Dopamine neurons gate the intersection of cocaine use, decision making, and impulsivity

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Conflict of interest: In the last 3 years, CAW has been retained as an expert witness by Hogan Lovells LLP, and received due compensation. The authors confirm they have no other conflicts of interest or financial disclosures to make.

Data Availability Statement: ˆThe data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Gambling disorder and drug addiction are superficially similar, and highly comorbid. Both clinical populations are highly impulsive and exhibit risky decision-making. Drug-associated cues have long been known to facilitate habitual drug-seeking, and the salient audiovisual cues embedded within modern gambling products may likewise encourage problem gambling. The dopamine (DA) neurons of the ventral tegmental area (VTA) are exquisitely sensitive to drugs of abuse, uncertain rewards, and reward-paired cues, and may therefore be the common neural substrate mediating synergistic features of both disorders. To test this hypothesis, we first gained specific inhibitory control over VTA DA neurons by transducing a floxed inhibitory DREADD (AAV5-hSyn-DIO-hM4D(Gi)-mCherry) in rats expressing Cre recombinase in tyrosine hydroxylase neurons. We then trained rats in our cued rat gambling task (crGT), inhibiting DA neurons throughout task acquisition and performance, before allowing them to self-administer cocaine in the same diurnal period as crGT sessions. The trajectories of addiction differ in women and men, and the DA system may differ functionally across the sexes, therefore we used male and female rats here. We found that inhibition of VTA DA neurons improved decision making and impulse control in males, but surprisingly worsened decision making in females, yet prevented cocaine-induced deficits in decision making in both sexes. Inhibiting VTA DA neurons nevertheless drove both sexes to consume more cocaine. These findings show that chronic dampening of DA signalling can have both protective and deleterious effects on addiction-relevant behaviours, depending on biological sex and dependent variable of interest.

Introduction

The choice to use illicit, addictive drugs such as cocaine is inherently risky, and often impulsive. It is therefore perhaps unsurprising that high levels of risky decision making and impulsivity increase vulnerability to substance use disorder (SUD) [1-3]. Risky decision making and impulsivity are also strongly associated with gambling disorder (GD), a behavioral addiction often comorbid with affective disorders, impulse control problems, and chemical dependencies. Playing casino-inspired sound and light cues when wins are revealed increased risky choice in human subjects, suggesting such audiosensory cues promote a cognitive style associated with greater addiction liability [4]. Pairing sugar pellet wins with flashing lights and sounds in a rat gambling task (rGT) also dramatically increased the number of rats preferring the risky options at baseline, and also increased the number of animals experiencing cognitive impairment as a result of cocaine self-administration [5,6].

The fact that rats trained to "gamble" on heavily-cued reinforcement schedules make more risky choices, appear more motivated to take cocaine, and are also more vulnerable to cocaine-induced increases in risky choice, may indicate risky decision-making and cocaine-taking cross-sensitise. As such, the ability of reward-concurrent cues to facilitate cocaine use and problem gambling may be underpinned by similar neurobiological mechanisms. The addition of cues to the rGT also potentiated the increase in motor impulsivity caused by chronic dopamine agonist administration, indicating that pairing uncertain rewards with salient cues could predispose animals to greater impulse control problems on-task when the dopamine system is challenged [7]. Considerable evidence suggests that the activity of mesolimbic dopamine (DA) neurons, originating in the ventral tegmental area (VTA), plays a critical role in the reinforcing properties of psychostimulant drugs, probabilistic rewards, and reward-paired cues [8-10]. Although

it is fairly undisputed that both these unconditioned and conditioned rewards elicit dopamine release, it is less clear whether addiction vulnerable individuals have hypo- or hyper-reactive dopamine systems [11,12].

Male rats that exhibited a relatively blunted locomotor response to cocaine subsequently developed a preference for the risky options on the rGT. Animals trained on the cued version of the rGT, in which risky choice is greater on average, also had lower levels of DA efflux in the nucleus accumbens after training [6]. Although repeated administration of cocaine results in locomotor sensitization (i.e. a greater behavioural and neurochemical response to each sequential injection of drug), basal DA levels actually drop[13]. It therefore seems that repeated exposure to either heavily-cued probabilistic rewards or psychostimulant drugs, both of which acutely induce DA release, leads to a down-regulation of basal dopaminergic activity. While this may form part of an adaptive, homeostatic response to excessive stimulation, such suppression may lead to hyper-reactivity to subsequent exposure to these reinforcers, through either receptor up-regulation or other neuroplastic mechanisms [14-17].

We therefore used chemogenetics to selectively decrease activity of VTA DA neurons during performance of the crGT. We predicted this manipulation would reduce preference for the risky options at baseline, reduce motor impulsivity, and also prevent cocaine self-administration from driving risky choice. Females are thought to have more sensitive DA systems, but recent data suggest DA antagonists are less effective at modulating decision making on the rGT in female rats [18,19]. Drug addiction also takes a different trajectory in females [20,21]. We therefore predicted that the effect of suppressing VTA DA neuron activity may be greater in males than females.

Methods

Subjects

Subjects were 32 male (transgene positive (TG+): n = 16; transgene negative (TG-): n = 16) and 32 female (TG+: n = 16; TG-: n = 16) transgenic rats, bred in house against a Long-Evans background (Charles River, St. Constant, QC), that expressed cre recombinase (Cre) in neurons containing tyrosine hydroxylase (Long-Evans-TG(TH-Cre)3.1Deis, RRRC #00659; Rat Resource and Research Centre, RRRC, Columbia, MO). Rats were pair- or trio-housed in a climate-controlled colony room on a reverse 12 hours light-dark cycle (lights off 08:00; temperature 21°C). All housing conditions and testing procedures were in accordance with the guidelines of the Canadian Council on Animal Care, and all protocols were approved by the Animal Care Committee of the University of British Columbia.

Experimental timeline

Over the course of a 2-week period, rats underwent stereotaxic surgery for delivery of the viral vector. All rats recovered with their cage mates for 4-6 weeks, and two weeks prior to commencement of crGT training, were food restricted to 85% of their free feeding weight. crGT began began 4-6 weeks after surgery. Thirty minutes prior to each pre-training and crGT session, rats received IP injections of CNO (1.0 mg/kg). After 35 crGT sessions, rats were implanted with jugular vein catheters and randomly assigned to cocaine or saline self-administering groups. After allowing 1 week for recovery, rats again began daily crGT sessions, with CNO still administered prior. After 5 baseline sessions of crGT, rats began cocaine self-administration sessions, which took place from 17:00 – 19:00. crGT testing was conducted the following morning from 09:00 – 09:30. CNO was still administered to all rats prior to the crGT sessions.

CNO has been shown to effectively modulate behaviour and activate DREADDs for 40-70 minutes [22,23]. As such, by spacing the CNO injections and self-administration sessions apart by 8.5 hours, we minimised the chances that CNO would be active during the cocaine/saline self-administration sessions. After 10 consecutive days of cocaine self-administration with concurrent crGT sessions, rats were euthanized and their brains were processed for immunohistochemistry (Figure 1).

Stereotaxic Surgery

We induced anesthesia with 5% isofluorane in oxygen (2 L/min flow rate) before securing rats in a stereotaxic frame and bilaterally infusing AAV5-hSyn-DIO-hM4D(Gi)-mCherry (0.5 uL/hemisphere; UNC Vector Core) into the VTA (AP: -5.5, ML:+/- 0.6, DV:-8.2 from skull) at a rate of 0.10 uL/min using standard stereotaxic techniques. We left the injector tip in place for a further 5 minutes to allow for diffusion of the bolus. All rats received 5 mg/kg ketaprofen every 12 hours for 48 hours following surgery. Animals were then maintained on standard rat chow (14 g/day/male; 11 g/d/female), plus the sugar pellets earned in the task (~5 g per day). Water was available ad libitum at all times. Behavioural testing began at least one week following the start of food restriction.

