice preferences on the cued rGT. Inhibiting this pathway throughout task acquisition accelerated the development of risk preference in risky rats during the early learning phase but also increased selection of the most optimal choice later in training. Activating this pathway had little effect on the development of choice preferences. Once the task was learned, decreasing activity in the nigrostriatal pathway increased risky choice, whereas activation only weakly impacted choice preferences.

In keeping with a prominent role for nigrostriatal dopamine signalling in motor function, inhibition also generally slowed motor output during task acquisition, as indicated by lower motor impulsivity, and higher omissions and choice latencies. Inhibition during task performance also decreased the number of trials completed. Such observations increase confidence that the chemogenetic inhibition was effective. However, generally speaking, effect sizes were small, and this was particularly true for the activation experiments. This may be partly explained by the use of the DREADDs technique, which modulates neuronal activity to a lesser degree than techniques such as optogenetics or inactivation via GABA

TABLE 1 Nigrostriatal manipulation during task performance: Other variables.

Dose	Dose (mg/kg)	Premature responses	Choice latency	Collect latency	Omissions	Trials completed
Inhibition Tg+	0	11.77 ± 2.49	1.51 ± 0.17	1.18 ± 0.05	0.67 ± 0.67	63.44 ± 5.37
	1.0	10.51 ± 3.24	2.18 ± 0.31	1.40 ± 0.21	3.00 ± 1.00	44.25 ± 5.20
	3.0	11.05 ± 2.73	2.38 ± 0.35	1.28 ± 0.14	2.67 ± 0.88	40.31 ± 6.06
Inhibition control	0	11.69 ± 1.95	1.77 ± 0.16	1.22 ± 0.05	0.43 ± 0.20	71.96 ± 5.10
	1.0	7.56 ± 1.32	1.75 ± 0.15	1.23 ± 0.06	0.86 ± 0.34	75.15 ± 4.76
	3.0	11.70 ± 1.89	1.78 ± 0.16	1.25 ± 0.05	1.57 ± 0.53	73.43 ± 5.94
Activation Tg+	0	23.91 ± 3.44	1.65 ± 0.21	1.08 ± 0.07	2.77 ± 0.62	75.46 ± 9.77
	0.1	27.86 ± 4.42	1.78 ± 0.23	1.07 ± 0.06	3.44 ± 0.70	72.30 ± 9.25
	1.0	18.03 ± 2.82	1.86 ± 0.24	1.20 ± 0.43	3.83 ± 0.77	77.11 ± 9.67
	3.0	19.57 ± 3.61	1.98 ± 0.25	1.25 ± 0.06	4.11 ± 0.81	79.76 ± 10.12
Activation control	0	21.49 ± 3.42	1.84 ± 0.22	1.33 ± 0.05	2.39 ± 0.51	70.33 ± 8.23
	0.1	22.16 ± 3.32	1.84 ± 0.22	1.30 ± 0.05	2.61 ± 0.45	72.37 ± 8.56
	1.0	22.33 ± 3.90	1.96 ± 0.23	1.75 ± 0.08	3.00 ± 0.48	69.93 ± 8.41
	3.0	24.30 ± 4.17	1.93 ± 0.24	1.34 ± 0.06	2.70 ± 0.52	69.07 ± 8.27

Note: Mean values of all other task variables (% premature response, choice latency, collect latency, omissions, trials completed) following acute CNO dosing for experimental and control rats in the inhibition and activation experiments. Data are mean ± SEM.

agonists. Inhibitory DREADD receptors have been reported to dampen neuronal firing rates to about 60% of basal levels (Chang et al., 2015; Smith et al., 2016). However, that is also a strength of this technique, as it may emulate naturally occurring differences in average neural activity, as opposed to completely silencing or hyperstimulating a particular neuronal subgroup.

