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ARTICLE

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Selective chemogenetic inactivation of corticoaccumbal projections disrupts trait choice impulsivity

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Impulsive choice has enduring trait-like characteristics and is defined by preference for small immediate rewards over larger delayed ones. Importantly, it is a determining factor in the development and persistence of substance use disorder (SUD). Emerging evidence from human and animal studies suggests frontal cortical regions exert influence over striatal reward processing areas during decision-making in impulsive choice or delay discounting (DD) tasks. The goal of this study was to examine how these circuits are involved in decision-making in animals with defined trait impulsivity. To this end, we trained adolescent male rats to stable behavior on a DD procedure and then re-trained them in adulthood to assess trait-like, conserved impulsive choice across development. We then used chemogenetic tools to selectively and reversibly target corticostriatal projections during performance of the DD task. The prelimbic region of the medial prefrontal cortex (mPFC) was injected with a viral vector expressing inhibitory designer receptors exclusively activated by designer drugs (Gi-DREADD), and then mPFC projections to the nucleus accumbens core (NAc) were selectively suppressed by intra-NAc administration of the Gi-DREADD actuator clozapine-n-oxide (CNO). Inactivation of the mPFC-NAc projection elicited a robust increase in impulsive choice in rats with lower vs. higher baseline impulsivity. This demonstrates a fundamental role for mPFC afferents to the NAc during choice impulsivity and suggests that maladaptive hypofrontality may underlie decreased executive control in animals with higher levels of choice impulsivity. Results such as these may have important implications for the pathophysiology and treatment of impulse control, SUDs, and related psychiatric disorders.

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INTRODUCTION

Humans and animals are required to make decisions between immediate and delayed rewards in order to survive (i.e., foraging theory). Deciding between rewards of different delays to receipt is referred to as intertemporal choice and is marked by various gradations of choice impulsivity, or the propensity to choose rewards available now over those available later. The process underlying impulsive choice is commonly referred to as delay discounting (DD), as individuals discount the value of rewards as a function of the delay to their delivery [1, 2]. For instance, most people would consider a free large pizza today more valuable than five free large pizzas in a year and a wide body of research demonstrates DD of rewards by both humans and animals [3, 4]. Excessive levels of DD, however, result in impulsive choices and are associated with several psychiatric disorders, including attention-deficit/hyperactivity disorder, impulse control disorders, and substance use disorder (SUD) [3, 5–9]. Further, choice impulsivity may directly contribute to the pathophysiology of these disorders (e.g., immediate drug use vs. family and job stability). For instance, steeper discounting as delays to reward increase predicts enhanced acquisition and escalation of cocaine self-administration in rats and is associated with greater severity of SUD symptoms and reduced success of recovery in humans [10–13]. Despite this, the neurobiological mechanisms underlying DD remain unclear.

DD has both trait-like (enduring) and state-like (unfixed) characteristics which may underlie both innate and acquired vulnerabilities to SUD [14, 15]. Among its trait-like characteristics, DD is heritable [16–18] and has robust test-retest reliability [14, 19–22], and subjects demonstrate similar levels of discounting across tasks [23, 24] and reinforcers [14, 25]. Conversely, DD is also readily altered by short-term manipulations, such as drug administration [26–28]. Even with the robust trait-like characteristics of DD, comparisons of DD across the lifespan have yielded mixed results. While several studies show a decline in DD as individuals age from adolescence to adulthood and from young adulthood to older adulthood [29–36], other research demonstrates no difference in DD across the lifespan [32, 34, 37–40], or

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even an increase in DD [41]. Further, very few reports test the same subjects across development, making it difficult to determine if DD is a stable lifelong trait within subjects and thus if DD phenotypes (e.g., high vs. low levels of choice impulsivity) convey similar risk to the development of SUD across life stages.

Human studies suggest a role for corticostriatal circuits in DD. For instance, corticostriatal activity is shown to be inversely related to choice impulsivity [42–45], with greater DD associated with reduced frontal cortical control over both ventral and dorsal striatum [46–48]. Yet animal research interrogating corticostriatal circuits during DD has not provided a clear conclusion. Several investigations show that lesions or pharmacological manipulation of the striatum disrupts DD in rodents [49–58]; in particular, lesions of the nucleus accumbens core (NAc), the accumbens subregion shown to be involved in processing delay to reinforcement, increase delay aversion [50, 59]. However, it is unclear how frontal cortical regions may influence striatal processing during DD. Human research demonstrates a role for both orbitofrontal cortex (OFC) [60, 61] and anterior cingulate cortex (ACC) [42, 45] in intertemporal choice. Notably, OFC is conserved in rodents, while human ACC aligns most with rodent medial prefrontal cortex (mPFC) [62–64]. Rodent research illustrates that the role of OFC in DD is complex; showing either decreased DD following inactivation, increased DD after inactivation, differences based on subregion (i.e., medial versus lateral OFC), or no effect [65–71]. In rodent studies, DD is enhanced by prefrontal 6-OH-DA dopamine depletion in juvenile rats [72], pharmacological disruption of dopaminergic signaling in mPFC in adult rats [73], or viral over-expression of D1 receptors in mPFC [74]. However, while Churchwell and colleagues (2009) showed that pharmacological inactivation of mPFC increased impulsive choice on a single delay T-maze procedure, several studies show that complete lesions of the mPFC do not affect DD in rats during a multi-delay instrumental choice procedure [50, 75–77]. Therefore, while a wide body of research has observed cortical and striatal contributions to DD, a causal role of corticostriatal projections in control over impulsive choice has not been established.

Consequently, the current study had two main goals: (1) to assess DD in adolescence and adulthood in the same subjects to directly compare intertemporal choice across the lifespan and (2) to investigate the role of corticostriatal projections in trait-like DD. To accomplish this, adolescent male rats were trained daily on a DD task in which delay to the larger reinforcer increases across the session. Stable DD behavior was recorded in adolescence and again in adulthood to allow comparison of impulsive choice across developmental stages in the same animals and determine potential trait-like choice impulsivity. Following stable DD behavior in adulthood, rats received bilateral infusion of an inhibitory DREADD virus (Gi-DREADD) in the prelimbic region of the mPFC and bilateral guide cannulae aimed at the NAc. Systemic delivery of the Gi-DREADD actuator clozapine-n-oxide (CNO) allowed for the assessment of temporary mPFC inactivation on DD behavior, while intra-NAc infusion of CNO allowed for the interrogation of mPFC-NAc projections in impulsive choice.

