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Effects of Manipulating Prefrontal Activity and Dopamine D1 Receptor Signaling in an Appetitive Feature-Negative Discrimination Learning Task

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Healthy cognition requires inhibitory modulation of associative learning; conversely, impaired inhibitory discrimination is implicated in behavioral disorders. The medial prefrontal cortex (mPFC) and its dopamine innervation are key to understanding inhibition and impulsivity. We therefore examined the role of prelimbic and infralimbic cortices in within-subjects appetitive feature-negative learning using microinfusions of (a) the gamma-aminobutyric acid-A receptor agonist muscimol (0.25 μg in 1.0 μl; $N = 35$), (b) the dopamine D1 receptor agonist SKF-81297 (0.1 μg in 1.0 μl; $N = 33$), and (c) the dopamine D1 receptor antagonist SCH-23390 (5 μg in 1.0 μl; $N = 35$). A conditioned stimulus (CS) was followed by food, but on trials on which the CS (A+) was compounded with the inhibitory cue (AX−), the food delivery was canceled. Difference scores (CS–preCS responding) were used to measure learning. All three experiments showed the feature-negative discrimination (A+/AX−), as decreased responding to AX− versus A+. This discrimination was reduced but preserved following muscimol infusions in Experiment 1. Similarly, in Experiments 2 and 3, infusions of SKF-81297 and SCH-23390 were both without effect on the acquisition of the discrimination. Like muscimol, SCH-23390 reduced difference score responding, consistent with nonspecific effects on the (expression of) learning. Thus, there was no evidence to suggest that inactivation of prelimbic or infralimbic cortices impaired feature-negative discrimination learning and no evidence for dopaminergic modulation of such learning in the medial prefrontal cortex either. These results are discussed in the context of the nonspecific effects of the infusions and the overall inconsistent performance in summation and retardation tests of conditioned inhibition.

Keywords: Appetitive associative learning, dopamine D1 receptor, feature-negative discrimination, muscimol, medial prefrontal cortex

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Preliminary analyses of the results of Experiments 1 and 2 (prehistology) were presented in poster format at the Federation of European Neuroscience Societies (FENS) in 2022 by Rebecca Hock and Naana Owusu-Amoah, respectively. The abstract details were as follows: "Roles of prelimbic and infralimbic prefrontal cortices in an appetitive inhibitory discrimination learning task" (Hock et al., 2022; *FENS Forum Abstracts*, p. 1453) and "Infusions of a dopamine D1 receptor agonist into the prefrontal cortex and appetitive inhibitory discrimination learning" (Owusu-Amoah et al., 2022; *FENS Forum Abstracts*, p. 5842). These abstracts are available online at https://kenesvm.azureedge.net/public/general/FENS2022.pdf. Data are available from Hock et al. (2024).

Rebecca M. Hock played a lead role in data curation, investigation, and methodology, a supporting role in writing–review and editing, and an equal role in conceptualization, formal analysis, and writing–original draft. Naana Owusu-Amoah played a lead role in visualization and a supporting role in investigation, methodology, project administration, writing–original draft, and writing–review and editing. Lauren Waite played a supporting role in investigation, methodology, validation, and writing–review and editing. Charlotte Muir played a supporting role in investigation, methodology, project administration, validation, and writing–review and editing. Carl W. Stevenson played a supporting role in funding acquisition, investigation, methodology, supervision, validation, writing–original draft, and writing–review and editing. Charlotte Bonardi played a lead role in methodology and validation, a supporting role in funding acquisition, investigation, resources, supervision, writing–original draft, and writing–review, and editing, and an equal role in data curation and formal analysis. Helen J. Cassaday played a lead role in funding acquisition and supervision, a supporting role in data curation, project administration, and visualization, and an equal role in conceptualization, formal analysis, writing–original draft, and writing–review and editing.

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Inhibitory modulation of associative learning is an important component of healthy cognition, as the capacity to inhibit thoughts and behaviors is a determinant of impulse control (Sosa & dos Santos, 2019). The inhibition of learned associations has been most extensively investigated in aversively motivated procedures, where fear and the absence of fear have high translational relevance in the context of safety learning, anxiety, and posttraumatic stress disorder (Christianson et al., 2012). Similarly, in appetitively motivated procedures, impairment of inhibition can cause unchecked associations to disturb thought processes, producing in this case incoherence and impulsivity, with translational potential for obesity (Chen et al., 2018), as well as for drug (Guillory et al., 2022) and behavioral addictions such as gambling disorder (Ioannidis et al., 2019).

Maladaptive levels of impulsivity are characteristic of a variety of psychiatric disorders, in addition to substance use, with evidence for the importance of neurodevelopmental factors and dopamine (DA) modulation in the mesocorticolimbic system (Sanchez & Bangasser, 2022). In particular, the prefrontal cortex (PFC) is implicated in impulsive decision making, as measured by a variety of behavioral tasks (Kim & Lee, 2011). For example, inhibition of the contextual control of amphetamine sensitization provides a model for drug addiction, and such inhibition is impaired by inactivation of the medial prefrontal cortex (mPFC), resulting in a loss of contextual control. Specifically, muscimol infusion in mPFC reduced the capacity of conditioned inhibitors to block the expression of sensitized locomotion, that is, increased impulsivity (Guillory et al., 2022). Such evidence is consistent with the wider role of the mPFC in the organization of goal-directed behavior and cognitive control (Friedman & Robbins, 2022; Mair et al., 2022). Moreover, neuromodulation in prelimbic (PL) versus infralimbic (IL) subregions of mPFC has dissociable effects in various cognitive tasks and with some mixed findings in associative learning studies of latent inhibition (aversively motivated; Cassaday et al., 2014).

The simplest form of associative learning is excitatory and occurs when an experimental conditioned stimulus (CS) such as a tone or a light is paired with the unconditioned stimulus (UCS) such as foot shock or food. Tests of inhibitory learning require the learning of a discrimination in which the inhibitory stimulus counters the effect of the excitatory conditioning. For example, in a feature-negative discrimination, an established CS (A+) continues to be followed by the UCS when presented on its own, but on trials on which the CS is compounded with the inhibitory cue (AX−), the otherwise expected UCS does not occur. Pavlovian conditioned inhibition tasks use the feature-negative discrimination procedure, followed by confirmatory summation and/or retardation tests (Rescorla, 1967, 1969). Simpler differential inhibition designs involve presentations of the CS (A+) and the inhibitory cue (X−), with only the excitor followed by the reinforcer.

Aversively motivated differential inhibition (A+/X−) involves mPFC, as demonstrated in safety signaling and fear discrimination procedures in both rodent (Corches et al., 2019; Saul'skaya & Sudorgina, 2016; Sudorgina & Saul'skaya, 2016) and human studies (Weber et al., 2016). Moreover, there is evidence for bidirectional regulation by (projections from) DA neurons in the ventral tegmental area (VTA): Safety signaling increased activation of VTA DA neurons, while impaired safety signaling has been associated with the lack of VTA DA neuron activation (Yan et al., 2019).