4.1 | Chemogenetic activation of SNc dopamine neurons had minimal effects

Alternatively, the smaller effect sizes for the activation experiments may suggest that increased dopamine activity along this pathway has only minimal effects on behaviour or is more tightly regulated than inhibition, such that any impacts on behaviour are minimized. Based on our immunohistochemistry results, excitatory hM3 and inhibitory hM4 DREADDs expression was comparable across experiments, indicating that the marginal behavioural effects of activating SNc dopamine neurons cannot be attributed to differences in viral transduction. However, it remains possible that the downstream effects of additional excitatory g-protein coupled receptor insertion trigger compensatory mechanisms in this specific neuronal population. Although we can find no evidence of this in the literature at the time of writing, there have been remarkably few studies published that document the effects of excitatory DREADDs targeted to SNc dopamine neurons. Although we did not directly measure neuronal activity following CNO administration for these experiments, chemogenetic activation of VTA DA neurons using identical methods as reported here is highly efficacious in our hands (Hynes et al., 2024). Nevertheless, we cannot entirely rule out that the chemogenetic approach used here may have not sufficiently activated dopamine neurons in the SNc. Future studies may shed light on the reason for these discrepancies, but the current data suggest that potentiating SNc dopamine activity using this technique has only minimal effects on decision making, at least in females.

4.2 | Dissociable regulation of motor activity and decision making by dopaminergic SNc neurons

At first glance, the accelerated development of risky choice induced by nigrostriatal inhibition may seem at odds with reduced motor impulsivity, given that risky choice correlates with premature responding on the rGT at the population level (Barrus et al., 2015). Studies investigating the extent to which dopaminergic activity in the dorsal striatum is involved in other aspects of impulsivity such as impulsive choice have been inconsistent (Magnard et al., 2018; Tedford et al., 2015). In the present study, reductions in motor impulsivity were correlated with increased choice latencies but not impaired decision making at the start of training, indicating that the impact of nigral inhibition on impulsivity was a result of motor slowing rather than better response inhibition. We also note that decision-making effects were constrained to a subset of rats that preferred the risky options by the end of training, whereas reduced premature responding was apparent in all rats, further suggesting that different mechanisms are at play. Indeed, other studies have demonstrated that the neural mechanisms of risky choice and motor impulsivity can be dissociated on the rGT (Barrus & Winstanley, 2016; Betts et al., 2021; Chernoff et al., 2021; Hathaway et al., 2021). By contrast, others have found that denervation of this pathway can impair response inhibition when the time required to withhold responses is 20 s (Engeln et al., 2016). Ostensibly, motor deficits would be less apparent in these conditions compared with the 5-s ITI on the rGT, as the rat would have more time to navigate around the box and make a prepotent response before the waiting time is done. It may be that a similar effect would be observed on the rGT if the ITI was sufficiently lengthened. Nevertheless, under the current conditions, it appears that nigrostriatal inhibition can induce motor slowing, particularly in early training before motor patterns are fully developed, but does not necessarily impact response inhibition. The lack of effect on premature responding in the performance phase experiments lends strength to this conclusion.

As changes in decision making do not correlate with motor slowing, the neural mechanisms underlying these two processes may be separable. Studies on patients with Parkinson's disease (PD) have found similar results. Dopamine replacement therapies used to treat PD can lead to the development of impulse control disorders (Voon et al., 2009). In rodent models, treatment with the D2/D3 agonist pramipexole can increase risk taking as measured by probabilistic discounting, in addition to improving motor symptoms (Rokosik & Napier, 2012). Pharmacotherapy such as mirtazapine can ameliorate decision-making deficits while leaving improvements to motor symptoms intact (Holtz et al., 2016), suggesting there is some degree of dissociation between the neural mechanisms of cognitive and motor performance mediated by mesostriatal pathways.

4.3 | Changes in decision making following inhibition of SNc dopamine neurons inconsistent with delayed transition to habitual control