MATERIALS AND METHODS

Animals

Subjects were male Long-Evans rats bred at the University of Maryland School of Medicine. Many studies investigating sex differences in intertemporal choice show that male and female rodents and humans exhibit similar levels of DD [78, 79], have conserved corticostriatal activity during task performance [47], and express indistinguishable test-retest reliability [41, 80]. Adolescent rats (PND 30–55) were group-housed, and adult rats (PND 55+) were pair-housed until stereotaxic surgery in adulthood (PND ~ 120–130); they were then singly housed to protect surgical implants. When littermates were used, they were distributed across experimental and control groups. All housing rooms were maintained at 24 °C and 40–50% humidity under a 12-h light/dark cycle (lights on at 0700 h). Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Maryland School of Medicine.

Apparatus

See Supplementary Methods.

DD task

In adolescence and adulthood, food was removed from the homecage the evening before initiation of training or re-testing to facilitate acquisition of the task. Afterwards, daily food pellets earned on the task were supplemented up to 16 ± 2 g of chow after the session. This feeding schedule resulted in adolescent animals maintaining a typical free-feeding body weight throughout adolescence (based on feeding regimen by [29] as well as growth charts published by Charles River Laboratories) and adult animals maintaining 85 ± 5% free-feeding body weight.

Figure 1A illustrates the study timeline. During adolescence and early adulthood, rats were tested in 2 sessions per day. Following re-stabilization on the DD task after surgery, rats were thereafter tested once per day. Initially, adolescents underwent nosepoke training under a fixed ratio 1 (FR1) schedule of reinforcement during 45-min sessions; in addition to response-contingent pellets delivered by a nosepoke at either port, a single, noncontingent pellet was delivered every 5 min. Once nosepoking produced ≥50 reinforcers in both sessions, rats were trained to discriminate among two ports that produced 1 ("small") vs. 3 ("large") pellets under an FR1 schedule of reinforcement until they allocated ≥80% of responses to the large alternative port.

Rats then progressed to a within-session increasing delay procedure [2, 28]. Sessions consisted of 4 blocks of 2 sample trials followed by 12 choice trials (Fig. 1B). The start of each trial was signaled by houselight and active nosepoke port(s) light illumination. In the 2 sample trials, rats were exposed to both choice alternatives in random order. If the left port was signaled, a response in that port produced a single pellet immediately (i.e., small-immediate alternative). If the right port was signaled, a response in that port produced three pellets after a delay of T-s (i.e., large-delayed alternative) during which the stimulus light above the port flashed (2 Hz) until pellet delivery. Choice trials allowed rats to choose between the small-immediate and large-delayed alternatives. During the first block, the delay (T) to the large-delayed alternative was 0-s, and it increased across successive trial blocks (5, 10, and 20-s). Trials were separated by a 45-s compensating inter-trial interval (ITI) that ensured equal trial spacing regardless of the alternative chosen. Sessions terminated following completion of all trials or 60 min, whichever occurred first. Changes in delay gradient shape were quantified using the area-under-the-curve (AUC) method where a decrease in AUC indicated an increase in impulsive choice [81]. Stable discounting behavior (i.e., less than 10% change in AUC for 3 consecutive sessions with no successive increases or decreases in AUC across the 3 sessions) was averaged in adolescence (~PND 50–53) and again in adulthood following stereotaxic surgery but prior to chemogenetic testing (PND ~ 150–153) ($n = 16$).

Virus injection and cannula implantation surgery

Under isoflurane anesthesia, rats ($n = 16$) were bilaterally transduced with 0.5 µl/side of pAAV-CaMKIIa-hM4D(Gi)-mCherry (gift from Bryan Roth, University of North Carolina Vector Core, Chapel Hill, NC) [82] into the prelimbic region of mPFC (+2.7 AP, ±0.5 ML, −2.5 DV relative to skull surface at Bregma) to primarily target excitatory projection neurons [83]. Bilateral guide cannulae (26 G, Plastics One, Roanoke, VA) were targeted at the NAc (+1.3 AP, ±1.4 ML, −5 DV), and a stainless steel obdurator (33 G, Plastics One) was inserted in each cannula [83]. Projections of the prelimbic cortex project preferentially to the NAc core [84–86]. An additional group of animals was implanted with guide cannula (as above) but not transduced with virus in order to control for stereotaxic implantation and to examine the intrinsic pharmacological effects of CNO (NIDA Drug Supply Program, RTI International, Research Triangle Park, NC). Rats were administered topical antibiotic and analgesic ointment as well as injectable analgesics for 48 h following surgery and were allowed at least 7 days of recovery.

Systemic and intracranial pharmacology

Following surgery, rats were allowed to restabilize on the DD task. In separate sessions, the same group of rats underwent systemic (1.5–3.0 mg/kg, IP; $n = 9$) and intra-NAc (1.5 µg/side, IC; $n = 16$) injections of CNO or