Aversively motivated feature-negative discrimination learning (A+/AX−) has also been shown to depend on mPFC in the rat, using both reversible inactivation studies (muscimol in IL, but not PL; Sangha et al., 2014) and electrophysiological studies (in vivo recordings in IL; Ng & Sangha, 2023). Similarly, fMRI studies of human children (with and without anxiety disorders) performing an aversive noise prediction task in a related design (AB+/AX−) confirmed the role of ventral mPFC (Harrewijn et al., 2021). In contrast, appetitively motivated feature-negative procedures have confirmed the role of the PL (but not the IL) region of mPFC in the acquisition of feature-negative discriminations, using both neurotoxic (MacLeod & Bucci, 2010) and electrolytic lesion methods in the rat (Meyer & Bucci, 2014).

In an appetitively motivated conditioned inhibition procedure, excitotoxic lesions to the IL were without effect on the acquisition of the underpinning A+/AX− discrimination and summation test performance. However, these lesions impaired performance in the retardation test of conditioned inhibition (Rhodes & Killcross, 2007). Thus overall, the evidence for the role of mPFC in inhibitory learning is good, although mixed with respect to the relative contributions of PL and IL subregions. Moreover, there have been relatively few appetitively motivated studies, only one of which confirmed conditioned inhibition by summation and retardation test (Rhodes & Killcross, 2007).

In the present study, three experiments used the same within-subjects appetitive discrimination (A+/AX−) to establish X as an inhibitor of excitatory responding to A. In each case the feature-negative discrimination training was followed by both summation and retardation tests for conditioned inhibition. According to strict criteria, feature-negative discrimination learning is necessary, but not sufficient, for the demonstration of what should be termed conditioned inhibition (Rescorla, 1967, 1969). It was an open question as to whether summation and retardation tests would be passed in the present study. However, it has been argued that feature-negative discriminations of the form A+/AX− provide a good proxy for conditioned inhibition, so the requirement to pass both summation and retardation tests may be overly stringent (Sosa & dos Santos, 2019; Sosa & Ramírez, 2019).

Reversible inactivation provides a useful complement to lesion approaches to distinguish the roles of PL and IL in inhibitory learning (Guillory et al., 2022). Moreover, reversible inactivation can be restricted to key experimental stages of the behavioral procedure, and compensatory processes following lesions are not an issue within the time frame of the study (Vaidya et al., 2019). However, any lack of effect of regional inactivation would not preclude a modulatory role for DA within these mPFC areas of interest, and both too little and too much activity can impair mPFC-mediated cognitive performance (Pezze et al., 2014).

DA D1 receptors are implicated in latent inhibition (aversively motivated; systemic drug studies; Diaz et al., 2015; Nelson et al., 2012), trace conditioning (appetitively motivated; microinfusions in mPFC; Pezze et al., 2015), and contextual fear conditioning (microinfusions in dmPFC; Stubbendorff et al., 2019). Their role in appetitively motivated inhibitory learning is yet to be examined.

Therefore, the same experimental design was used to examine the role of PL and IL in appetitively motivated feature-negative learning using microinfusions of (a) the gamma-aminobutyric acid-A receptor agonist muscimol, (b) the dopamine D1 receptor agonist SKF-81297, and (c) the dopamine D1 receptor antagonist SCH-23390. Drugs were administered by microinfusions prior to feature-negative training and summation tests. Retardation tests were conducted drug-free.

Thus, there was no state change between the training and summation test. Measuring the drug-free retardation test expression of conditioned inhibition allowed us to isolate performance-mediated effects.

Method

Transparency and Openness

Sample sizes were estimated based on Mead's equation (Mead, 1988) which, taking stimulus counterbalancing into account, suggested a minimum sample size of 8 per group to show the within-subjects behavioral effects. Power analysis of our pilot data using G*POWER (Erdfelder et al., 1996) suggested similar sample sizes. Assuming $\alpha = .05$, we have obtained moderate to large effect sizes ($f > .25$) and satisfactory power (power >.8) with sample sizes of approximately 8 per group for the design used in the present study. Each of the infusion groups started larger (minimum 12 per group) to allow for losses due to surgical complications and histological exclusions. The data exclusions and the criteria for data exclusion are reported below. The data files are available in the University of Nottingham Research Data Repository at https://doi.org/10.17639/nott.7366. Data were analyzed using SPSS Version 28.0.1.1 and are shared in SPSS format (no code required). The study's design and its analysis were not preregistered.

Animals

Experimentally naïve male Wistar rats (Charles River Laboratories, United Kingdom) were group-housed in two-level "double-decker," individually ventilated cages (462 mm X 403 mm X 404 mm; Tecniplast, United Kingdom) across the three experiments. These were kept under temperature- and humidity-controlled conditions (21 °C ± 1.5 °C; 50% ± 8%) and alternating 12-hr light/12-hr dark cycles (all procedures were conducted during the light phase). Rats initially had ad libitum water and food access (Teklad Global 18% Protein Rodent Diet 2018C; Envigo, United Kingdom). Two days prior to the start of behavioral training and testing, the animals were placed on food restriction while maintaining >80% free-feeding weight. The daily food ration given to the rats was a minimum of 4 g rodent diet per 100 g body weight (maximum 8 g/100 g—adjusted as required for keeping bodyweights in line with projected growth), calculated as the total weight of all animals in a cage/100× 4. As 80% was the lowest weight limit, we aimed to keep the rats at 85% of the projected body weight (calculated on a weekly basis and based on the projected growth rate for free-feeding Wistar rats). The primary experimenters handled all rats prior to the commencement of any procedures, during a 10-day acclimatization to the environmental conditions of the animal unit.

All procedures were conducted in accordance with the requirements of the U.K. Animals (Scientific Procedures) Act 1986, Project Licence number PPL P4C629C86. Thirty-seven male Wistar rats were used in Experiment 1. Two of the rats had to undergo a Schedule 1 method of humane killing due to surgical complications; thus, the experiment was run with 35 rats (prior to histological exclusions). The rats weighed between 151 g and 175 g on arrival and 225–314 g at the time of surgery. Thirty-eight male Wistar rats were used in Experiment 2. Five of the rats had to undergo a Schedule 1 method of humane killing due to surgical complications; thus, the experiment was run with 33 rats (prior to histological exclusions). The rats weighed between 151 g and 175 g on arrival and 255–298 g at the time of surgery. Thirty-six male Wistar rats were used in Experiment 3. One of the rats had to undergo a Schedule 1 method of humane killing due to surgical complications; thus, the experiment was run with 35 rats (prior to histological exclusions). The rats weighed between 151 g and 180 g on arrival and 250–331 g at the time of surgery.