Apparatus

The crGT and cocaine self-administration were each conducted in 16 separate banks of operant conditioning chambers (30.5□×□24□×□21 cm; Med Associates, St. Albans, VT, USA), located in separate rooms. Each chamber was enclosed within a ventilated sound-attenuating cabinet (Med Associates Inc, VT). The operant boxes were equipped with a fan to provide

ventilation and to mask extraneous noise. Set in the curved wall of each box was an array of five holes. Each nose-poke unit was equipped with an infrared detector and a yellow light-emitting diode stimulus light. Sucrose pellets (45 mg, Formula P; Bio-Serv) could be delivered at the opposite wall via a dispenser. The cocaine self-administration chambers were identical to the crGT chambers with the exception of an additional cantilevered drug deliver arm and vascular access tether system plus externally mounted variable rate syringe pump. Online control of the apparatus and data collection was performed using code written by CAW in MEDPC (Med Associates) running on standard IBM-compatible computers.

The Cued Rat Gambling Task (crGT)

All rats were trained on the cued rat gambling task (crGT), as described previously [5]. During each 30-minute rGT session, rats sampled between four response holes, each of which was associated with distinct magnitudes and probabilities of sucrose pellet rewards or time-out punishments (see Figure 1). The optimal approach in the crGT is to favour options which deliver smaller per-trial gains but lower time-out penalties; consistent choice of the smaller reward options was advantageous due to more frequent rewards, but also less frequent and shorter time-outs, with the two-pellet choice (P2) resulting in the most reward earned per unit time. A 2-s audiovisual cue was presented concurrent with reward delivery on each option. The cue increased in complexity with the size of the reward: P1 win: P1 hole flashes for 2s at 2.5 Hz, monotone; P2 win: P2 hole flashes for 2s at 2.5 Hz, tone changes pitch once after 1s; P3 win: P3 hole flashes at 5 Hz for 1s, followed by flashing of the two neighboring holes in one of two patterns chosen at random, traylight flashes concurrently at 0.2 Hz for 2s, three different tones

used, changing pitch every 0.1 s, in one of two patterns chosen at random; P4 win: P4 hole flashes at 5 Hz for 1s, followed by flashing of all five holes in one of four patterns at random, traylight flashes concurrently at 0.2 Hz for 2s, six different tones used, changing pitch every 0.1 s, in one of four patterns chosen at random.

Responses made during the ITI were recorded as premature responses, a measure of impulsive action, which resulted in the illumination of the house light and a 5 s time-out penalty after which a new trial could be initiated. If a response was not made into one of the 4 holes during the 10 s stimulus presentation, the trial would be registered as an omission, after which point another trial would begin. Animals received on training session per day, 5-7 days per week, until statistically stable, asymptotic levels of performance were observed (35 sessions).

Jugular catheter implantation

Once stable behaviour had been established on the crGT, animals were implanted with a intravenous catheter to enable drug self-administration. Specifically, we aseptically implanted catheters constructed of Silastic silicone tubing (Dow Corning via VWR International, Edmonton, AB, Canada), attached to back-mounted cannulae (Plastics One, Roanoke, VA, USA), into the right jugular vein as per our previous published methods [6]. We passed the catheters through the skin subcutaneously and externalized the cannulae between the scapulae. Following surgery, the catheter was filled with a lock solution (Kelly give the recipe) and the animals were left to recover for 5-7 days, after which crGT testing resumed and cocaine self-administration began.

crGT-concurrent cocaine self-administration

Animals were trained to lever press for cocaine hydrochloride (0.50 mg/kg/infusion, dose calculated as the salt and dissolved in sterile 0.9% saline; Medisca Pharmaceuticals, British Columbia, Canada) or saline vehicle over 10 daily 2-hr sessions (Calu et al., 2007; Ferland and Winstanley, 2017). At the start of each self-administration session, two free infusions of solution were given to fill catheters and indicate drug was available. Rats were presented with two levers, one active and one inactive, with an illuminated cue-light situated over the active lever. Using a fixed ratio (FR1) schedule, responses on the active lever would result in a single 4.5 s infusion in concert with the cue light flashing (50 Hz) and a novel 20 kHz tone (this tone was not used in the crGT). Following the infusion, animals underwent a 10 s time-out during which drug would not be delivered, the cue light and tone were not presented, but levers would remain extended and responses monitored. Responses on the active lever during infusions and timeouts were recorded and interpreted as preliminary cocaine "seeking" behaviors. Inactive lever presses, while monitored, had no programmed consequences. Animals were limited to 30 infusions per hour to prevent overdose.

Histology

In order to visualize DREADD expression in VTA DA neurons, we double-labelled 35um sections coronal sections with primary antibodies against mCherry (Cat#ab205402; Abcam; Toronto, ON, Canada; 1:700 for 48 hours) and tyrosine hydroxylase (Cat#AB152; Millipore Sigma; Oakville, ON, Canada; 1:100 for 24 hours) for 24 hours. Sections were then washed in PBS and incubated with secondary antibodies conjugated to Alexa Fluor® 488 (Cat#A-21103) and Alexa Fluor® 633 (Cat#A-11034) (Thermo Fischer Scientific; Burnaby, BC, Canada; 1:500 for both). Sections were then cover-slipped under HARLECO® Krystalon™

mounting medium (Thermo Fischer Scientific; Burnaby, BC, Canada) and visualized using a SP8 WLL confocal microscope (Leica Microsystems, Germany). For quantification of DREADDs expression, we adopted a method similar to previous published methods [19,24,25]. We defined the anterior and posterior bounds of DREADD expression (i.e., < 1 mCherry+ cell) in coronal sections and counted cells from 1-3 sections within these bounds immediately anterior or posterior to the DREADD infusion site. In both hemispheres, we quantified the number of neurons somatically co-expressing mCherry and TH, as well as those expressing mCherry alone. We averaged the number of cell bodies identified across hemispheres and sections for individual rats. We confirmed, but did not quantify, terminal expression in the nucleus accumbens and medial prefrontal cortex.

Statistical Analysis

We analyzed the following crGT variables per session: score [(P1+P2) – (P3+P4)], percent choice of each option (number of PX chosen/ total number of choices x 100), percentage of premature responses (number of premature responses/ total number of trials initiated x 100), sum of omitted responses, sum of trials completed, and average latencies to choose an option and collect reward. For cocaine self-administration analyzed number of infusions of achieved, number of responses on the active lever, and number of responses on the inactive lever. We used SPSS Statistics 25.0 (IBM; Chicago, IL, USA) to conduct all analyses. We performed repeated measures ANOVA over 35 sessions of task performance with sex and transgene status as between-subjects factors. We followed up significant within-subjects omnibus outcomes by comparing rates of task acquisition via linear contrasts. Significant between-subjects effects were up with independent samples t-tests. Total cocaine consumed and active responses made was

calculated as the sum of all infusions achieved or responses made over the 10 day testing period. Because clinical and pre-clinical research suggests that impulsivity and decision making share a common latent construct [3,26,27], we wished to control for the relationship in some of the present analyses. In this particular group of rats, premature responding accounted for 7% of the variance in decision making score (r_{59} = -0.256, p = 0.025). For all ANCOVA analyses, we set the covariate as the average score over the last 5 days of task acquisition.

Results

Histology

Figure 2A&B show evidence of somatic and axonal DREADD expression in VTA DA neurons. Similar to what others have reported with this transgenic viral approach, we show that the majority of DREADD-expressing neurons co-expressed TH [% co-labelled soma = 71.62 +/- 8.42 (SEM)] . The vast majority of DREADD-expressing cell bodies were located in the VTA (Figure 2C&D; region: $F_{1,55} = 53.502$, $p < 0.000$; VTA vs. SNc: $t_{(fem)27} = 2.826$, $t_{(male)30} = 5.332$). Females and males did not differ in the number of DREADD expressing cells (sex: $F_{1,55} = 0.144$, $p = 0.705$). Though we did observe some DREADD expression in TG- animals, such instances were relatively few (TG: $F_{1,55} = 34.303$, $p <0.000$). We excluded from all analyses rats that expressed mCherry only unilaterally (n = 1 TG+ male) as well as rats expressing a bilateral average of fewer than 2 mCherry positive neurons in the VTA (n = 3 TG+ females). One TG- female died post-operatively.