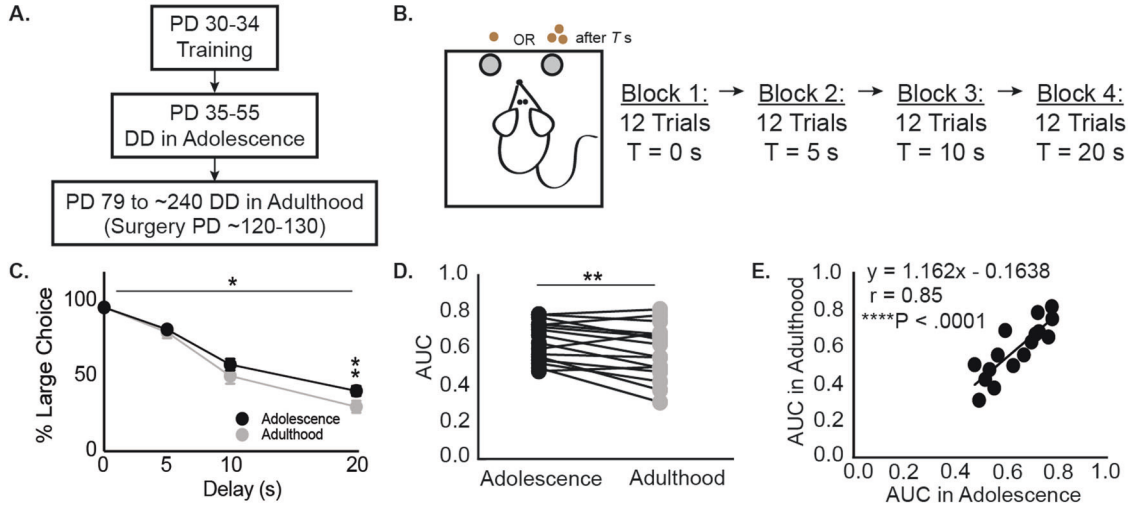

Fig. 1 Delay discounting is stable across development. A Experimental timeline indicating postnatal day (PD) on which animals began nose-poke training for food pellets and the DD task. **B** Illustration depicting the DD task structure. During each session, rats cycled through 2 forced-choice and 12 free-choice trials of each delay in ascending order (0, 5, 10, and then 20 s), with 1 food pellet available immediately or 3 pellets available after a delay. **C** % choice of the large reinforcer in adolescence (~PND 50–53) and adulthood (~PND 150–153) at each delay ($n = 16$). Two-way repeated measures ANOVA shows that animals discounted delayed rewards in both their adolescence and adulthood ($P < .001$); however, in adulthood they exhibited greater DD ($P = 0.012$), specifically at the 20-s delay ($P = 0.004$). **D** AUC in adolescence and adulthood similarly illustrated greater DD in adulthood ($P = 0.009$). **E** Scatter plot and fit line (Y = 1.162*X - 0.1638) showing a significant positive correlation between impulsive choice in adolescence and in adulthood, as measured by AUC, evidencing relative stability in level of DD across development. Error bars indicate +/− SEM. *$P \leq 0.05$, **$P < 0.01$, ****$P < 0.0001$.

saline vehicle ($n = 14$). Doses of CNO and vehicle were counterbalanced between animals within each block. Two rats did not complete vehicle injections due to blocked IC cannulae. After each test session animals were given at least 3 sessions to re-establish stable discounting behavior. To determine if CNO infusion itself in the absence of Gi-DREADD transduction affects impulsive choice, a separate cohort of rats received bilateral cannula aimed at the NAc but no transfection with Gi-DREADD virus ($n = 10$). Once they achieved stable DD performance in adulthood, the effects of intra-NAc CNO were assessed. For IP injections, CNO or vehicle was delivered in a volume of 1 ml/kg 30 min before testing. For IC infusions, bilateral infusion cannulae (33 G, 1.5 mm projection) were connected to a 5 µl Hamilton syringe via PE50 tubing back-filled with CNO. Infusion cannulae were inserted through the guide cannulae 1 min prior to CNO delivery, and CNO was delivered in a volume of 0.5 µl/side over 2 min using a motorized syringe pump. Infusion cannulae were left in place for 5 min after infusion.

Histology

After behavioral experiments, animals were anesthetized with a fatal dose of pentobarbital and transcardially perfused. Brains were extracted, sectioned into 40 µm coronal sections, and viewed using a fluorescent confocal microscope to confirm hM4Di-mCherry expression in cell bodies of the mPFC and terminals in the NAc, as well as cannula placement in the NAc. Rats included in data analysis had viral expression confined to the prelimbic cortex [83] and bilateral NAc cannulae placement within a confined range of the targeted NAc coordinates (+/− 0.25 mm AP, +/− 0.3 mm ML, and + 0.25 mm DV) (Fig. 2).

Slice electrophysiology

To verify inhibitory function of the DREADD virus following CNO, a subset of rats were deeply anesthetized with isoflurane, and their brains were rapidly removed and submerged in ice-cold, modified artificial cerebrospinal fluid (aCSF; in mM: 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose). 250 µm thick coronal slices were made containing the NAc (Leica VT 1200 vibratome, Leica Biosystems, Deer Park, IL) and stored in 95% oxygen, 5% carbon dioxide (carbogen)-bubbled aCSF (in mM: 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose) at 32 °C for 30 min. Hemisected slices were placed in a recording chamber with aCSF containing 50 µM picrotoxin at 30 ± 1 °C. Whole-cell patch-clamp recordings were made from NAc medium spiny neurons (MSNs) using boroscillate glass micropipettes (World Precision Instruments, Sarasota, FL) in the 2–5 MΩ range filled with a high $CsMeSO_3$ internal solution (in mM: 120 $CsMeSO_3$, 5 NaCl, 10 tetraethylammonium-Cl, 10 HEPES, 5 QX-314, 1.1 EGTA, 0.3 Na-GTP, and 4 Mg-ATP). Cells were voltage clamped at −60 mV using a Multiclamp 700B amplifier (Molecular Devices, San Jose, CA), and excitatory postsynaptic currents (EPSCs) were evoked every 20 s using a concentric bipolar stimulating electrode (World Precision Instruments) located approximately 100 µm from the cell. Signals were filtered at 2 kHz and digitized at 10 kHz. Following a stable 5-min baseline period, aCSF containing either 10 µM CNO or vehicle was applied to the slices. Cells were discarded from analysis if the series resistance changed more than 15% throughout the recording.

Data analysis

See Supplementary Methods.

RESULTS

Delay discounting in adolescence and adulthood is positively correlated

To examine impulsive choice across development, performance on the DD task during adolescence and again in adulthood was assessed. Fig. 1C shows delay gradients for the same animals in adolescence and adulthood. A significant main effect of delay [$F(3,45) = 207.207$, $P < .001$, $\eta p^2 = 0.932$] illustrates that animals robustly discounted delayed rewards across adolescence and adulthood. Interestingly, animals exhibited greater discounting of delayed rewards in adulthood as compared to adolescence [main effect of developmental stage $F(1,15) = 8.251$, $P = 0.012$, $\eta p^2 = 0.355$; delay x developmental stage interaction $F(3,45) = 3.321$, $P = 0.028$, $\eta p^2 = 0.181$]. Post-hoc comparisons between groups at each delay showed that rats chose significantly fewer rewards at the 20-s delay [$t(15) = 3.372$, $P = 0.004$, Cohen's $d = 0.843$] but exhibited similar levels of discounting at the other delays [corrected $\alpha = 0.0125$; 0-s, $t(15) = -0.019$, $P = 0.985$, Cohen's $d = 0.005$; 5-s, $t(15) = 0.498$, $P = 0.626$, Cohen's $d = 0.124$; 10-s, $t(15) = 2.578$, $P = 0.021$, Cohen's $d = 0.644$]. Paired-samples *t*-test revealed a significant difference between AUC in adolescence and adulthood [Fig. 1D; $t(15) = 2.983$, $P = 0.009$, Cohen's $d = 0.746$]. Fig. 1E illustrates a significant positive correlation between delay gradient AUC in adolescence and adulthood [$r = 0.85$, $P < 0.0001$], evidencing that, although DD tends to increase as training