Stereotaxic Implantation of Guide Cannulae Into the Medial Prefrontal Cortex

Procedures for implantation and infusion are detailed elsewhere (Pezze et al., 2014, 2015, 2017). Rats were anesthetized with isoflurane delivered in oxygen (induction: 5%; maintenance: 1%–3%) and were secured in a stereotaxic frame. The rats received peri- and postoperative analgesia to minimize pain: 0.4 ml/kg Metacam (0.5 mg/ml meloxicam; Boehringer Ingelheim Vetmedica GmbH; preoperatively and postoperatively for 4 days), 0.013 ml/kg Buprecare (0.3 mg/ml buprenorphine; Animalcare Ltd; preoperatively), and lidocaine hydrochloride (Hameln Pharma Ltd; at the incision site once the cannulae were secured). EMLA cream 5% (lidocaine 2.5%, prilocaine 2.5%; AstraZeneca) was applied to the ear bars, and eye lubricant (Lubrithal, Dechra) was applied to prevent corneal desiccation during surgery. Once the skull was exposed, bregma and lambda were located and aligned. Bilateral infusion guide cannulae (the "mouse" model C235GS-5-1.2 of Plastics One, Bilaney, United Kingdom) consisted of a 5-mm plastic pedestal that projected two parallel 26-gauge metal tubes, 1.2 mm apart and extending 5 mm from the pedestal for the PL and 6 mm for the IL. These were implanted through small holes bored in the skull. The guide cannula tips for the PL and IL were placed at the following coordinates: (PL) +3 mm anterior, ±0.6 mm lateral from bregma and −4.0 mm ventral from the skull surface; and (IL) +3 mm anterior, ±0.6 mm lateral from bregma and −5.0 mm ventral from the skull surface. Dental acrylic and stainless-steel screws were used to secure cannulae to the skull. Double stylets (33-gauge; Plastics One) were inserted into the guides (with no protrusion below guides), and the guides were closed with a dust cap. After surgery, rats had at least 7 days to recover from surgery and regain presurgical bodyweight before food restriction and behavioral testing. During the recovery period, rats were monitored and habituated to the manual restraint necessary for the drug microinfusions and throughout the experiment were injected daily with antibiotic suspension (Synulox; 14% amoxicillin; Zoetis).

Microinfusion Procedure and Drugs

The 33-gauge injectors (Plastics One) were inserted into the cannulae guides while rats were gently restrained. The two injector tips projected 0.5 mm below the guides targeted to either the PL or IL, and the two injector ends were each connected to a 5-μl syringe mounted on a microinfusion pump via polyethylene tubing. Over 1 min, bilateral infusions of either sterile saline vehicle (0.9% NaCl; control) or the drug solution were administered (0.5 μl/side). To verify the successful infusion of solution into the brain, the movement of an air bubble included in the tubing was monitored. After the initial 1 min, the injector remained in place for another 1 min to allow tissue absorption of the infusion bolus. Then the

injectors were removed, and the stylets were replaced. Behavioral testing began 10–15 min after the infusion.

In Experiment 1, the rats received a bilateral infusion of saline or 0.125 μg muscimol (gamma-aminobutyric acid-A receptor agonist; Sigma-Aldrich). In Experiment 2, the rats received a bilateral infusion of saline or 0.05 μg SKF-81297 (D1 receptor agonist; Tocris Bioscience). In Experiment 3, the rats received a bilateral infusion of saline or 2.5 μg SCH-23390 hydrochloride (D1 receptor antagonist; Tocris Bioscience). In each case, the infusion drug doses were based on those used in previous studies (Pezze et al., 2014, 2015; Stubbendorff et al., 2019).

Behavioral Testing

Apparatus

Six identical operant chambers (20 × 24 × 30 cm; ENV-008; Med Associates, St. Albans, VT, United States) encased in ventilated sound-attenuating boxes (74 × 38 × 60 cm; Med Associates CT-ENV-016MX) were used. Extraneous noise was masked by a fan in each box. Each operant chamber was equipped with two circular 2.8 W LED stimuli lights positioned to the left and right of a receptacle (Model ENV-203). The right light was illuminated throughout its scheduled presentations, whereas the left one was always pulsed (0.33 s on and 0.33 s off). A 2.8-W houselight was positioned 11 cm above the receptacle and was always switched on for all experiments. In reinforced trials, two sucrose reward pellets (45 mg; 5TUT, TestDiet) were dispensed into the receptacle. Located on the top left of the opposite wall, a speaker enabled the delivery of two 70 dB auditory stimuli: a white noise stimulus and a 10-Hz clicking stimulus. The flooring of the chamber consisted of 20 inactive shock bars spaced 1 cm apart, located above a tray of sawdust. Responses were recorded as photobeam breaks caused by nose poking into the reward receptacle.

All experimental data were recorded by a Viglen Genie (OS: Windows XP) computer connected via an interface to the operant boxes and running MED-PC IV (Med Associates).

Behavioral Design

The behavioral design was similar to that described by Waite et al. (2021). The animals were first trained that each of two auditory stimuli (A and C) predicted sucrose delivery. Additional trials were then introduced in which A was accompanied by a visual stimulus (X); no sucrose was delivered on these AX− trials. In this way, X signaled the omission of the sucrose otherwise signaled by A and so should acquire inhibitory properties and the ability to counteract the effects of stimuli predicting sucrose. Therefore, the first indicator of inhibitory learning would be lower rates of responding during AX presentations than during A presentations in the feature-negative acquisition stage. Two further tests were then conducted to try to rule out alternative explanations of the results (Rescorla, 1967, 1969). In the summation test, the ability of X to suppress the level of conditioned responding elicited by an alternative excitatory stimulus (C) was evaluated. This was achieved by comparing levels of responding to C and C in compound with the putative inhibitor X (weak control) or CX and C in compound with a novel, visual control stimulus Y (strong control). The comparison with CY controls for external inhibition. Both X and Y could cause external inhibition, but only X can be inhibitory. CX− versus CY− provides a strong control because Y, being less familiar, will cause more external inhibition than X. If X had acquired inhibitory properties, then CX should elicit lower levels of conditioned responding than C and CY presentations. The summation test was followed by retardation testing, in which the rate of acquisition of excitatory conditioning to putative inhibitor X and control stimulus Y was compared. If X was an inhibitor, it should acquire associative strength more slowly than Y. The behavioral measure was in each case nose poke responses per minute (RPM).

Procedure

Prior to the behavioral procedure, there were 4 days of home cage exposure to the sucrose pellets reward to reduce neophobia. Sucrose pellets were placed in each cage in a glass dish at the same time as the daily ration of the rodent diet.

Rats were individually assigned to a conditioning box for the duration of the experiment and completed the below experimental stages. Throughout rats were run in batches of up to 6 and received a single training session each day. All stimuli were presented for 20 s and preceded by a 20 s pre-CS period during which responding was also recorded. The intertrial interval was 40 s plus a 40 s variable interval component (from an exponential distribution) taking the intertrial interval up to an average of 80 s. The different trial types in each of the experimental phases (shown in Table 1 and summarized below) were presented in a semirandom order.