Decision making profile:

In light of our recent report that females and males respond differently to manipulations of the DA system in the crGT and trend sex-wise interaction with decision making score in the present data (TG x sex: $F_{1,55} = 3.639$, $p = 0.062$), we analyzed the decision making profile of males and females separately. In support of our initial hypothesis, we demonstrated that chemogenetic inhibition of VTA DA neurons during acquisition of the crGT results in more optimal decision making, at least in males (Figure 3B: linear session x TG: $F_{1,29} = 4.664$, $p = 0.039$). Specifically, linear regression analysis revealed that TG- males (r = -0.861 points/day) underwent a more rapid decline in overall decision making score than TG+ males (r = -0.6524

points/day; comparison of slopes: $F_{1,66} = 294.5$, $p < 0.01$). However, when rates of premature responding were covaried in the score analysis for males, the omnibus ANOVA gave a non-significant outcome (linear session X TG: $F_{1,28} = 0.538$, $p = 0.469$).

In stark contrast to the males, TG+ females developed a significantly *lower* score over time than TG- females, indicative of greater risky choice (Figure 3A, session X TG: $F_{34,884} = 1.728$, $p = 0.006$). Using linear regression analyses to evaluate the change in score over session, we confirmed that TG+ females showed a more severe decline (r = -1.235 points/day) in overall decision making score than TG- females (r = -0.352 points/day) (comparison of slopes: $F_{1,66} = 59.13$, $p < 0.001$). Covarying premature response rate did not nullify the within-subjects effect in females, but did result in the detection of a between-subjects difference ($F_{1,25} = 4.478$, $p = 0.044$), which first emerged on session 14 and remained significant for the remainder of testing (TG- vs. TG+ -- s1 to s13: all ts < 0.700, all ps > 0.491; s14: $t_{26} = 2.115$, $p = 0.044$; s35: $t_{26} = 1.926$, $p = 0.033$).

When relative choice of each of the four options was analysed over acquisition of the task in males and females, we again found evidence to suggest CNO was affecting decision making differently across sex, dependent on TG status (choice x session x TG x sex: $F_{102,5508} = 1.403$, $p = 0.005$). In males, CNO did not differentially modulate choice of P1 (Figure 4A; session X TG: $F_{34,986} = 1.014$, $p = 0.447$), P3 (Figure 4C; session x TG: $F_{34,985} = 0.418$, $p = 0.997$), or P4 (Figure 4D; session x TG: $F_{34,986} = 0.583$, $p = 0.683$). However, TG+ males did acquire a preference for the optimal choice P2 at a faster rate than TG- males (Figure 4B; linear session x TG: $F_{1,29} = 6.623$, $p = 0.015$). Subsequent linear regression analyses indicated that TG+ males improved over time (r = 0.1385 points/session), while TG- males got worse (r = -0.078 points/session; comparison of slopes: $F_{1,66} = 5.374$, $p = 0.024$).

We also found further evidence of riskier decision making in females when activity of dopaminergic VTA neurons was dampened; TG+ females displayed a reduced preference for P2 (Figure 5B; session X transgene: $F_{34,850} = 1.924$, $p = 0.001$) and an increased preference for P4 (Figure 5D; session X TG: $F_{34,850} = 2.891$, $p < 0.001$; TG: $F_{1,25} = 4.257$, $p = 0.050$). These observations were further substantiated by subsequent linear regression analyses. Whereas TG- females showed an expected increased preference for the optimal choice P2 over time ($r = 0.300$), TG+ females exhibited a longitudinal *decrease* ($r = -0.242$) ($F_{1,66}=32.89$, $p<0.001$). TG+ females also exhibited a rapid increase in the preference for P4 over time ($r = 0.337$), whereas TG- females progressively decreased choice of this option ($r = -0.096$; comparison of slopes: $F_{1,66} = 101.00$, $p < 0.0001$). In summary, these results suggest that chemogenetically inhibiting dopaminergic neurons within the VTA caused severe deficits in decision making in females, and marginal improvements in males .

Motor impulsivity

The omnibus ANOVA analysing premature responding revealed a between-subjects effects of transgene status ($F_{1,55} = 10.356$, $p = 0.002$) and of sex ($F_{1,55} = 12.257$, $p = 0.001$), again leading us to analyze females and males separately. Concordant with our hypotheses, TG+ males showed reduced premature responding throughout testing (Figure 6B; TG: $F_{1,29} = 9.581$, $p = 0.004$); these differences emerged on the 5th session of crGT training and continued throughout (TG- vs. TG+ -- s1-s4 : all ts < 0.967, all ps > 0.171; s5: $t_{29} = 2.218$, $p = 0.018$; s35: $t_{29} = 0.699$, $p = 0.050$). Transgene status did not impact premature responding in females (Figure 6A; $F_{1,26} = 1.803$, $p = 0.191$).

Additional variables

Although females were generally slower to make a choice than males, TG+ rats of both sexes were slower to choose an option (Figure S1; all rats: TG: $F_{1,55}$ = 11.268, p = 0.001), sex: ($F_{1,55}$ = 12.218, p = 0.001); TG, females: TG: $F_{1,26}$ = 4.246, p = 0.049; males: $F_{1,29}$ = 7.298, p = 0.011). TG+ animals of both sexes also made more omissions, although this only reached trend-level significance in males (Figure S2, all rats: TG: ($F_{1,55}$ = S3.101, p = 0.001; sex: $F_{1,55}$ = 9.240, p = 0.004); TG, -females: ($F_{1,26}$ = 8.966, -males: $F_{1,29}$ = 3.832, p = 0.060). These data may suggest that inhibiting VTA DA neurons lead to a general decrease in response vigour. However, TG+ male rats were only marginally slower to collect reward than TG- animals, and female TG+ rats were even faster in this regard than their TG- counterparts (Figure S3; all rats: sex x TG: $F_{1,55}$ = 6.660, p = 0.003; TG, -females: $F_{1,26}$ = 3.119, p = 0.089, -males: $F_{1,29}$ = 4.010, p = 0.055). Although female TG+ animals completed fewer trials, this likely results from the longer average trial length resulting from the more frequent, lengthy time-out penalties incurred through persistent choice of P4, rather than a drop in motor output (Figure S4; all rats: session x TG x Sex: $F_{34,1870}$ = 1.475, p = 0.038; TG, -females: $F_{1,26}$ = 6.356, p = 0.018, -males: $F_{1,29}$ = 0.016, p = 0.899).

Cocaine self-administration

A planned contrast revealed a linear effect of session for animals self-administering cocaine ($F_{1,37}$ = 36.518, p < 0.000) but not saline ($F_{1,12}$ = 3.698, p = 0.079). Although CNO was no longer active during the self-administration sessions, TG+ rats across both sexes self-administered significantly more cocaine (Figure 7A; TG: $F_{1,37}$ = 5.094, p = 0.030; sex: $F_{1,37}$ = 0.825, p = 0.370; TG x sex: $F_{1,37}$ = 0.012, p = 0.915)); this difference was most pronounced in

the first 2 and last 3 sessions of self-administration (TG- vs. TG+ -- s1: t_{39} = 2.201, p = 0.017; s2: t_{39} = 1.734, p = 0.046; s8: t_{39} = 2.152, p = 0.019; s9: t_{39} = 2.309, p = 0.013; s10: t_{39} = 2.550, p = 0.008; s3-s7: all ts < 1.432, all ps < 0.080). Though the omnibus ANOVA detected no difference in active responses between TG+ and TG- animals, (Figure 7B; active responses - TG: $F_{1,37}$ = 2.518, p = 0.121 ; sex: $F_{1,37}$ = 0.810, p = 0.374; TG X sex: $F_{1,37}$ = 0.279, p 0.600), exploratory post-hoc tests revealed greater responding in TG+ rats in the last 2 sessions (TG- vs. TG+ -- s1-s8: all ts < 1.624, all ps > 0.056; s9: t_{39} = 2.275, p = 0.015; s10: t_{39} = 2.264, p = 0.015). Responding upon the inactive lever were unaffected by the manipulation (TG: $F_{1,37}$ = 0.161, p = 0.690 ; sex: $F_{1,37}$ = 1.220, p = 0.277; TG X sex: $F_{1,37}$ = 1.746, p = 0.195).