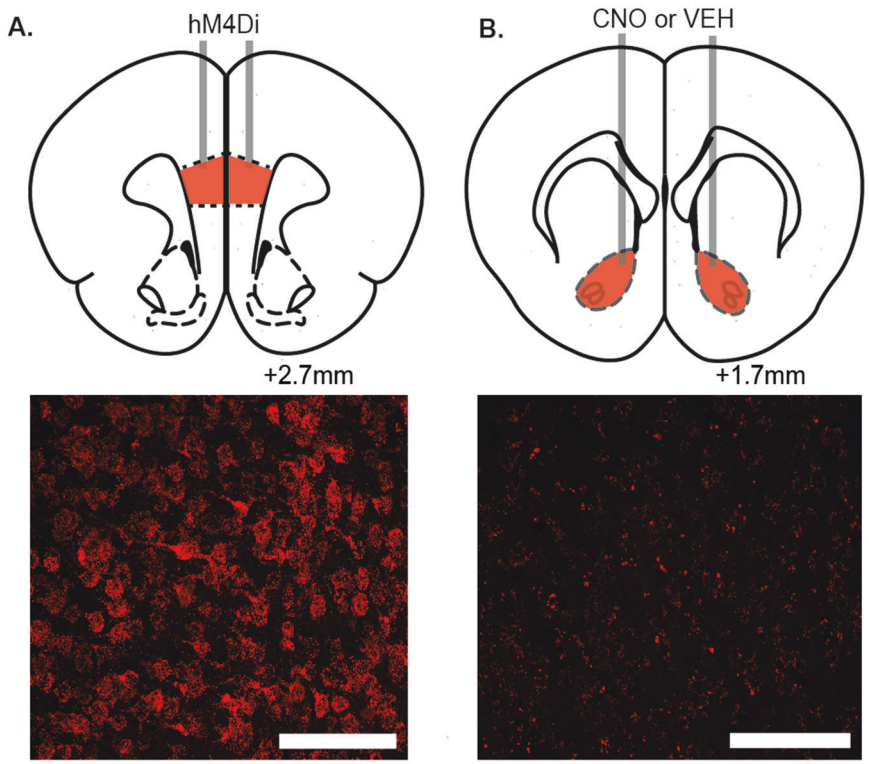

Fig. 2 Histological verification of Gi-DREADD expression. A Top panel shows illustration of bilateral hM4Di virus infusion into mPFC (coronal section +2.7 mm from Bregma) [83]. Bottom panel shows mCherry fluorescence of Gi-DREADD transduced cell bodies in mPFC, scale bar represents 100 μm. **B** Top panel shows illustration of cannula placement in NAc (coronal section at +1.7 mm from Bregma) [79]. Rats included in data analysis had viral expression confined to the prelimbic cortex [83] and bilateral NAc cannulae placement within a confined range of the targeted NAc coordinates (+/− 0.25 mm AP, +/− 0.3 mm ML, and +0.25 mm DV). Bottom panel shows mCherry fluorescence of Gi-DREADD-expressing mPFC terminals in NAc, scale bar represents 100 μm.

continues into adulthood, each animal maintains relative stability of choice impulsivity across development.

Chemogenetic inactivation of mPFC does not affect impulsive choice

In adulthood, rats transduced with Gi-DREADD in the mPFC received systemic (IP) injection of vehicle and each of 2 doses of CNO to determine if DREADD-induced inactivation of mPFC altered impulsive choice. There was no significant change in DD task performance from baseline following IP vehicle or CNO at either dose [Fig. 3A; $F(3,24) = 0.909$, $P = 0.452$, $\eta p^2 = 0.102$], suggesting no effect of mPFC inactivation on impulsive choice. These data align with previous work showing no effect of mPFC lesion or inactivation on DD [50, 75, 76].

CNO-mediated inactivation of excitatory mPFC-NAc projections increases impulsive choice

In order to directly assess the role of excitatory projections from mPFC to NAc in impulsive choice, vehicle or CNO was microinfused bilaterally into the NAc to selectively inactivate mPFC afferents (Fig. 3B). During baseline and following intra-NAc vehicle, selection of the large-delayed reinforcer decreased as delays increased [main effect of delay $F(3,39) = 75.258$, $P < 0.000$, $\eta p^2 = 0.853$]. However, vehicle did not significantly alter impulsive choice at any delay relative to baseline [no main effect of treatment, $F(1,13) = 0.026$, $P = 0.875$, $\eta p^2 = 0.002$; no treatment x delay interaction, $F(3,39) = 0.791$, $P = 0.507$, $\eta p^2 = 0.057$] and did not affect AUC between sessions [Fig. 3C; $t(13) = 0.489$, $P = 0.633$, Cohen's $d = 0.131$]. Interestingly, intra-NAc CNO robustly increased impulsive choice across the session, as shown by a decrease in AUC [Fig. 3E; $t(15) = 3.578$, $P = 0.003$, Cohen's $d = 0.895$]. All animals decreased their choice of the large-delayed reinforcer as delays increased, and CNO similarly decreased choice of the large reinforcer at each delay (Fig. 3D) [main effect of delay, $F(3,45) = 199.354$, $P < 0.000$, $\eta p^2 = 0.930$]. However, administration of CNO significantly reduced choice of the large-delayed reinforcer compared to baseline [main effect of CNO, $F(1,15) = 15.280$, $P = 0.001$, $\eta p^2 = 0.505$; no delay x CNO interaction, $F(3,45) = 2.742$, $P = 0.54$, $\eta p^2 = 0.155$]. Critically, planned comparisons confirmed that CNO had no effect on choice of the large reinforcer at the 0 s delay [$t(15) = 0.253$, $P = 0.507$, Cohen's $d = 0.170$], demonstrating no disruption in preference for the large-delayed reinforcer over the small-immediate reinforcer or the ability of animals to perform the task. There was also no difference observed following CNO treatment at the 5-s delay [corrected $\alpha = 0.0125$, $t(15) = 2.681$, $P = 0.017$, Cohen's $d = 0.670$]. However, at the two longest delays, CNO-induced inactivation of mPFC-NAc excitatory projections significantly reduced choice of the large-delayed reinforcer [corrected $\alpha = 0.0125$; 10-s, $t(15) = 2.846$, $P = 0.012$, Cohen's $d = 0.711$; 20-s, $t(15) = 3.091$, $P = 0.007$, Cohen's $d = 0.773$]. Finally, CNO did not affect consumption of food pellets or time to complete the task (data not shown).

No-virus control animals exhibited normal DD gradients (Fig. 3F), decreasing their choice of the larger reinforcer as the delay increased [$F(3,27) = 57.184$, $P < 0.001$, $\eta p^2 = 0.864$]. CNO infusion had no effect on DD performance [no main effect of treatment, $F(1,9) = 2.715$, $P = 0.134$, $\eta p^2 = 0.232$; no difference in AUC, $t(9) = 0.261$, $P = 0.800$, Cohen's $d = 0.083$], suggesting that CNO-induced increases in impulsive choice in experimental rats cannot be attributed to non-specific actions of intra-NAc CNO or its metabolites [87, 88].