Preexposure. Preexposure was conducted on the first day of testing; it included 24 trials, in sessions lasting approximately 48 min. The animals were exposed to each stimulus they would encounter during the behavioral testing six times. The auditory stimuli were white noise (A) and the click (C). The visual stimuli (X and Y) were

Table 1
Behavioral Testing Design

		Training		Tests	
	Preexposure	Excitatory training	Feature-negative acquisition	Summation	Retardation
Stimuli	A− (×6) C− (×6) X− (×6) Y− (×6)	A+ (×15) C+ (×15)	A+ (×10) C+ (×5) AX− (×20)	C− (×10) CX− (×10) CY− (×10)	A− (×5) X+ (×15) Y+ (×15)

Note. "−" denotes nonreinforced trials and "+" denotes reinforced presentations, which were followed by 2 sucrose pellets. Stimuli A and C were white noise and a continuous click, respectively. Stimuli X and Y were either a constant right light or flashing left light (counterbalanced).

counterbalanced; for half the rats in each group, X was the constant light, and Y was the flashing light, and for the remainder, it is the reverse. All stimuli were unreinforced (there were no reward pellets) during preexposure.

Excitatory Training. The rats underwent 6 days of excitatory training, in which they were exposed to the auditory stimuli (A and C) 15 times each; thus, there were a total of 30 trials per session, each of which lasted approximately 60 min. After each stimulus, two reward pellets were dispensed.

Inhibitory Acquisition. Feature-negative training was conducted for 4 days. The two auditory stimuli were reinforced when presented alone, but the AX compound was presented without reinforcement. There was a total of 35 trials: 10 reinforced A trials, five reinforced C trials, and 20 unreinforced AX trials.

Summation Test. There was 1 day of summation testing, in which the animals were presented with 10 unreinforced trials of C, CX, and CY each, 30 trials in total.

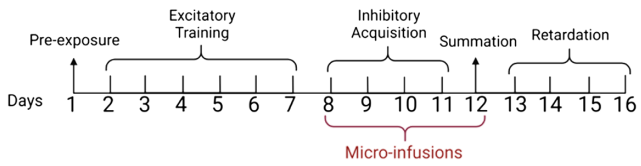
Retardation Test. Retardation testing was conducted for 4 days. The animals underwent a total of 35 trials: five unreinforced trials of A, 15 reinforced trials of X, and 15 reinforced trials of Y.

Microinfusions of the drug (full details above) were administered 10–15 min prior to behavioral testing, during the feature-negative acquisition phase, and on the summation test day. There was a total of 5 days of microinfusions. The procedural timeline is shown in Figure 1.

Perfusions and Histology: Verification of Cannula Placements

After the completion of the experiments, the rats were anesthetized with a lethal dose of pentobarbital (1–1.5 ml Dolethal; pentobarbital barbiturate, 200 mg/ml; Vetoquinol, United Kingdom) and transcardially perfused with 0.9% saline followed by 4% formaldehyde solution in saline. Brains were then extracted from the skull and postfixed in 4% formaldehyde, before being cut into 80-μm coronal sections on a vibratome (Leica VT1000 S), targeting the area encompassing mPFC. These sections were then mounted on microscope slides and stained with cresyl violet. Placements of the injector were determined using a light microscope and mapping onto coronal sections of a rat brain stereotaxic atlas (Paxinos & Watson, 2007).

Figure 1
Procedure Timeline



Note. The behavioral tests were conducted over 16 days. Preexposure was 1 day long, excitatory training occurred for 6 days, the feature-negative inhibitory acquisition phase occurred for 4 days, the summation test was 1 day, and the retardation phase was 4 days long. There were 5 days of microinfusions—during the feature-negative inhibitory acquisition phase and on the summation test day. See the online article for the color version of this figure.

Design and Analysis

Statistical analyses were conducted using IBM SPSS Statistics Version 28. Repeated measure analyses of variance were used to analyze the RPM measured as difference scores (CS–preCS RPM) at the various stages of each of the three experiments, with within-subjects factors of day (six levels at excitatory training, four levels at feature-negative discrimination training and retardation testing) and stimulus (two levels: A vs. C at excitatory training stage; A vs. AX at the feature-negative discrimination stage; C vs. CX and CX vs. CY at the summation test; and X vs. Y at the retardation test). The between-subjects factor was in each case infusion group (three levels: saline, PL drug, IL drug). As in previous studies, there was no difference between the saline injections at PL and IL coordinates, so these groups were combined for the analyses (Pezze et al., 2015, 2017). The main effect of infusion reported for summation is for all three levels of stimulus (C, CX, CY). Post hocs to follow up on the main effects of infusion were by Fischer's least significant difference test.

Results

The below reported analyses exclude the data for rats infused at cannula tips entirely misplaced outside of the target PL and IL subregions. Rats with misplacements between IL and PL were reassigned based on histological verification (resulting in larger PL than IL group sizes). The cannula tip placements for the included rats are shown in Figure 2. Rerunning the analyses for the key feature-negative discrimination learning stage with more stringent exclusion criteria, to also exclude rats with midline placements, or that were on the border of PL and IL, or borderline with some other structure, confirmed the same conclusions and with very similar patterns of results statistically. Hence, the PL and IL group allocations are based on the most liberal inclusion criteria, and we acknowledge that some infusions would have been partially effective in the target subregion.

We also checked for effects of replication, again at the key feature-negative discrimination learning stage. There were some effects of replication on overall response rates: in Experiment 1, $F(1, 27) = 6.192$, $p = .019$, and in Experiment 2, $F(1, 25) = 6.494$, $p = .017$. However, as replication did not interact with stimulus or infusion at the feature-negative discrimination stage, the below results are reported collapsed across the two replications of each experiment.