Effects of chronic CNO-mediated inhibition of VTA DA neurons on the ability of cocaine self-administration to drive risky choice

We have previously shown that 10 days of cocaine self-administration in the afternoons/evenings results in increased risky decision making on the crGT in males, as indicated by a decrease in score during the concomitant morning sessions. Although we observed the expected drop in score in TG- animals, this was not evident in TG+ rats, regardless of sex (Figure 7A, score: session X TG: $F_{14,532}$ = 2.963, p < 0.000; session x TG x sex: $F_{14,532}$ = 0.611, p = 0.708). We then collapsed data across sex and compared scores averaged over pre-cocaine (5 sessions) and post-cocaine (10 sessions) epochs of crGT performance, and confirmed a decrease in decision making score in TG- rats (Figure 8A; t_{21} = 3.220, p = 0.004) but not TG+ rats (Figure 8B; t_{19} = -0.630, p = 0.536). Saline self-administration did not alter decision making on the crGT (score: session X TG: $F_{14,140}$ = 1.213, p = 0.273).

Effects of chronic CNO-mediated inhibition of VTA DA neurons on the ability of cocaine self-administration to decrease motor impulsivity

We and others have also observed that concurrent cocaine self-administration can reduce premature responding in male rats, consistent with theories surrounding the efficacy of psychostimulants in the treatment of impulse control disorders [28,29]. After observing a session x sex interaction ($F_{1,39}$ = 5.010, p = 0.031) and a trend toward a between-subjects effect of transgene status ($F(_{1,39})$ = 3.348, p = 0.075), we conducted two post-hoc paired-samples t-tests, comparing the pre- and post-cocaine crGT training epochs at each level of sex and transgene status. As expected from our previous findings, we showed that male rats exhibit reductions in motor impulsivity following cocaine self-administration (t_{21} = 2.967, p = 0.004) yet females showed no reduction (t_{19} = 1.366, p = 0.094) (see Figure S4).

Effects of chronic CNO-mediated inhibition of VTA DA neurons on the ability of cocaine self-administration to modulate other crGT variables

Following cocaine self-administration, both female and male TG- rats began to complete fewer trials per session, while TG+ rats were unaffected (session X TG: $F_{14,532}$ = 2.155, p = 0.008; pre- vs. post-cocaine -- TG-: t_{21} = 2.850, p = 0.010; TG+: t_{20} = 0.912, p = 0.372). Fewer trials completed are often coincident with reductions in decision making score, as more losses result in more time out penalties, reducing the possible number of trials completed per session. Compared to TG+ rats, TG- rats became quicker to choose an option (session X TG: $F_{14,532}$ = 2.021, p = 0.013; pre- vs. post-cocaine – TG-: t_{21} = 4.946, p <0.000; TG+: t_{20} = 1.620, p = 0.121) and quicker to collect a reward following a win (session X TG: $F_{14,532}$ = 3.857; p < 0.001; pre- vs. post-cocaine – TG-: t_{21} = 5.382, p <0.000; TG+: t_{20} = 0.592, p = 0.561) Cocaine self-

administration did not result in any change in the number of trials omitted per session (all Fs < 2.212, all ps > 0.145).

Discussion

Here we report that chemogenetic inhibition of VTA DA neurons during acquisition and performance of the crGT improves decision making and impulsivity in male rats, while causing a longitudinal decline in decision-making ability in females. Despite these highly disparate effects across sex, this same manipulation protected both males and females against cocaine-induced deficits in decision making, and minimised the ability of cocaine-taking to reduce motor impulsivity, even though these animals were actually consuming more cocaine compared to Tg-controls. Collectively, these results point to a complex interplay between dopaminergic tone, risky decision making, and the behavioural effects of cocaine, and do not support the hypothesis that suppressing the mesolimbic DA system is universally beneficial or detrimental in terms of addiction vulnerability.

Before considering the potential significance of these findings, it is important to verify that the behavioural changes of interest are not the by-product of a general decrease in motor output, as may be expected from any manipulation that suppresses the DA system. During the crGT, TG+ve rats were slower to make a choice and collect reward, and also omitted more trials, indicative of reduced response vigour. However, only males showed a reduction in premature responses which accompanied a slight reduction in preference for the risky options over time, and these animals completed similar number of trials to their TG-ve counterparts. Female rats, on the other hand, showed no decline in premature responding and a significant increase in risky choice. It is therefore difficult to draw a link between indicators of motor slowing and decreases in motor impulsivity or risky decision-making patterns, as the former are clearly present in female rats without the latter.

In concordance with our initial hypothesis, we demonstrated a critical role for VTA DA neurons in cocaine-induced deficits in decision making. Specifically, we showed that self-administration of cocaine within the same diurnal period no longer resulted in an increase in risky choice when VTA DA neurons were inhibited during the crGT test session, even though rats were still self-administering ample cocaine. Following exposure to cocaine, recent data suggests DA neurons are rendered hypersensitive and more responsive to cues [30,31]. By chemogenetically inhibiting VTA DA neurons during task performance, we may have effectively dampened the ability of uncertain rewards and their accompanying audiovisual cues from driving the DA system, thereby preventing the descent into risky decision making.

Substantial evidence suggests that exposure to highly-cued, probabilistic schedules of reward, such as those used in the crGT, leads to biobehavioural sensitization of the DA system, similar to that observed following exposure psychostimulant drugs [17,32,33]. A recent study, for example, showed that exposure to an uncertain schedule of cued saccharine reward led to upregulation of striatal D_2 receptors equal to that seen following chronic amphetamine [32]. Accumbal DA levels in male rats trained on the cued rGT were significantly lower than those trained on the uncued version of the task [6]. This observation lead us to hypothesize that the repeated phasic DA bursts predicted to arise from daily streams of cued, uncertain rewards trigger an adaptive down-regulation of the DA system. We therefore predicted that chemogenetically inhibiting the mesolimbic DA system during each daily session should minimise the effect of the cues, and enable more optimal decision-making. While male rats did appear to adopt a slightly more optimal strategy, female rats instead dramatically increased preference for the risky options. The improved performance in males was also linked to the decrease in motor impulsive responses, such that the effect became non-significant when

premature response rates are co-varied in the analysis. As such, chronically dampening the mesolimbic DA system in male rats may reduce poor decisions mediated by impulsive "urges", but may not fundamentally improve outcome evaluation or action selection.

The marked increase in risky choice in females caused by chemogenetic inhibition of VTA DA projections, in the absence of any change in premature responding, was totally contrary to our predictions. However, a substantial body of evidence suggests that the mesocorticolimbic DA systems of females and males differ, both at baseline and in their respective responses to dopaminergic drugs [18,20,34]. Numerous microdialysis experiments have demonstrated that, at baseline, males exhibit higher tonic levels of accumbal dopamine than females [35,36]. Taken together with the evidence that chemogenetic manipulations of VTA DA neurons produce similar changes in VTA activity and terminal DA release across the sexes [37], it is possible that the chemogenetic manipulation implemented here rendered absolute DA levels lower in females, as compared to males. Consistent with the inverted-U function which describes the DAergic contributions to some behaviours [38], inhibition of VTA DA neurons may have reduced striatal DA to a level that promoted optimal performance in males, whereas in females, the manipulation may have reduced DA to detrimental levels [39]. Indeed, extremely low levels of striatal DA are associated with impaired cognitive flexibility as well as the affective state of reward deficiency [40,41]. In females, cognitive inflexibility could have impaired learning of the task by causing rats to perseverate on risky options while failing to sample and switch to more optimal strategies. Furthermore, a state of reward deficiency may have increased the allure of the rewards with the highest payouts and most salient cues, as these options would presumably result in the most phasic DA release.

In rationalizing the sex differences we observed here, it is also important to consider that the VTA DA neurons we targeted project to numerous extrastriatal targets, including the prefrontal cortex (PFC) [42]. Most complex cognitive behaviours, including decision making, are orchestrated in a large part by an interaction of dopaminergic processes between the PFC and striatum, and critically, the dopaminergic projection from the VTA to the PFC is denser in female rats than it is in males [43]. Inhibiting all dopaminergic projections from the VTA may have therefore had a greater inhibitory effect on prefrontal dopaminergic processes in females, compared to males. Local antagonism of prefrontal D_2 receptors causes significant deficits in probabilistic decision making [44]. It is therefore possible that the decision making deficits we observed here in females were mediated by a greater reduction in prefrontal DA. Future experiments will aim to evaluate whether this hypothesis is correct.