Recent evidence suggests that choice patterns on the cued rGT, at least in males, are less goal-directed than choice on the uncued rGT (Hathaway et al., 2021). More specifically, choice is insensitive to reinforcer devaluation when rats are trained on cue-outcome schedules that promote risky choice. It is thought that the control of behaviour can shift over time from the ventral striatum to the DMS to the DLS through a series of spiralling loops passing through the dopaminergic midbrain (Haber et al., 2000). For example, as drug seeking becomes more resistant to devaluation in rats, the locus of dopaminergic control shifts from the ventral to the dorsal striatum (Murray et al., 2012; Zapata et al., 2010). If this shift is evident in risk-preferring female rats performing the cued rGT, we would expect that dampening nigrostriatal signalling would promote optimal choice in this group by reinstating goal-directed control. If the development of a bias towards the risky options was underpinned by a premature transition from goal-directed to habitual performance, somewhat similar to a shift from exploration to exploitation, we would also expect the effect of inhibiting SNc dopamine to manifest earlier rather than later in training. Although we did observe an increase in preference for the best option, P2, when SNc dopamine neurons were inhibited during acquisition, this effect was most pronounced towards the end of training. The timing at which this effect emerges therefore does not support the hypothesis that cue-induced risky choice is driven by a more rapid switch to habitual control. The increased choice of P2 was also evident in all rats, including those that showed a strong preference for the best options. Again, these data argue against our hypothesis that risk-preferring rats would benefit the most from inhibition of SNc dopamine neurons. Finally, unlike chemogenetic inhibition of VTA dopamine neurons (Hynes et al., 2021), the effect on P2 choice was too small to result in an overall change in risk score. Therefore, although SNc dopamine signalling may promote suboptimal choice as training progresses, these data strongly suggest it is not the major determinant of overall choice bias.

Although it is possible that our failure to detect the effects we expected may be due to an inadequate sample size, the number of animals used was sufficient to detect robust effects on non-decisional variables, and these were largely as predicted from previous reports (i.e., inhibition of SNc dopamine reduces motor output while activation facilitates action; Benazzouz et al., 1993; Saunders et al., 2018). Comparable numbers of rats were also used in our previous work targeting the VTA, and these effect sizes were substantial. Furthermore, in the current study, we were able to detect significant differences in risk score specific to risk-preferring rats. However, the direction of this effect was opposite to our prediction: rather than reducing preference for the risky options early in training, rats developed this preference more rapidly and sustained this bias throughout training when SNc dopamine neurons were inhibited. This could be attributed to early onset of a preference for P3, which is associated with maximal uncertainty regarding the nature of the outcome ($p = 0.5$). The final score was nevertheless comparable across experimental and control rats, indicating that it was the rate at which the preference was developed, rather than the size of the preference itself, that was influenced by inhibiting SNc dopamine projections.

Chemogenetic inhibition of VTA dopamine neurons, in contrast, dramatically increased the level of risky choice in females throughout training, resulting in a significantly more negative final score (Hynes et al., 2021). Whereas VTA dopamine activity can therefore directly determine choice preference, the output of SNc DA neurons may alter the rate at which such preferences come to dominate choice, such that dampening this signal results in more rapid biases towards an option once it becomes preferred, as seen here with P2 and P3. Given that distinct sex differences have been observed following manipulation of dopaminergic activity within the VTA (Hynes et al., 2021), and that sex hormones such as oestradiol can regulate terminal activity within the dorsal striatum (Yoest et al., 2018), it may be the case that males would be differentially impacted by nigrostriatal inhibition and/or activation. Future studies may investigate whether this is indeed the case.

It is worth considering whether the differing effects observed in early versus late training could be explained by a shift in dopamine activity from the DMS to the DLS as the task is learned (Burton et al., 2015; Everitt & Robbins, 2013). Thus, the initial increase in risky choice may be caused by inhibition of dopaminergic activity within the DMS, which is critical for goal-directed behaviour and linking actions to outcomes (Balleine & Dickinson, 1998; Yin et al., 2005). Increased selection of the most optimal P2 option later in training could then be due to inhibition of dopamine release in the DLS, preventing choice patterns from stabilizing and becoming rigid or inflexible (Yin et al., 2004). However, if the control of behaviour really does shift from DMS to DLS as training proceeds, and more optimal choice results from dampening dopaminergic signalling to the DLS, then inhibition of the nigrostriatal pathway in the performance phase, after months of task training, should

similarly improve choice. We instead observed an increase in selection of the risky options, P3 and P4. It may be important to note that rats in this cohort were largely risk-preferring, with only a small number of optimal decision-makers, due to natural variation in performance across cohorts. This may have prevented the detection of differences between risky and optimal decision-makers. As such, the effect of inhibiting SNc dopamine neurons after the task had been acquired may mimic what was observed early in training, in which risk-preferring rats exhibited a more pronounced bias towards the risky options. Nevertheless, this result does not support the hypothesis that inhibition of SNc dopamine can increase preference for P2 later in training due to attenuation of dopaminergic signals to the DLS.