Experiment 1: Effects of Inactivation of Prelimbic or Infralimbic Cortex

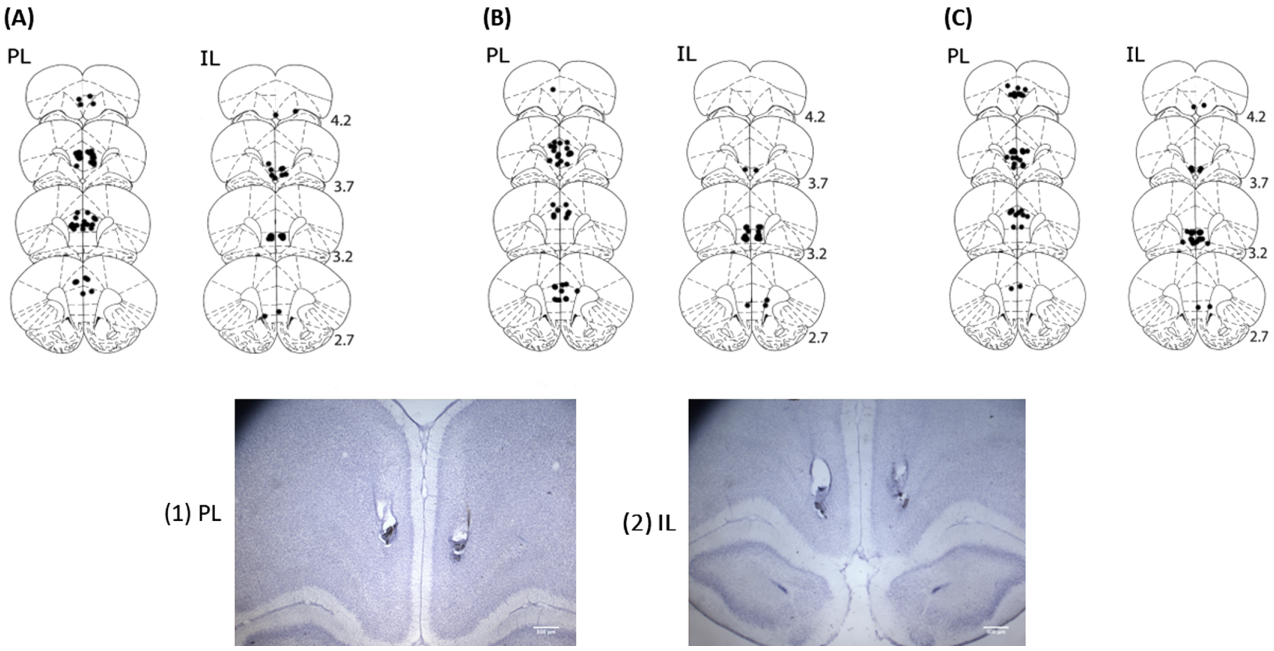
The sample sizes were $N = 12$ for the control group, $N = 13$ for the PL muscimol group, and $N = 8$ for the IL muscimol group. Figure 2A shows the range of included cannula placements.

Excitatory Training (Without Microinfusions)

There was an effect of days reflecting acquisition, $F(5, 150) = 42.848$, $p < .001$, and an effect of stimulus, $F(1, 30) = 4.439$, $p = .044$, because the response rate for A was overall higher ($M = 5.471 \pm 0.817$) than that of C ($M = 4.912 \pm 0.799$). However, there was no interaction between stimulus and days, $F(5, 150) = 1.133$, $p = .345$, and there were no effects involving infusion group-to-be, all

Figure 2

Approximate Locations of the Infusion Cannula Tips (Represented as Black Dots)



Note. Locations are shown in schematic coronal sections adapted from Paxinos and Watson (2007) for (A) the Experiment 1 muscimol infusions (B) the Experiment 2 SKF-81297 infusions and (C) the Experiment 3 SCH-23390 infusions, in prelimbic (PL) and infralimbic (IL) regions of medial prefrontal cortex. The numbers on the right of each section indicate the location of each section in millimeters anterior to bregma. The inset photographs are of coronal brain sections showing example placements targeted to (a) PL and (b) IL regions of the medial prefrontal cortex. Animals with entirely misplaced cannulae were excluded from the analyses and are not shown here. The borderline placements are shown (these animals were excluded only in the further analyses conducted to verify the conclusions drawn). See the online article for the color version of this figure.

*F*s < 1, indicating that at this preinfusion stage, the groups were matched for baseline responding. The small difference in responding to A versus C does not compromise the key results as the design does not require direct comparison between responding to A and C (A was used for the feature-negative discrimination test and C for the summation test).

Feature-Negative Discrimination Training (Following Microinfusions)

Figure 3A shows the acquisition of the discrimination in each of the infusion groups. There was a main effect of days, $F(3, 90) = 13.669$, $p < .001$, and there were interactions between days and infusion, $F(6, 90) = 3.293$, $p = .006$, and days and stimulus, $F(3, 90) = 2.825$, $p = .043$. There were also main effects of stimulus, $F(1, 30) = 15.323$, $p < .001$, and infusion, $F(2, 30) = 8.506$, $p = .001$. Response rates were overall higher to A ($M = 7.007 \pm 0.845$) than AX ($M = 5.346 \pm 0.708$), and preCS–CS responding was overall reduced following muscimol infusions in PL ($M = 5.227 \pm 1.168$; $p = .005$) or IL ($M = 2.937 \pm 1.489$; $p < .001$) compared with saline ($M = 10.366 \pm 1.216$). Statistically, there was no effect of the muscimol infusions on feature-negative discrimination learning because neither of the interactions involving stimulus and infusion was significant, maximum $F(2, 30) = 1.980$, $p = .156$. However, the infusions were not ineffective because the difference score RPM was overall reduced (for further details please see Supplemental Table S1).

Summation Test (Following Microinfusions)

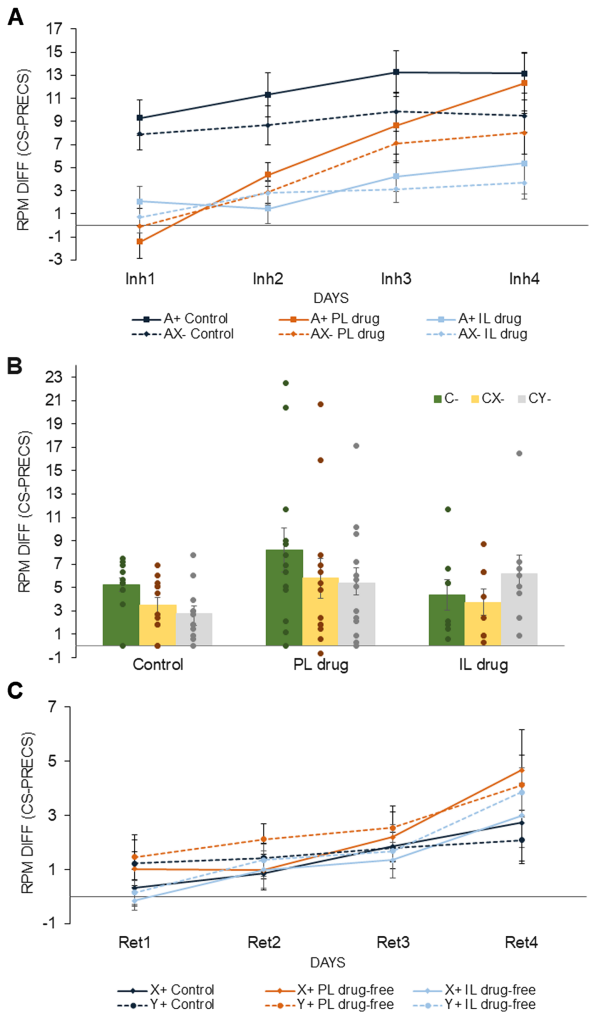
Figure 3B shows summation test performance for each of the infusion groups. The main effects of stimulus confirmed that response rates were higher, $F(1, 30) = 4.957$, $p = .034$, for C ($M = 5.943 \pm 0.851$) than CX ($M = 4.339 \pm 0.788$), but responding to CX was not lower than responding to CY ($M = 4.751 \pm 0.722$), $F(1, 30) = 0.486$, $p = .491$. The summation test performance profiles shown in Figure 3B suggest some qualitative differences in performance across the infusion groups. However, there were no interactions between stimulus and infusion for either comparison, maximum $F(2, 30) = 2.458$, $p = .103$. Similarly, there was no overall effect of infusion, $F(2, 30) = 1.472$, $p = .245$. Thus, the summation test was passed on the weak, but not the strong, control, and statistically there was no effect of muscimol infusions targeted to PL or IL on the summation test expression of inhibitory learning.