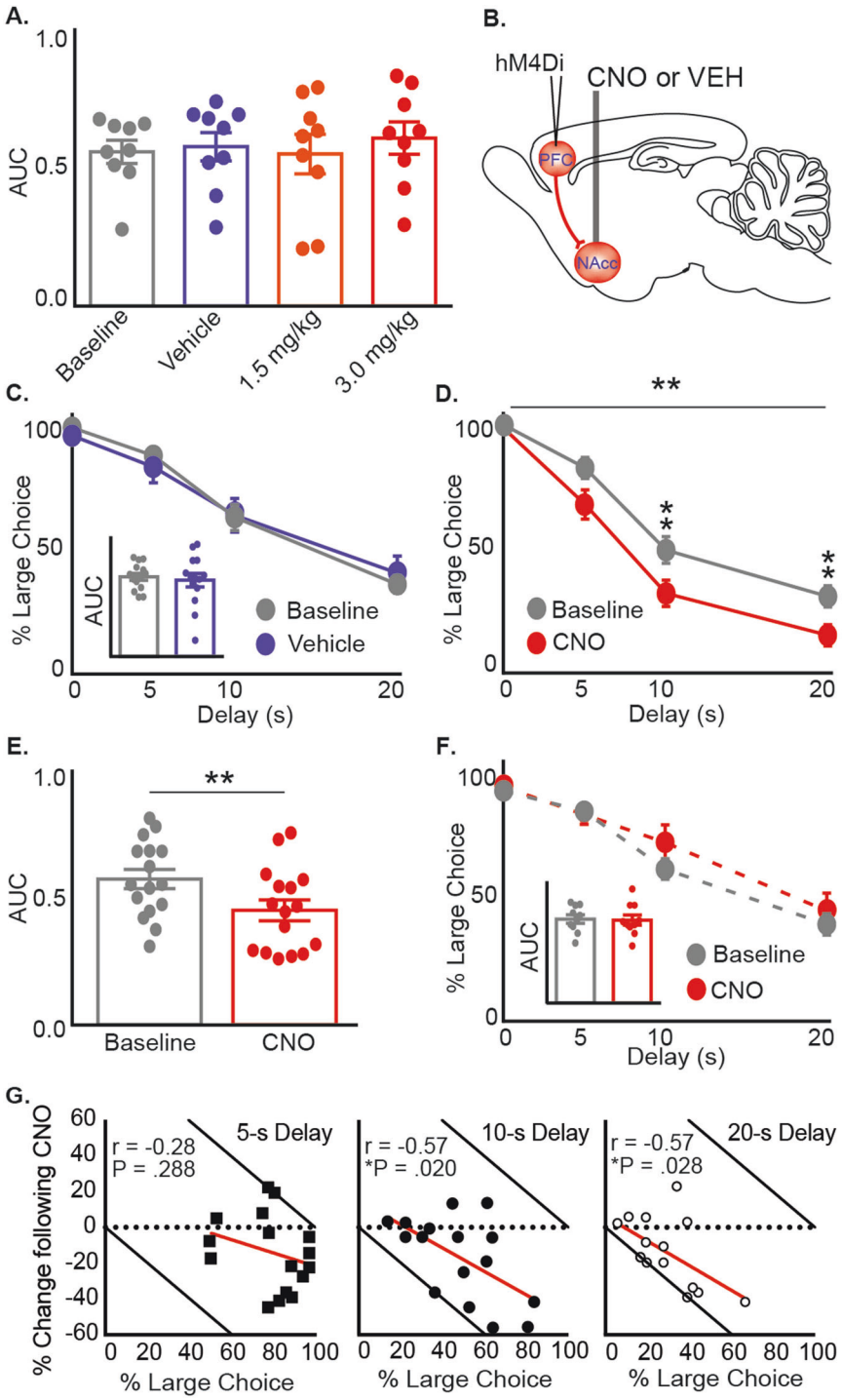

Fig. 3 Systemic DREADD inhibition of mPFC neurons does not affect impulsive choice, however, inhibition of mPFC-NAc projections increases impulsive choice. A Impulsive choice (AUC) was not different from baseline following systemic (IP) infusion of either dose of CNO or vehicle ($n = 9$; $P = 0.452$). Each dot represents a single subject. **B** Illustration of rat brain showing transduction of the inhibitory Gi-DREADD virus (hM4Di) in the mPFC and implantation of guide cannula in the NAc for infusion of CNO or vehicle. **C** Percent of trials in which a rat chose the larger delayed reward across delays during baseline and following intra-NAc vehicle infusion ($n = 16$). Inset shows no significant difference in DD task performance (measured by AUC) ($P = 0.633$). **D** CNO significantly increased impulsive choice across delays ($P = 0.003$). Planned comparisons showed that intra-NAc CNO did not affect choice of the larger reward when there was no delay (0-s), but significantly reduced large choice on 10-s ($P = 0.012$) and 20-s delay ($P = 0.007$). **E** Similarly, intra-NAc infusion of CNO significantly decreased AUC ($P = 0.003$). **F** Intra-NAc CNO infusion in rats not transduced with Gi-DREADD virus had no effect on choice of the larger-delayed reinforcer ($P = 0.134$). **G** Scatter plots and fit lines (red) showing correlation between the percent of large choices at baseline (i.e., baseline delay tolerance or choice impulsivity) and the change in percent large choice following CNO infusion for the 5-s (left), 10-s (middle) and 20-s (right) delays. Black lines are the constraints on the positive or negative CNO-mediated change that could occur given the baseline percent large choices. Significant negative correlations were identified for the 10-s ($r = -0.57$, $P = 0.020$) and 20-s ($r = -0.57$, $P = 0.028$) delays, but not for the 5 s delay ($P = 0.288$). Greater percent decreases in choice of the large alternative occurred in animals with higher percentage choice of the large alternative, i.e., animals with lower levels of trait choice impulsivity. Error bars indicate +/− SEM. *$P \leq 0.05$, **$P < 0.01$.