4.4 | Inhibition of SNc dopaminergic neurons may impair classification of RPEs

Studies on Parkinson's patients, in whom SNc dopamine neurons are progressively lost, may help explain these results. Individuals with PD exhibit increased reliance on external cues to guide attention, as well as decreased detection of prediction violations (Dirnberger & Jahanshahi, 2013; Trempler et al., 2020). Thus, initial increases in risky choice may be the result of biased attention towards the larger cues on the risky options, as well as reduced detection of the need to flexibly update choice patterns following lengthy time-out penalties. Learning rates should be highest during the initial sessions, when subjects are exploring the reinforcement contingencies associated with each outcome. This information should be used by the subjects to build an internal representation or model of the task's reinforcement structure (Daw et al., 2005; Dolan & Dayan, 2013). Exclusive selection of any option, including the most optimal, will inevitably result in delivery of time-out penalties on a set proportion of trials. Developing and then maintaining an optimal strategy therefore requires subjects to discern when a penalty should instigate a negative re-evaluation of that option's value, and therefore prompt a shift in choice patterns, and when to ignore it as a relatively rare event. If subjects are building an accurate model of the task, RPEs generated following penalties after selection of P1 or P2 should increasingly fall into the latter category and have been described as 'stability-demanding prediction errors' (Trempler et al., 2020). In contrast, RPEs following penalties incurred by selection of P3 or P4 should be considered 'flexibility-demanding prediction errors' that signal the need for adaptation.

Whether an RPE can be considered flexibility or stability-demanding depends critically on accurate probability estimates. Patients with PD can show deficits in both selecting or inhibiting actions, sometimes reacting to rare events as if they signal a need for immediate behavioural change, while also failing to switch strategy following repeated negative outcomes on set-shifting tasks (Cools et al., 2001; Wylie et al., 2009, 2010). One interpretation of these seemingly contradictory behavioural patterns—both too much and too little flexibility—is that subjects are struggling to differentiate between these two types of RPE, perhaps as a result of insufficient learning of the probability context. This may explain why dampening SNc dopamine signalling here produced both an increase in preference for P3 in some animals early in learning, followed by an increase in P2 later in training: once animals started to form a preference for a particular option, this choice became more likely to be repeated, like a snowball rolling downhill, perhaps due to a premature categorization of RPEs as stability-demanding.

As to why this manipulation did not amplify choice of P1 or P4, it is perhaps notable that choice of P1 and P4 were generally lower in experimental animals from the outset of free choice. These options are associated with either the smallest reward (P1) or the longest penalty (P4), both of which are delivered far more frequently than not. There is arguably less of a nuanced cost/benefit trade-off associated with these options, and they can be discounted without an accurate calculation of the probability of events.

Overall, we have shown that the nigrostriatal pathway contributes to the development of risk preference in females, as well as to the maintenance of choice patterns once the decision-making task is well learned, in addition to impacting measures of motor output. Gambling disorder is on the rise in women, and differences have been observed in the type of gambling that women partake in, the speed of their progression to problem gambling, and their motivations to gamble (González-Ortega et al., 2019). Thus, understanding the contribution of mesostriatal dopamine in females to factors associated with gambling disorder, such as deficits in cost/benefit decision making, could shed light on gender differences observed in clinical populations.

AUTHOR CONTRIBUTIONS

Brett Alexander Hathaway: Conceptualization; data curation; formal analysis; methodology; visualization; writing—original draft; writing—review and editing. **Andrew Li:** Data curation; methodology. **Hannah G Brodie:** Data curation. **Mason M Silveira:** Data curation; formal analysis. **Melanie Tremblay:** Conceptualization; data curation. **Yeon Soo Seo:** Data curation. **Catharine Antonia Winstanley:** Conceptualization;

funding acquisition; investigation; project administration; resources; software; supervision; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.16287.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in OSF at https://doi.org/10.17605/OSF.IO/2XUC9.

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