Retardation Test (Without Microinfusions)

There was a main effect of days, $F(3, 90) = 12.562$, $p < .001$. However, the expected effect of stimulus was not significant, $F(1, 30) = 3.308$, $p = .079$, and neither were there any effects

Figure 3

Difference Scores (CS-preCS responding) for the Experiment 1 Muscimol Study



Note. Mean responding computed as the average rate of responding (in responses per minute; RPM) on each day of the key experimental stages. Error bars show the standard error of the mean. All of the *y*-axis scales are adjusted so that the difference score RPM to the stimuli in the different infusion groups are as clear as possible. For purposes of comparison across stages and/or experiments, please note that there are differences in the RPM across the experimental stages as well as differences in the baseline RPM across the experiments. (A) The 4 days of the feature-negative inhibitory (Inh1–4) training phase (A+, AX−), following muscimol infusions that reduced CS–preCS responding in both PL, $p = .005$, and IL, $p < .001$ (full statistics are reported in the text). Overall AX < A RPM, $p < .001$, confirmed that the feature-negative discrimination was learned. (B) Summation test performance following muscimol infusions: responding to the transfer stimulus, with or without the inhibitor, and in comparison with the change in responding produced by compounding with a novel stimulus (C, CX, CY). Overall CX < C RPM, $p = .034$, confirmed that the summation test was passed on the weak control; CX < CY RPM would confirm that the summation test was passed on the strong control, but it was not, $p = .491$. (C) Responding on the 4 days of the retardation test (X+, Y+) conducted drug-free (no infusions). Overall X < Y RPM would confirm that the retardation test was passed, but it was not, $p = .079$. CS = conditioned stimulus; PL = prelimbic; IL = infralimbic. See the online article for the color version of this figure.

involving prior infusion group, all Fs < 1. Response rates to X were not lower than to the control stimulus Y. Rather, as shown in Figure 3C, there were modest levels of new learning to both X and Y, and this learning was not different in the groups previously infused with muscimol in PL or IL.

Experiment 2: Effects of D1 Agonist in Prelimbic or Infralimbic Cortex

The sample sizes were $N = 11$ for the saline control group, $N = 11$ for the PL-SKF group, and $N = 9$ for the IL-SKF group for feature-negative inhibitory acquisition. Figure 2B shows the range of included cannula placements. Procedural error on the final session (Day 4) of feature-negative training resulted in two rats from the control group receiving an incorrect training program. Feature-negative performance data for these subjects for Day 4 were analyzed using an average of the performance measures for training Days 3 and 4. These rats were excluded from subsequent summation and retardation testing (final saline control group $N = 9$).

Excitatory Training (Without Microinfusions)

There was an effect of days reflecting acquisition, $F(5, 140) = 37.744$, $p < .001$. There was no main effect of stimulus, $F(1, 28) = 0.947$, $p = .339$, and no interaction between stimulus and days, $F(5, 140) = 0.760$, $p = .580$. There was no effect of infusion group-to-be, $F(2, 28) = 0.733$, $p = .489$, indicating that at this preinfusion stage, the groups were matched for baseline responding.

Feature-Negative Discrimination Training (Following Microinfusions)

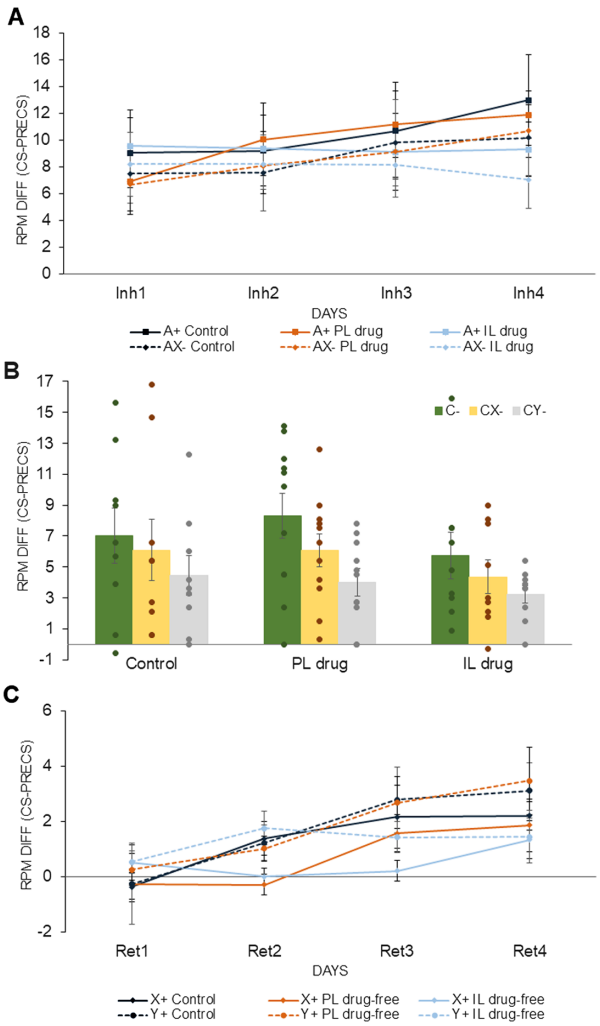
Figure 4A shows the acquisition of the discrimination in each of the infusion groups. There was a main effect of days $F(3, 84) = 3.047$, $p = .033$, but there were no interactions between days and infusion, $F(6, 84) = 1.383$, $p = .231$, or days and stimulus, $F(3, 84) = 0.557$, $p = .645$. There was a main effect of stimulus, $F(1, 28) = 26.298$, $p < .001$. Response rates were overall higher to A ($M = 9.938 \pm 1.378$) than AX ($M = 8.438 \pm 1.317$). Overall responding was similar following SKF-81297 infusions targeted to PL ($M = 9.327 \pm 2.24$) or IL ($M = 8.619 \pm 2.476$) compared with saline ($M = 9.618 \pm 2.24$), and there was no main effect of infusion statistically, $F(2, 28) = 0.046$, $p = .955$ (for further details please see Supplemental Table S1). Moreover, there was no effect of the SKF-81297 infusions on feature-negative discrimination learning because there were no significant interactions involving stimulus and infusion, both Fs < 1.