Trait impulsivity determines degree of change in impulsive choice following silencing of PFC-NAc projections

A large body of research indicates stable subpopulations in impulsive choice and suggests differences in underlying neurobiology [89–92]. To explore individual differences in the contributions of the mPFC-NAc pathway to DD, the change in proportion of large-alternative choices following CNO was assessed as a function of trait level of impulsivity or proportion of large-alternative choices during baseline (the average of the three sessions preceding CNO test day) (Fig. 3G). Pearson's correlation indicates that animals with lower levels of impulsivity, i.e., those with higher baseline choice of the large alternative at the 10-s and 20-s delays, exhibit larger decreases in these choices following intra-NAc CNO (5-s: $r = -0.2830$, $R^2 = 0.08009$, $P = 0.288$; 10-s: $r = -0.5745$, $R^2 = 0.3300$, $P = 0.020$; 20-s: $r = -0.5664$, $R^2 = 0.3208$, $P = 0.028$). Additionally, constraint lines (Fig. 3G, black lines) indicate that the majority of animals did not reach a floor in their % large choices following intra-NAc CNO treatment. Altogether these data demonstrate that mPFC-NAc excitatory projections mediate impulse control during the DD task differentially in animals with higher vs. lower levels of trait choice impulsivity.

Ex vivo confirmation of Gi-DREADD expression and function

Electrically-evoked EPSCs were measured using whole-cell patch-clamp recordings from NAc MSNs and compared to baseline following vehicle and CNO (Fig. 4A, B) [main effect of treatment: $F(2,22) = 11.288$, $P < 0.001$, $\eta^2 = 0.530$]. Post-hoc analyses showed that while EPSC amplitude following vehicle did not differ from baseline [$t(12) = 1.026$, $P = 0.163$, Cohen's $d = 0.548$], EPSC amplitude was significantly reduced following CNO treatment when compared to both baseline and vehicle [corrected $\alpha = 0.0167$; $t(14) = 7.917$, $P < 0.001$, Cohen's $d = 3.990$; $t(14) = 2.752$, $P = 0.008$, Cohen's $d = 1.387$]. To confirm a CNO-induced reduction in the probability of presynaptic vesicular release, paired-samples *t*-test revealed a significant reduction in PPR following CNO (Fig. 4C, D) [$t(8) = 2.437$, $P = 0.0408$, Cohen's $d = 0.812$]. Altogether, these data support inhibitory function of CNO-mediated Gi-DREADD activation on mPFC afferents to NAc.

DISCUSSION

We utilized a DD task to assess impulsive choice across development and to interrogate the functional role of the mPFC and its afferents to the NAc in impulsive decision-making. First, we found that DD performance in adolescence was positively correlated with performance in adulthood, suggesting relative phenotypic delay tolerance or aversion throughout the lifespan. Second, specific Gi-DREADD-mediated inactivation of mPFC-NAc projections, but not global inactivation of the mPFC, reversibly increased DD. Finally, increases in impulsive choice due to mPFC-NAc inactivation were negatively associated with characteristic baseline levels of delay discounting; namely, animals with lower levels of choice impulsivity were more affected by silencing mPFC input to the NAc than animals with higher levels. These data together suggest that phenotypic intertemporal choice may be, in part, mediated by mPFC input to the NAc.

Relative stability of DD from adolescence to adulthood aligns with a wide body of research demonstrating long-term, stable "trait-like" levels of impulsive choice in humans and animals [14, 19, 20, 41]. Although it is possible that DD behavior observed here is not the result of trait-like choice impulsivity but rather due to fixed patterns of responding on the task established through extended training, the propensity for DD behavior to become fixed or habitual over training is currently unexplored. Additionally, while our animals displayed relative stability over development (i.e., rats that were more/less impulsive during adolescence were more/less impulsive during adulthood), results did not support absolute stability across developmental stages (i.e., rats were overall less impulsive during adolescence than in adulthood). Previous direct comparisons of DD between adolescents and adults also yielded mixed results. While a number of studies show a tendency for adolescents to exhibit more impulsive choice [29, 30, 33, 34, 93], other research demonstrates no differences [34, 37–40]. Interestingly, McClure and colleagues (2014) found that rats in early adolescence (PND 28–42) were less impulsive than when tested again in the transition from late adolescence to early adulthood (PND 58–64) [41, 94–96]. Disparate findings among previous work and present findings may be attributable to individual differences between cohorts [97]. The use of a within-subjects design in the current study allowed for sensitive comparisons of DD across development to uncover an increase in impulsive choice in adulthood that would not have reached significance with a between-subjects design. Of course, it is possible that extended experience with the reinforcer altered its value, reducing choice of the large reinforcer at longer delays in adulthood. However, it is important to note that animals were motivated to earn reinforcers, completed each session, and consumed all earned pellets. Altogether, these data evidence relative trait-like levels of impulsive choice within subjects and provide a framework to investigate neural systems contributing to intertemporal choice.

Here we show that chemogenetic silencing of the mPFC using a Gi-DREADD under the CaMKII promoter had no effect on DD performance, replicating the results of previous lesion and pharmacological inactivation studies [50, 75, 76]. However, present results also indicate that Gi-DREADD-induced inhibition of the mPFC-NAc pathway reliably increases impulsive choice. Thus, while global inhibition of the CaMKII-expressing neurons of the prelimbic mPFC had no effect on DD, selective attenuation of afferents from the prelimbic cortex to the NAc core resulted in robust increases in DD. Other published studies demonstrate similar phenomena. For example, Giertler and colleagues (2003) showed that intra-NAc infusion of amphetamine significantly reduced reaction time in a conditioned lever release task, while temporary inactivation of the NAc with lidocaine had no effect on reaction times [98]. Similarly, disruption of dopamine signaling in the mPFC with intra-mPFC infusion of a D2 receptor agonist or antagonist is shown to increase impulsive choice, while the current study and others show no effect of mPFC inactivation [50, 75, 76]. There are at least two potential possibilities through which this may occur: (1) complete disruption of mPFC activity allows for other regions to control behavioral output, and (2) mPFC activity during DD promotes both impulsive and self-controlled choices through different efferent pathways and thus complete inhibition has no net effect on behavior. While the current study was not designed to test these hypotheses, previous research supports a combination of these possibilities. Indeed, brain regions other than mPFC are shown to similarly exert control over the NAc during DD. For instance, inhibition of OFC-NAc projections increases impulsive choice [99], suggesting an alternative pathway that may be relied upon to maintain self-control following mPFC inactivation. Also, while no published studies evidence an mPFC efferent pathway that promotes impulsive choice, Li and colleagues (2020) found that two parallel mPFC pathways mediate impulsive action in mice. They show that optogenetic inhibition of mPFC projections to the subthalamic nucleus (STN) severely impairs performance on a go/no-go task (i.e., increases impulsive action), whereas inactivation of mPFC projections to the lateral hypothalamus improves performance [100]. Thus, it remains a target for future research to determine how mPFC inactivation and mPFC-NAc pathway inactivation differentially affect DD.