Chronic inhibition of VTA DA neurons increased cocaine intake, which matches the behavioural effect of administering low doses of DA antagonists[45]. It is important to note that rats were no longer under the influence of CNO while self-administering cocaine, as the drug administration sessions took place over eight hours after injection of CNO. Nevertheless, this pattern of behaviour is consistent with enduring reductions in tonic DA levels. Indeed, others have demonstrated pharmacologically that chronic DA antagonism leads to long-lasting reductions in tonic extracellular DA levels[46,47]; these same neurobiological changes are thought to underlie the anhedonia associated with addiction in humans with lower striatal D2R levels associated with an increased propensity to self-administer cocaine[48,49]. Based on this evidence, the phenotype resulting from our present manipulation may have rendered animals relatively under-responsive to the dopaminergic stimulation produced by cocaine, leading animals to self-administer more drug in order to obtain desirable brain cocaine concentrations.

An alternative, and more speculative hypothesis concerns the potential synergy between cocaine self-administration and risky choice. We found previously that healthy animals trained on the crGT *either* self-administered more cocaine than animals trained on the uncued task, *or* made more risky decisions while self-administering cocaine in the same diurnal period [6]. These data may suggest that making risky choices could somehow substitute for cocaine. Compared to control animals, rats that underwent inhibition of VTA DA neurons during daily crGT sessions experienced less on-task dopamine elevation. These rats may therefore consume more cocaine in an effort to boost accumbal DA levels, as sufficient stimulation may not have been achieved through the task

Through combining daily cognitive behavioural test sessions with cocaine self-administration, the current study sought to evaluate how dampening DA signalling affected the synergistic interactions between drug-taking, impulsivity, and risky decision making in males and females. Although such an approach may seem unnecessarily complicated, the number of behaviours analysed still represents a small fragment of the cognitive processes that human drug users employ each day. By relying exclusively on simple behavioural models of addiction disorders, we risk drawing overly simplistic conclusions. The findings reported here strongly suggest that the ability of cocaine-taking to subsequently affect measures of both risky choice and impulsivity results from a disinhibition or hypersensitivity of the mesolimbic DA system; when we suppressed activity in this pathway, cocaine self-administration no longer altered these behaviours on the crGT. However, this same inhibition resulted in greater drug-taking, suggesting that any attempt to impede dopaminergic signalling in order to improve the cognitive sequelae of drug abuse may have unwanted consequences. Furthermore, although dampening dopaminergic activity during acquisition of the crGT had a generally positive impact on impulse

control and decision making in male animals, this same manipulation drastically enhanced risky choice in females. Given that a rise in preference for uncertain options is a predictor of drug use[50], any putative therapeutics designed to prevent a "hyperdopaminergic state" from developing and contributing to the generation of addictions may be counter-productive, particularly in females. Despite decades of research into the relationship between DA signalling and addiction, questions clearly remain as to how activity within the DA system prevents or facilitates the addicted state. Combining complex behavioural models with sophisticated neural manipulations in animals will hopefully help to resolve these pressing issues, and lead to translationally meaningful results.

Author Contributions

TJH & CAW designed the experiment. TJH & KMH performed the surgeries. TJH, KMH, BAH, SAE, & CSC conducted the behavioural testing. CDH, CSC, & SK conducted the histology. CSC & TJH did the confocal imaging. GDB & BR provided statistical consultation. TJH & CAW wrote the paper.

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Figure 1. Hynes et al.

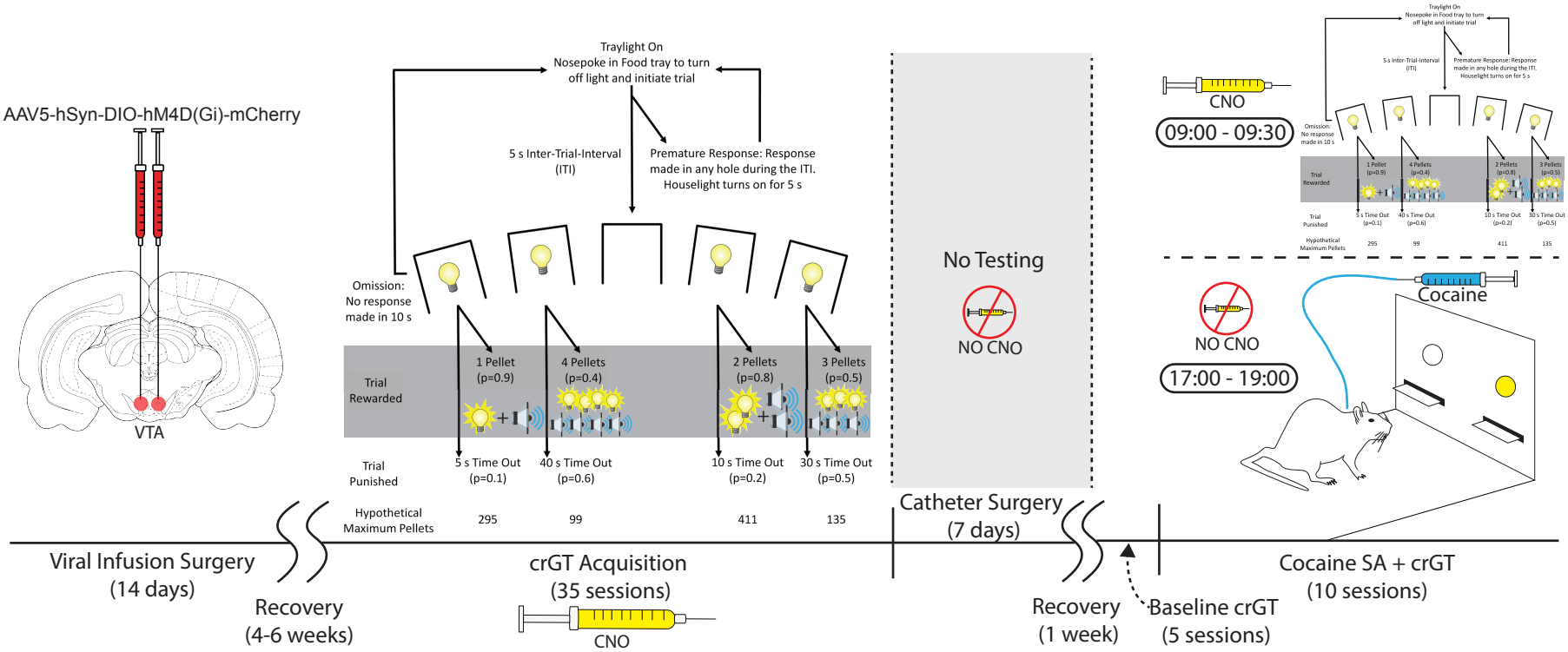

Figure 1. Experimental Timeline. The inhibitory DREADD AAV5-hSyn-DIO-hM4D(Gi)-mCherry was sterotaxically delivered into the bilateral VTA of female and male TH::Cre rats, which were then given 4-6 weeks to recover with their cage mates with ad libitum food. Rats were then food restricted and began daily crGT training, where CNO (1.0 mg/kg; i.p.) was delivered 30 minutes prior to the commencement of each session. After completing 35 sessions of crGT, rats were implanted with jugular vein catheters, singly housed, and allowed to recover in their home cages for one week. Rats then completed 5 baseline sessions of crGT with CNO on board, before entering into a phase of concurrent cocaine self-administration and crGT training. Each morning, CNO was administered 30 minutes prior to crGT training. Following each crGT session, rats were returned to their cages for ~8 hours and, later that evening, placed in different operant boxes and allowed to self-administer intravenous cocaine for 2 hours (0.5 mg/kg/infusion; FR1). After 10 consecutive days of concurrent crGT and cocaine self-administration, rats were euthanized, and their brains were processed for immunohistochemistry.