Summation Test (Following Microinfusions)

Figure 4B shows summation test performance for each of the infusion groups. There was a main effect of stimulus for the weak control, $F(1, 26) = 4.695$, $p = .04$; responding to CX ($M = 5.507 \pm 0.809$) was overall lower than that seen to C ($M = 7.019 \pm 0.915$), confirming that summation was passed on the weak control. Analysis of variance for the strong control showed a significant effect of stimulus, $F(1, 26) = 8.779$, $p = .006$. However, as responding to CX exceeded responding to CY ($M = 3.892 \pm 0.543$), the summation test was not passed on the strong control. There was no overall effect of infusion, $F(2, 26) = 0.583$, $p = .565$, and there were no interactions

Figure 4

Difference Scores (CS-preCS Responding) for the Experiment 2 SKF-81297 Study



Note. Mean responding computed as the average rate of responding (in responses per minute; RPM) on each day of the key experimental stages. Error bars show the standard error of the mean. All of the *y*-axis scales are adjusted so that the difference score RPM to the stimuli in the different infusion groups are as clear as possible. For purposes of comparison across stages and/or experiments, please note that there are differences in the RPM across the experimental stages as well as differences in the baseline RPM across the experiments. (A) The 4 days of the feature-negative inhibitory (Inh1–4) training phase (A+, AX−), following drug infusions. Overall AX < A RPM, $p < .001$, confirmed that the feature-negative discrimination was learned (full statistics are reported in the text). (B) Summation test performance following drug infusions: responding to the transfer stimulus, with or without the inhibitor, and in comparison with the change in responding produced by compounding with a novel stimulus (C, CX, CY). Overall CX < C RPM, $p = .04$, confirmed that the summation test was passed on the weak control; CX < CY RPM would confirm that the summation test was passed on the strong control, but it was not because CX > CY RPM, $p = .006$. (C) The 4 days of the retardation test (X+, Y+) were conducted drug-free (no infusions). Overall X < Y RPM confirmed that the retardation test was passed, $p = .018$. CS = conditioned stimulus; PL = prelimbic; IL = infralimbic. See the online article for the color version of this figure.

between stimulus and infusion, both Fs < 1. Thus, there was no effect of SKF-81297 infusion in PL or IL on the summation test expression of inhibitory learning.

Retardation Test (Without Microinfusions)

There was a main effect of days, $F(3, 78) = 9.373$, $p < .001$, and main effect of stimulus, $F(1, 26) = 6.402$, $p = .018$. As shown in Figure 4C, response rates to X ($M = 0.855 \pm 0.259$) were overall lower than those seen in response to the control stimulus Y ($M = 1.598 \pm 0.362$), so the retardation test was passed. There was no overall effect of prior infusion group, $F(2, 26) = 0.382$, $p = .686$, and there were no interactions involving stimulus and prior infusion group, both Fs < 1.

Experiment 3: Effects of D1 Antagonist in Prelimbic or Infralimbic Cortex

The sample sizes were $N = 11$ for the saline control group, $N = 14$ for the PL-SCH group, and $N = 8$ for the IL-SCH group for the feature-negative inhibitory acquisition. Figure 2C shows the range of included cannula placements.

Excitatory Training (Without Microinfusions)

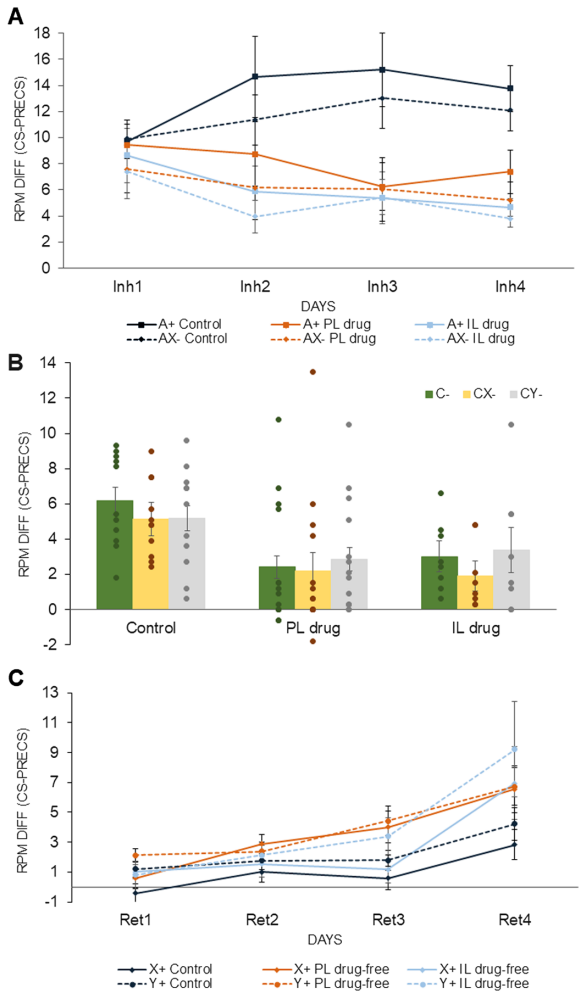
There was an effect of days reflecting acquisition, $F(5, 145) = 64.287$, $p < .001$, but no effect of stimulus, $F(1, 29) = 0.122$, $p = .730$, with no significant difference in response rates to stimulus A versus C. There was no effect of designated infusion group, $F(2, 29) = 0.563$, $p = .576$, indicating that at this preinfusion stage the groups were matched for baseline responding. Data capture was lost for one rat on Day 6, and this rat is therefore excluded from the excitatory training analysis. However, this rat was correctly conditioned, and its data are therefore included in the subsequent analyses reported below.

Feature-Negative Discrimination Training (Following Microinfusions)

Figure 5A shows the acquisition of the discrimination in each of the infusion groups. There was no main effect of days, $F(3, 90) = 0.418$, $p = .741$, but there was a significant main effect of stimulus, $F(1, 30) = 17.105$, $p < .001$, driven by overall higher response rates to A ($M = 9.137 \pm 1.074$) than AX ($M = 7.755 \pm 0.934$). There was an interaction between days and infusion, $F(6, 90) = 2.444$, $p = .031$; the mean response rates for controls increased from Day 1 to Day 4, while response rates for both drug groups decreased from Day 1 to Day 4. There was also a main effect of infusion, $F(2, 30) = 4.220$, $p = .024$, with significantly higher overall response rates for controls ($M = 12.462 \pm 1.695$) than for each of the SCH-23390 infusion groups, both PL drug ($M = 7.1 \pm 1.502$; $p = .025$) and IL drug ($M = 5.637 \pm 1.987$; $p = .014$). Statistically, there was no effect of the SCH-23390 infusions on feature-negative discrimination learning as there were no significant interactions involving stimulus and infusion, maximum $F(6, 90) = 0.802$, $p = .571$. However, the infusions were not ineffective, as the difference score RPM was reduced (for further details please see Supplemental Table S1).