Downstream of the cortex, minor projections of the prelimbic cortex target the accumbens shell subregion of the ventral striatum, but the vast majority preferentially target the accumbens core [84–86]. The core promotes learning and selection of delayed

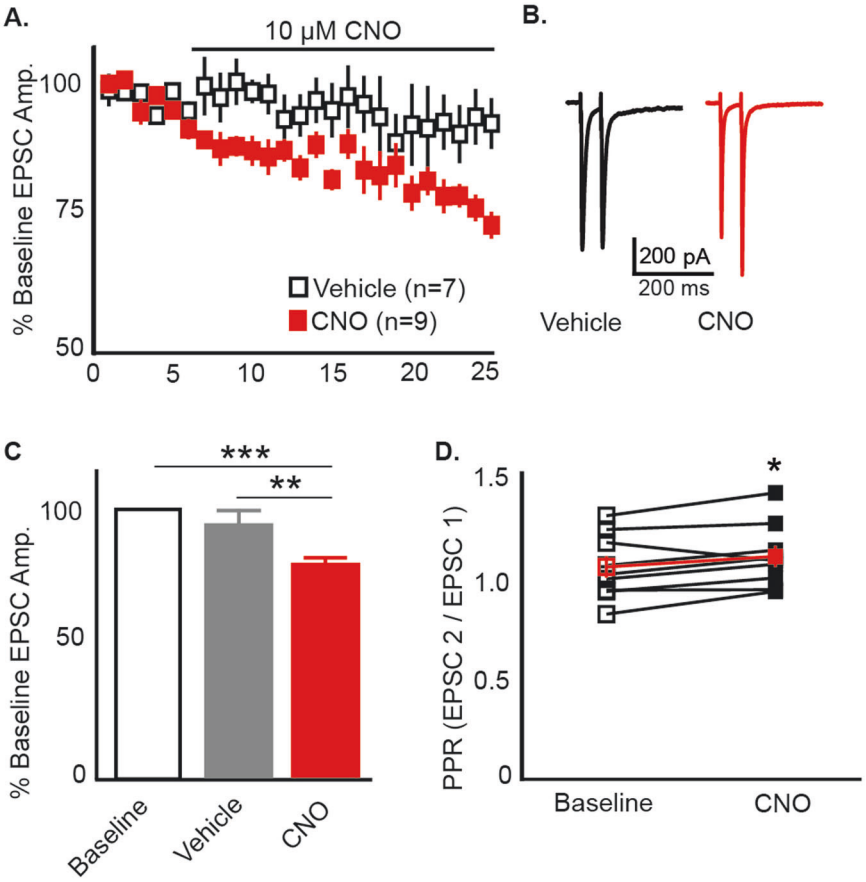

Fig. 4 Whole-cell patch-clamp recordings from nucleus accumbens medium spiny neurons (MSNs) verify hM4Di function. A Graph showing mean % of baseline EPSC amplitude over 25 min for 16 cells following bath application of 10 μM CNO ($n = 9$) or vehicle ($n = 7$). CNO application depresses EPSC amplitude as compared to vehicle. **B** Representative traces following vehicle (left) or CNO (right). **C** Statistical analysis of baseline (first 5-min) compared to the last 5 min of recording. CNO significantly reduces EPCS amplitude as compared to baseline ($P = < 0.001$) and compared to the last 5 min of recording in the vehicle and CNO recordings ($P = 0.008$). **D** Paired-pulse ratio increases following CNO application over baseline ($P = 0.041$), suggesting that CNO application decreases the probability of glutamate release. Each point represents a single cell; red points indicate mean +/− SEM. Error bars indicate +/− SEM. *$P \leq 0.05$, **$P < 0.01$, ***$P < 0.001$.

rewards [50, 101, 102], and its lesioning or inactivation results in steeper delay discounting [50, 103, 104]. Permanent lesions of the accumbens shell, however, have not affected intertemporal choice [103, 104]. Recent studies outline differentially patterned dopamine release in both the core and shell subregions during a DD task [78], and reversibly inactivating the core or the shell during a T-maze DD task resulted in waiting impairments [105]. Therefore, there may be a mediating role for the shell in addition to the core in DD. In the present study, we cannot entirely rule out inactivation of Gi-DREADD-expressing afferents targeting the shell from the prelimbic cortex. However, given that the majority of prelimbic afferents project to the core and only a proportion of all prelimbic afferents expressed Gi-DREADD, we believe our CNO injections targeting the core were acting upon terminals in this subregion. Dissecting the contributions of accumbens subregions in choice impulsivity is an opportunity for future investigation.

The observed increased preference for the small-immediate reward most likely reflects an intolerance to delay following mPFC-NAc inhibition. Intertemporal choice requires neural computations of reward size, delay duration, and preference for a small-immediate or large-delayed reward [106]. Here we show that inhibition did not affect preference for the large reward at the 0-s delay, suggesting that this pathway did not affect discrimination of reward size or preference for a larger reward when there was no delay to receipt. Preserved preference for the large reward at the 0-s delay also indicates that mPFC-NAc inhibition did not affect baseline motivation for food [107]. Additionally, rats completed all test sessions and ate all pellets, suggesting that motivation for food rewards remained intact across the test session. As delays progressed across the session, CNO decreased choice of the large reward to a similar degree at both the 10- and 20-s delay. Thus, it is possible that mPFC-NAc projections exert control over intolerance to longer delays, rather than intolerance to delay based on computations of reward value and delay length. Future research using additional delays or an adjusting delay procedure may provide further evidence of the precise role of this pathway. It is also possible that corticostriatal silencing reduced choice for the large-delayed reward by increasing subjective overestimation of the delay until reward, shifting the DD curve down at both 10-s and 20-s delays [108]. Humans and animals with poor interval timing also demonstrate greater impulsive choice [41, 106, 109–111]. However, rodent studies using lesions or temporary inactivation of mPFC or NAc do not support a causal role for these structures in time estimation required for DD [52, 112, 113]. Thus, these data align with previous research showing that both regions integrate information about reward size and delay to reward receipt [78, 114–118], and extend these findings to show that activity of projections from mPFC to NAc promote self-control during intertemporal choice.