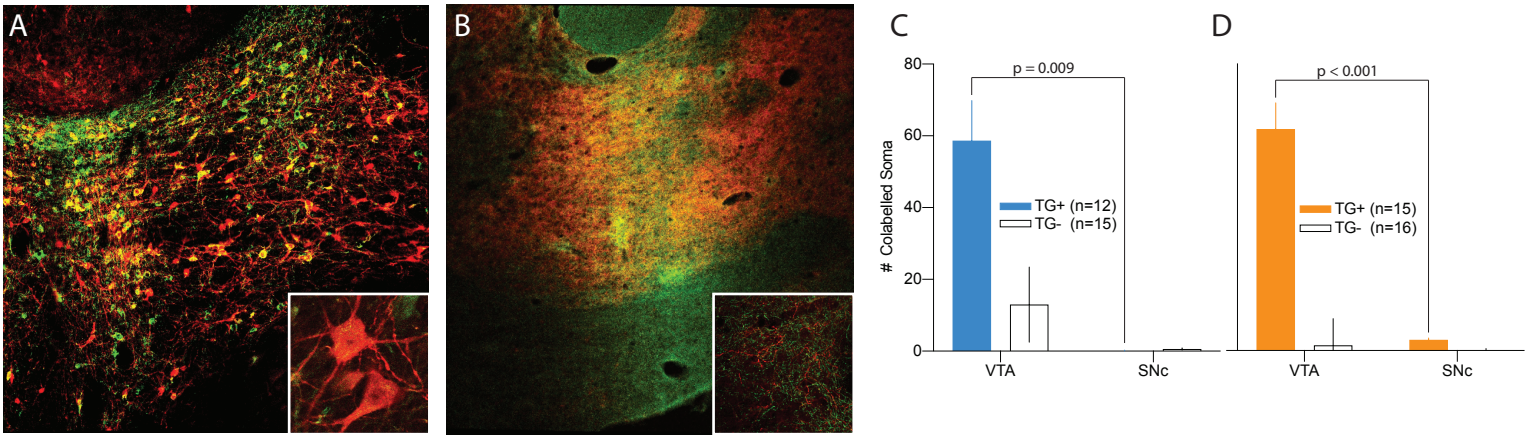

Figure 2. DREADD expression in VTA DA neurons. (A) Shown in green are putative dopaminergic [i.e., tyrosine hydroxylase (TH)] positive cell bodies of the VTA. Cell bodies shown in red are mCherry-tagged, indicating DREADD expression. Inset shows robust colabelling with TH and mCherry in cell bodies and axonal processes. (B) Striatal section showing dense DAergic innervation (TH; green) along with DREADD expression (mCherry, red). Inset shows DREADD expressing DAergic terminals. (C) In female and (D) male rats, the majority DREADD expressing DAergic neurons were found in the VTA, with minimal expression in TG- animals. p-values indicate the outcome of independent samples t-tests.

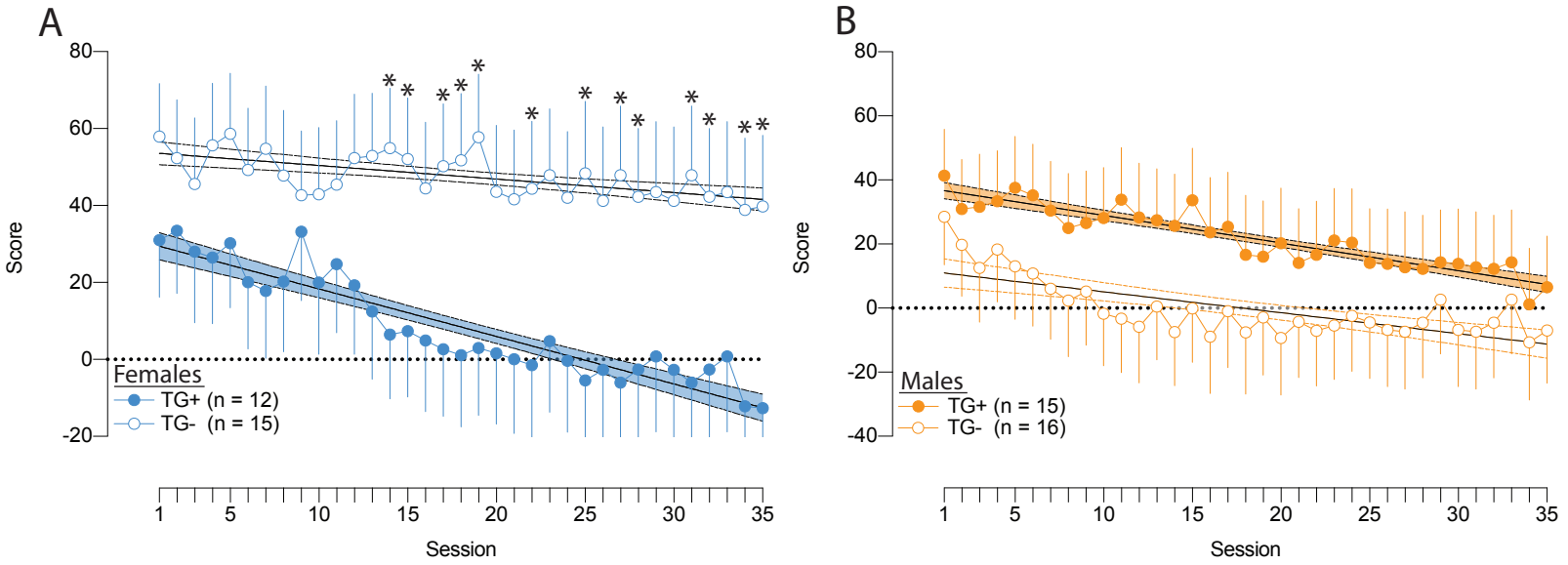

Figure 3. Longitudinal decision making score in female and male rats during crGT training. (A) TG+ females showed a steeper longitudinal decline in decision making ability than TG- females, following chronic administration of CNO prior to each crGT training session. By session 14 of crGT training, TG+ females performed significantly worse than TG- females. (B) Male TG+ rats showed a showed a significantly slower decline in decision making ability than TG- rats, but did not significantly differ in absolute score, following acquisition. Data points represent group means +/- 1SEM. Linear regression line is shown intersecting data points and is bound by dashed lines, marking the 95% CIs. "*" indicates sessions on which independent samples t-tests showed TG+ and TG- rats to be significantly different.