mPFC-NAc inhibition significantly reduced choice of the large-delayed reward to a greater extent in rats with lower levels of baseline impulsivity, suggesting reliance on this pathway for

control over DD may be stronger in animals with greater delay tolerance. In support of this, research demonstrates individual differences in DD in both humans and animals, with high and low impulsive subjects exhibiting differing structural and functional neurobiology [97, 119]. Neuroimaging studies show that individuals with high levels of impulsive choice have reduced corticostriatal functional connectivity and structural integrity of white matter compared to low impulsive individuals [46–48]. The current data align with these findings, highlighting a role for corticostriatal projections in DD and substantiate a mechanistic role for this projection in impulsive choice. Rodent studies have not specifically interrogated mPFC-NAc pathway during DD, although several studies have delineated neurobiological differences in both mPFC and NAc in animals differing in trait impulsivity. For instance, high impulsive rats have reduced D2 mRNA expression in prelimbic cortex and D2 receptor availability in the NAc [120–122], as well as blunted NAc dopamine release during DD [116] compared to low impulsive counterparts. Interestingly, similar dopaminergic dysfunction is observed in SUD, and it is hypothesized to result in decreased valuation of natural rewards (e.g., food) as well as increased impulsive choice through enhanced salience of immediate rewards [120, 123, 124]. The current data substantiate a role for mPFC-NAc projections in control over intertemporal decision-making and suggest that this pathway may be recruited to a greater degree in self-controlled versus impulsive individuals. Nevertheless, the current data cannot rule out the possibility that the observed greater increases in DD in rats exhibiting lower levels of impulsive choice at baseline may be due to greater parametric space for downshifts in discounting curves compared to animals exhibiting higher impulsive choice. However, the majority of animals did not reach a floor in their % large choices following CNO silencing of mPFC-NAc projections.

It is important to note that the DD behavior we analyze here may have been shaped by the design of the task, such as the use of cues or the order of delay presentation. Similarly, task design may have influenced the effects of neurobiological manipulations on behavior. In the current experiments, when rats responded for the delayed reward a cue light above the nosepoke port flashed at 2 Hz throughout the duration of the delay until reward delivery. Such delay-spanning cues are reported to enhance learning in a DD task, and with over-training they can begin to function as a conditioned reinforcer [65, 125, 126]. Zeeb and colleagues (2010) examined how the use of delay cues alters OFC involvement in DD. Briefly, they found that when the delay was cue-signaled, pharmacological inactivation of the OFC increased impulsive choice in low-impulsive animals; however, when the delay was unsignaled, OFC inactivation decreased impulsive choice in high-impulsive animals [65]. Cardinal et al. (2001) found no effects of mPFC lesions on DD using an unsignaled delay procedure, and here we replicated these results using a task in which the delay to reward was cue-signaled, suggesting that effects of mPFC inactivation may not be influenced by delay-spanning cues [50]. It remains a potential target for future research to determine how mPFC-NAc inactivation affects DD in an unsignaled DD procedure.

Like delay-spanning cues, the order in which delays are presented can also affect intertemporal choice. In our experiments, each session contained four blocks of trials with delay progressively increasing across blocks (i.e., ascending delays). Human research suggests that presenting delays in an ascending order results in greater discounting of rewards over delays as compared to descending order; however, behavior in both procedures is correlated [127, 128]. Rodent studies are mixed, with some reports showing differences in intertemporal choice as a function of the order of delay presentation [129, 130] and others showing no effect of delay order on behavior [131]. A number of rodent studies, however, do show that amphetamine administration, either systemically or directly into the NAc, decreases impulsive choice when delays are ascending but increases impulsive choice when delays are descending [54, 132, 133]. The authors of these studies argue that amphetamine reduces cognitive flexibility, biasing an animal to perseverate on their initial choice. When the initial choice was for a larger reinforcer at a short delay (as in ascending delays) choice across the session is biased towards choice of the larger reinforcer. If, in our investigation, chemogenetic inhibition of mPFC-NAc projections similarly reduced cognitive flexibility, we would assume this would decrease DD in our ascending procedure. However, instead we show greater discounting of the delayed reward. Thus, this suggests that decreased activity in this pathway results in reduced control over DD and greater impulsive choice.

Finally, a growing body of human and animal research presents conflicting data on sex differences in intertemporal choice [22, 134]. For instance, studies in human subjects either show that men exhibit greater control over delay discounting [135–138], that women exhibit greater control [139], or that both men and women discount delayed rewards at similar rates [139, 140]. Further, a recent meta-analysis of 28 papers shows no significant differences in delay DD between men and women [79]. Undoubtedly, in rodents, intertemporal choice has been more well-characterized in males, due to decades of using male rodents as "standard" in preclinical research. In studies that have compared DD behavior in male and female rodents, several studies do not substantiate sex differences in intertemporal choice [29, 78, 141]. Those studies that have identified sex differences in DD show that males are more impulsive than females when using a procedure in which delays are presented at random across the session [80], and females are more impulsive than males when using an adjusting delay procedure in which delays decrease after responses on the small/immediate lever and increase after responses on the large/delayed lever [142]. Further, evidence suggests that female rats express greater DD following amphetamine administration [141], and female rats bred to be low saccharin (LoS) preferring similarly exhibit greater DD than LoS male rats [142]. Thus, while sex differences may not be found at baseline levels of intertemporal choice, they may be unveiled by experimental manipulations and within subgroups of subjects. In the current study, we used male rats to examine baseline DD behavior across adolescence to adulthood and to determine a role for mPFC-NAc projections in intertemporal choice. We found that DD behavior was highly correlated in adolescence and adulthood evidencing relative stability and that mPFC-NAc inhibition increased impulsive choice in male rats. Based on previous research using similar within-session increasing delay procedures [78, 141] we expect we would observe similar effects in females; however, this remains an objective of future research.

In conclusion, here we show that impulsive choice assessed with DD (1) is conserved from adolescence to adulthood in rats and (2) requires mPFC-NAc pathway activation. These findings have clinical relevance as excessive DD is positively correlated with SUD, ADHD, and impulse control disorders [3, 5–9]. Further, mPFC, NAc, and projections between these structures are shown to be involved in these disorders. For instance, both ACC and NAc exhibit altered size and thickness in individuals with SUD [143], and reduced functional connectivity in ACC-NAc projections is correlated with greater relapse vulnerability [144]. Further, in rodents, optogenetic or chemogenetic silencing of the mPFC-NAc pathway attenuates both drug- and cue-primed reinstatement of drug seeking [145–147]. Therefore, these data provide integral knowledge for the understanding the neural underpinnings of DD and identify the mPFC-NAc pathway as a potential target for the treatment of excessive impulsive choice across the lifespan.

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AUTHOR CONTRIBUTIONS

JMW, NEZ, and JFC conceived of and designed the study; JMW, NEZ, VMA, HMD, and MHP carried out the experiments; JRS, LYZ, BNM, and JFC provided essential intellectual input on study design, analysis, and data interpretation; JMW, NEZ, and JRS analyzed the data; JMW and NEZ wrote the initial draft of the manuscript; all authors contributed to, edited, and approved of the final manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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