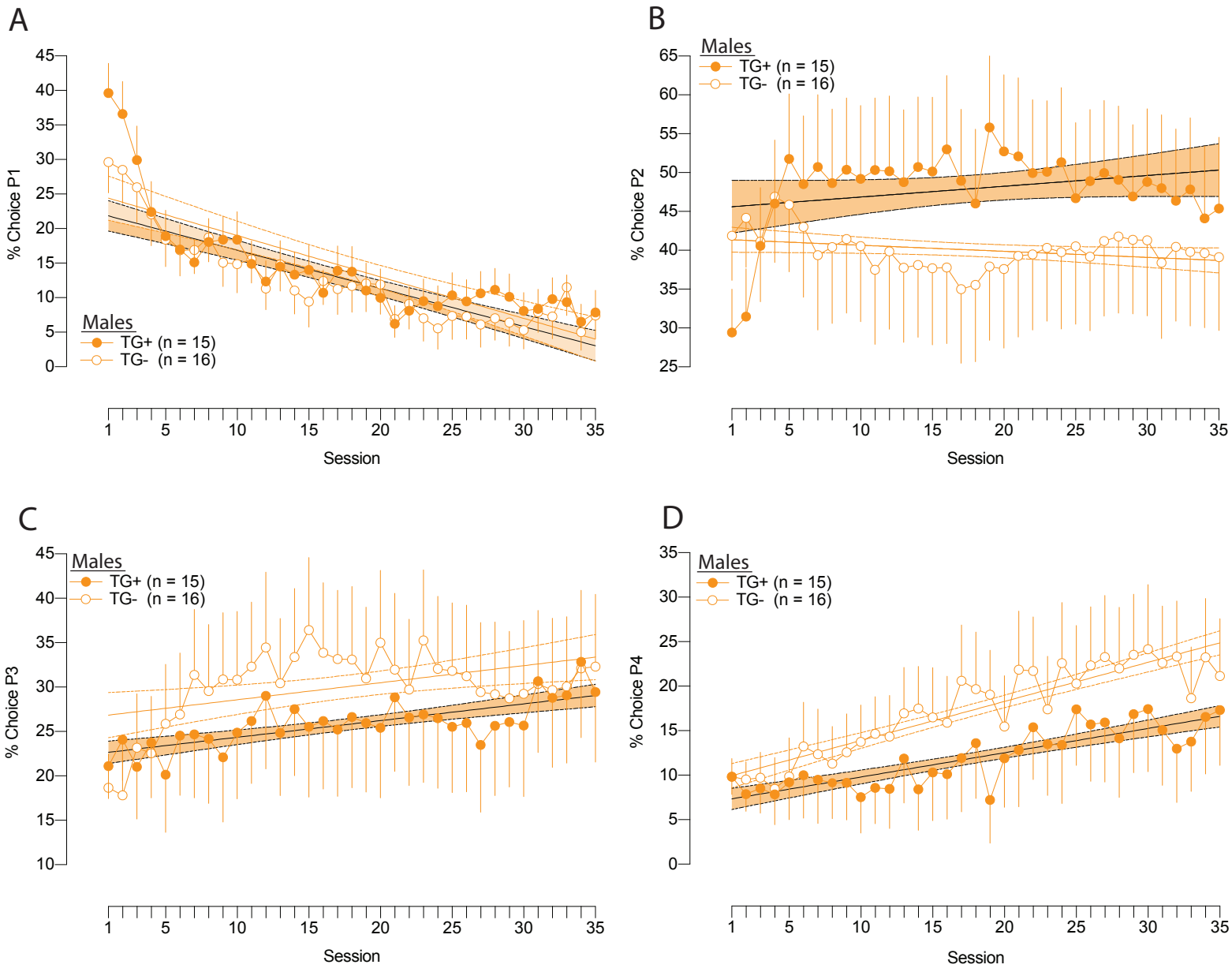

Figure 4. Longitudinal choice profile in male rats during crGT training. (A) TG+ and TG- males did not differ in the choice of P1. (B) TG+ males acquired a preference for the optimal choice, P2, more rapidly than TG- males. (C) TG+ and TG- males did not differ in their preference for P3 or (D) P4. Data points represent group means +/- 1SEM. Linear regression line is shown intersecting data points and is bound by dashed lines, marking the 95% CIs.

Figure 5. Hynes et al.

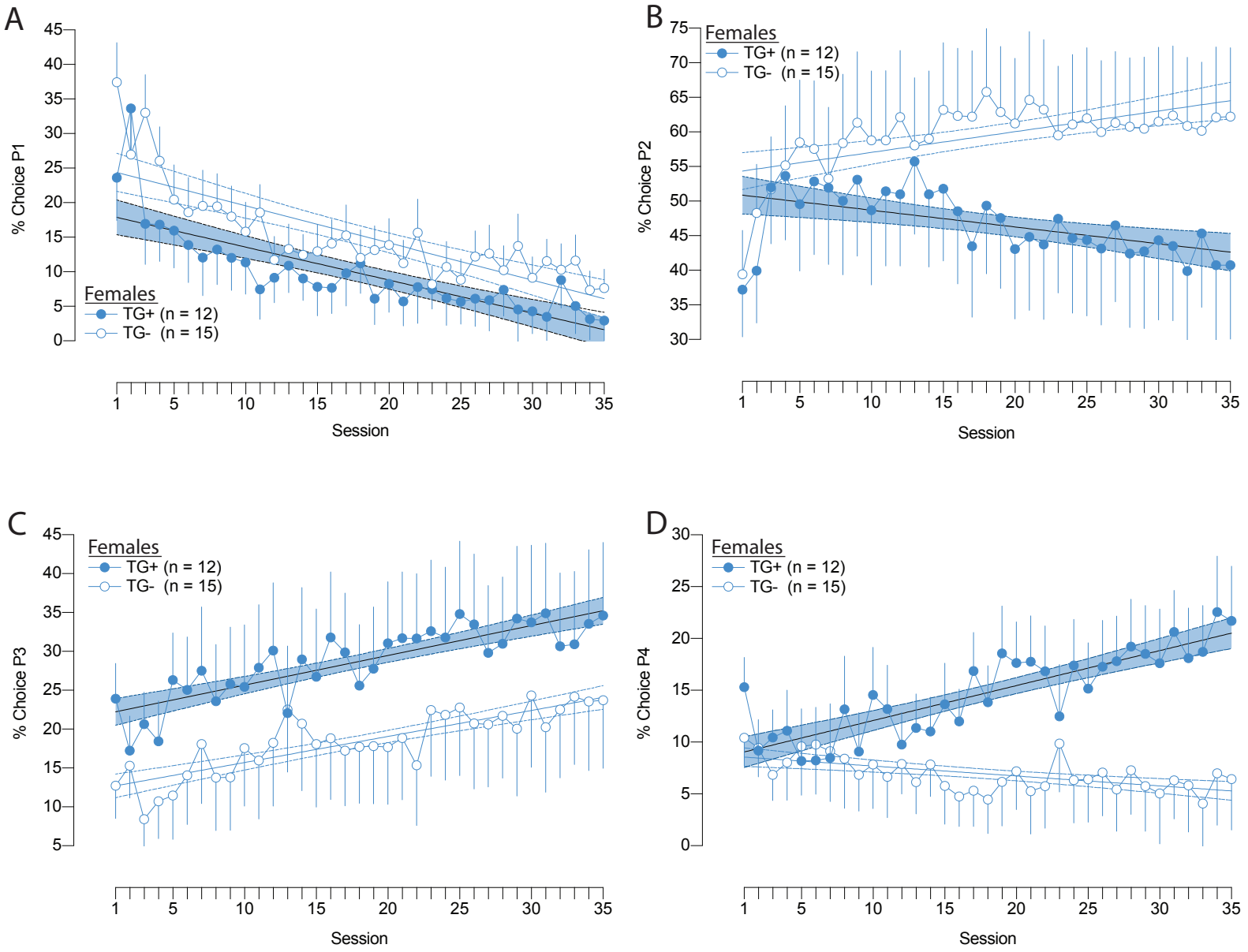

Figure 5. Longitudinal choice profile of female rats during crGT training. (A) TG+ females did not differ from TG- females in the propensity to choose the option P1. (B) TG- females acquired a preference for the optimal option P2 more rapidly than TG+ females. (C) TG+ and TG- females did not differ in the preference for P3. (D) TG+ females acquired a preference for the disadvantageous option P4 more rapidly than their TG- counterparts. Data points represent group means +/- 1SEM. Linear regression line is shown intersecting data points and is bound by dashed lines, marking the 95% CIs.

Figure 6 Hynes et al

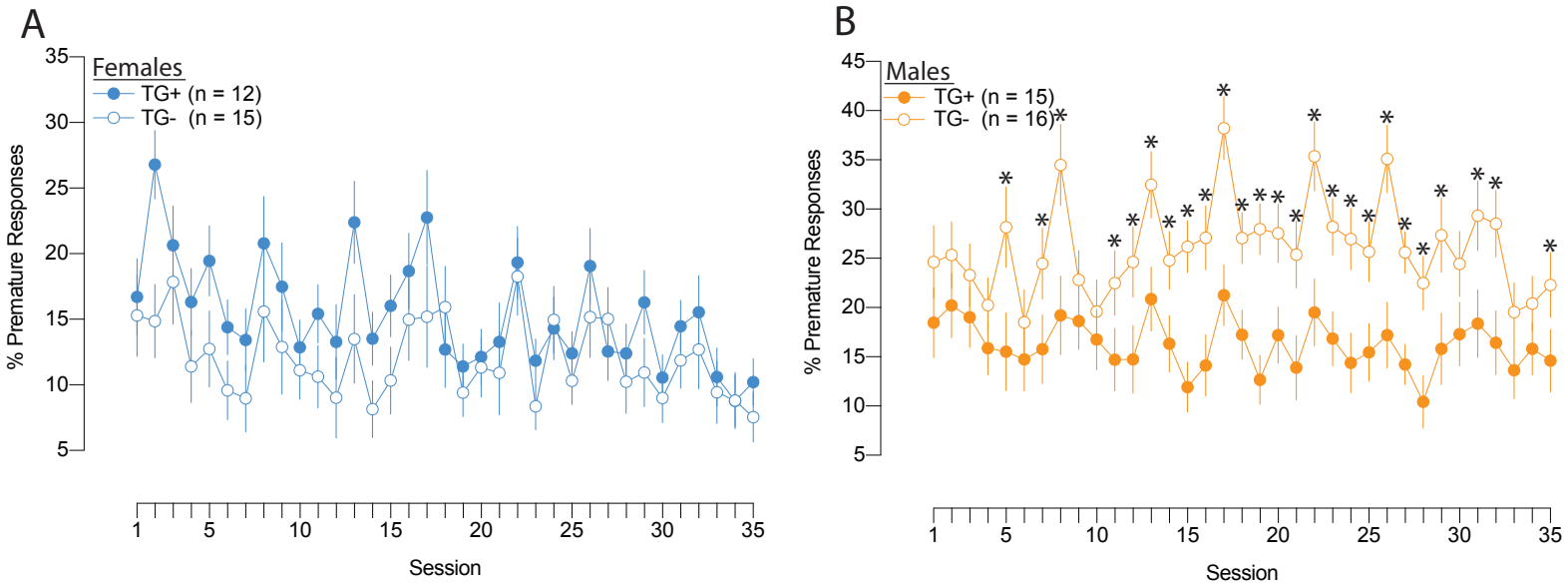

Figure 6. Longitudinal impulsivity in female and male rats during crGT training. (A) TG+ and TG- females did not differ from one another in the rate of premature responding, following chronic administration of CNO prior to each crGT training session. (B) Beginning on session 5 and lasting the duration of training, TG+ rats made impulsive responses than TG- rats following chronic CNO administration prior to each rGT training session. Data points represent group means +/- 1SEM. "*" indicates sessions on which independent samples t-tests showed TG+ and TG- rats to be significantly different.

Figure 7. Hynes et al.

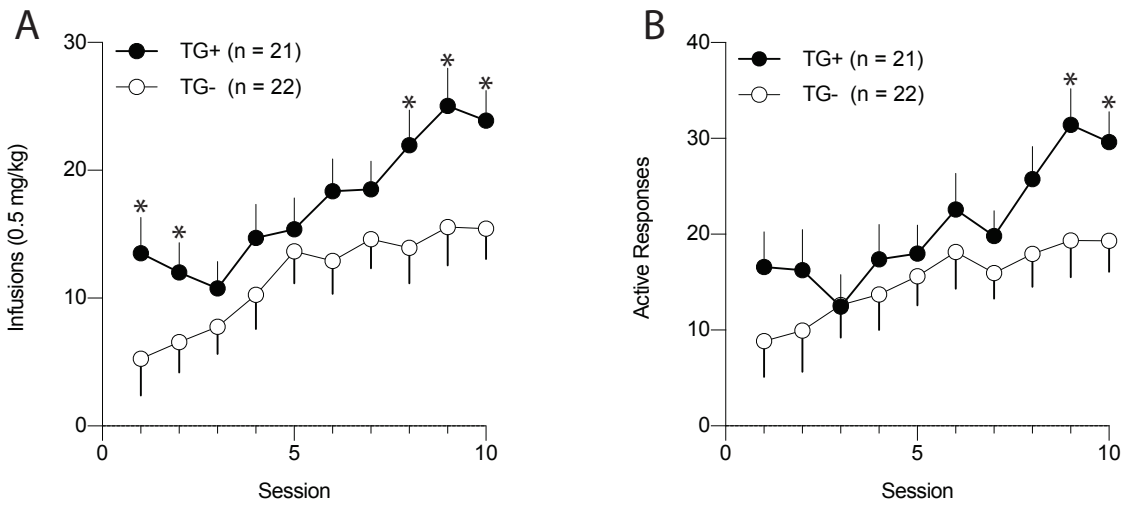

Figure 7. Cocaine self-administration. (A) Despite CNO no longer being active TG+ rats took more infusions of cocaine than TG- rats in the early and late sessions of the 10-day cocaine self-administration period. (B) In the last 2 session of cocaine self-administration, TG+ rats made more responses upon the active lever than TG- rats. "*" indicates sessions on which independent samples t-tests showed TG+ and TG- rats to be significantly different. Data shown as means +/- 1SEM.

Figure 8. Hynes et al.

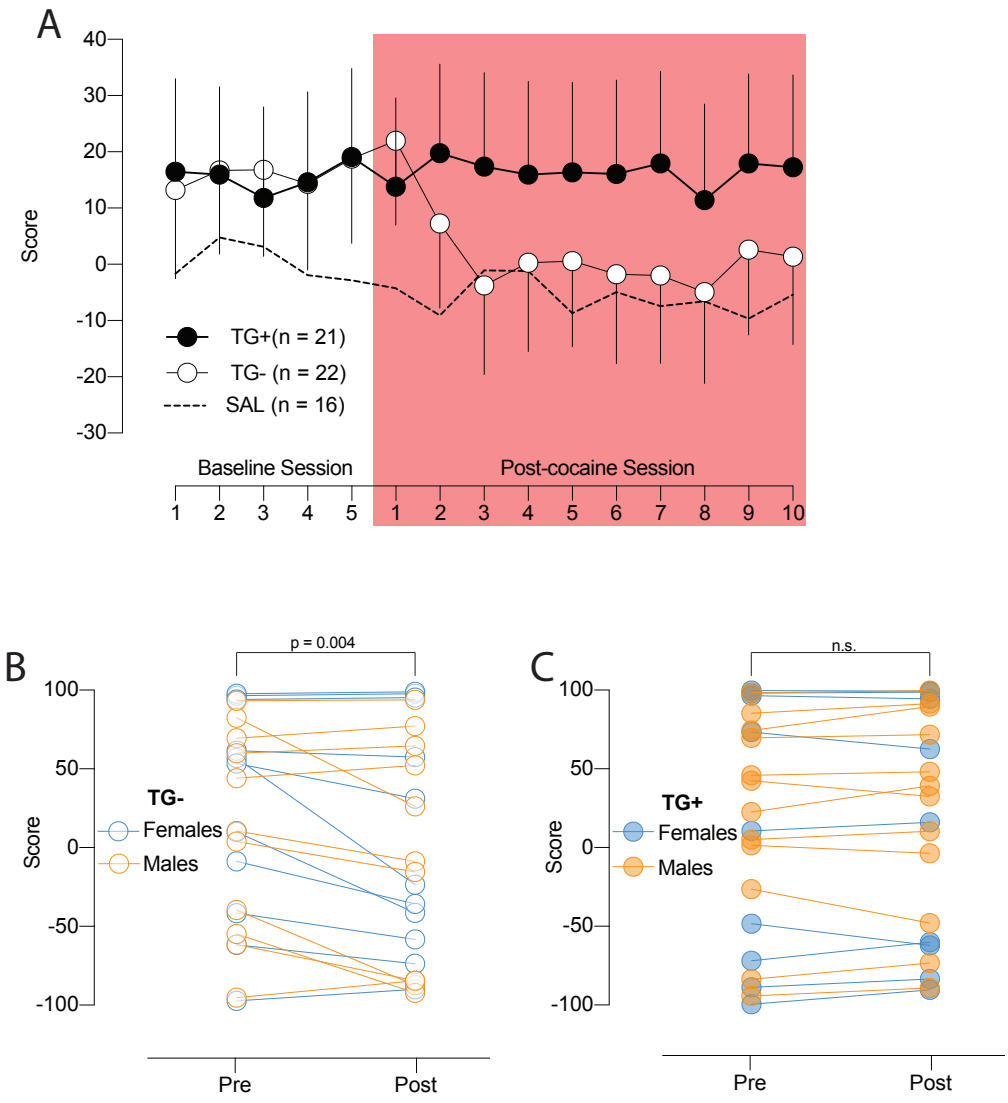

Figure 8. Concomitant crGT performance and cocaine self-administration. (A) TG- rats underwent an expected decline in decision making following engagement in cocaine self-administration, while such cocaine-induced deficits were not observed in TG+ rats. Rats that self-administered only saline (SAL) did not exhibit decision making deficits. Data shown as means +/- 1SEM. (B) Compared to their pre-cocaine baseline decision making score, male and female TG- rats underwent a significant decline following cocaine-exposure. (C) TG+ rats did not differ in decision making score pre- vs. post-cocaine exposure. Individual rats are represented by individual data points, with the connecting line showing the direction of change in score. p-values indicate the outcome of paired samples t-tests.