Figure 5

Difference Scores (CS-preCS Responding) for the Experiment 3 SCH-23390 Study



Note. Mean responding computed as the average rate of responding (in responses per minute; RPM) on each day of the key experimental stages. Error bars show the standard error of the mean. All of the *y*-axis scales are adjusted so that the difference score RPM to the stimuli in the different infusion groups are as clear as possible. For purposes of comparison across stages and/or experiments, please note that there are differences in the RPM across the experimental stages as well as differences in the baseline RPM across the experiments. (A) The 4 days of the feature-negative inhibitory (Inh1–4) training phase (A+, AX−), following drug infusions, which reduced CS–preCS responding in both PL, $p = .025$, and IL, $p < .014$ (full statistics are reported in the text). Overall AX < A RPM, $p < .001$, confirmed that the feature-negative discrimination was learned. (B) Summation test performance following drug infusions, which reduced CS–preCS responding in both PL, $p = .012$, and IL, $p < .043$: responding to the transfer stimulus, with or without the inhibitor, and in comparison with the change in responding produced by compounding with a novel stimulus (C, CX, CY). Overall CX < C RPM would confirm that the summation test was passed on the weak control, but it was not, $p = .078$; CX < CY RPM would confirm that the summation test was passed on the strong control, but it was not, $p = .058$. (C) The 4 days of the retardation test (X+, Y+) were conducted drug-free (no infusions). Overall X < Y RPM confirmed that the retardation test was passed, $p = .005$. CS = conditioned stimulus; PL = prelimbic; IL = infralimbic. See the online article for the color version of this figure.

Summation Test (Following Microinfusions)

Figure 5B shows the summation test performance for each of the infusion groups. The main effects of stimulus confirmed that response rates to CX were nonsignificantly lower than response rates to C, $F(1, 30) = 3.902$, $p = .058$, and responding to CX was not lower than to CY, $F(1, 30) = 3.32$, $p = .078$. Moreover, there were no interactions between stimulus and infusion for either comparison, both Fs < 1. However, there was an overall effect of infusion, $F(2, 30) = 4.029$, $p = .028$, with significantly higher overall response rates for controls ($M = 5.491 \pm 0.838$) than for each of the SCH-23390 infusion groups PL drug ($M = 2.486 \pm 0.743$; $p = .012$) and IL drug ($M = 2.763 \pm 0.983$; $p = .043$). Thus, the summation test was not passed on either the weak or the strong control, and there was no effect of SCH-23390 infusions in PL or IL on the summation test expression of inhibitory learning. However, the infusions were not ineffective, as shown in Figure 5B; difference score responding was overall reduced.

Retardation Test (Without Microinfusions)

There was a main effect of days, $F(3, 90) = 29.497$ $p < .001$, and of stimulus, $F(1, 30) = 8.982$, $p = .005$. As shown in Figure 5C, response rates to stimulus X ($M = 2.392 \pm 0.413$) were overall lower than those seen in response to the control stimulus Y ($M = 3.265 \pm 0.419$); hence, the retardation test was passed. There were no significant interactions by days; more importantly, there were no effects involving prior infusion group, maximum $F(2, 30) = 2.705$, $p = .083$ (for the main effect).

Discussion

In three experiments, there was no evidence to suggest that inactivation of PL or IL mPFC impaired feature-negative discrimination learning and no evidence for dopaminergic modulation of such learning in these mPFC subregions either. Average response rates were higher to A+ than AX−, reflecting robust feature-negative discrimination in all three experiments. However, performance in summation and retardation tests of conditioned inhibition was inconsistent. Some nonspecific effects of the infusions were taken into account by the within-subjects comparisons of responding to the different stimuli: feature-negative discriminations were preserved in infusion groups with overall reduced rates of responding. These nonspecific effects were unwanted but provide some positive control for the effectiveness of the infusion procedures.

In Experiment 1, the difference score RPM was overall reduced following muscimol infusions in PL or IL compared with saline. However, the A+ versus AX− discrimination was not significantly impaired following muscimol infusions. Thus, counter to expectations, the results of Experiment 1 did not provide any evidence that PL and IL areas of mPFC contribute differently to feature-negative discrimination learning because inactivation in these regions did not differentially affect the acquisition of the A+/AX− discrimination. Analyses of performance at summation did not reveal direct or indirect effects of muscimol on the test expression of conditioned inhibition either. Retardation test performance was insufficient to confirm conditioned inhibition in Experiment 1.

Similarly, in Experiments 2 and 3, infusions of the DA D1 agonist SKF-81297 and the DA D1 antagonist SCH-23390 at the same

mPFC coordinates were also without effects on the acquisition or retardation test expression of inhibitory learning. In Experiment 3, summation test performance was insufficient to confirm conditioned inhibition, but the retardation tests were passed in both Experiments 2 and 3. Thus, we find no evidence for dopaminergic modulation of inhibitory learning in mPFC. A previous study found that the same dose of SKF-81297, administered in IL and PL using the same methods and in the same laboratory, impaired trace conditioning in an appetitive task (Pezze et al., 2015). It must be noted that—in the present study—SKF-81297 infusion had no significant effect on conditioned responding whatsoever. However, no trace interval was in use. The overall effects in reducing the difference score RPM seen after infusion with SCH-23390 (and muscimol in Experiment 1) suggest that the infusions were not generally ineffective in the present study.

These effects on response rate provide some positive control for the effectiveness of the microinfusion procedures, but at the same time their lack of effect on the discriminations as such can be clearly distinguished statistically. Rather than comparing responding to the different stimuli (A vs. AX; C vs. CX; CX vs. CY; X vs. Y) between groups, the rats are required to differentiate between the stimuli within the learning sessions, so the effects of infusion are matched. Thus, the within-subjects design is powerful, providing experimental control for any systematic differences in responding between infusion groups (as well as individual variability) but necessitated the use of a variety of experimental stimuli including some use of visual stimuli (for X and Y). These visual stimuli include temporal (flashing vs. constant) as well as positional cues (the right light vs. the left light), which make them sufficiently salient to detect, even for albino rats (Waite et al., 2021). However, while the feature-negative discrimination was robust in all three experiments, there were some inconsistencies in performance at the follow-up tests used to confirm conditioned inhibition, which rely on the discrimination of X and Y.

Summation and Retardation Tests

The summation test was passed by the weak control (CX < C RPM) in Experiments 1 and 2 (but not 3), but not with the strong control (CX < CY RPM) in any of the three experiments. In contrast, the retardation test was passed in Experiments 2 and 3, but not in Experiment 1. The combination of summation and retardation tests has been suggested to be definitive to confirm conditioned inhibition because, between them, the two tests should rule out explanations of apparent inhibition based on too much or too little attention to the inhibitor (Hearst, 1972; Rescorla, 1969).

However, two-test procedures have not been universally adopted, and, more recently, it has been argued that the requirement for these stringent tests should be relaxed (Sosa & dos Santos, 2019; Sosa & Ramírez, 2019). Already studies of feature-negative (A+/AX−) discrimination (in the absence of follow-up summation and/or retardation tests) have proven very useful in studies of fear discrimination (in which the inhibitor amounts to a safety signal; Cassaday et al., 2023). In the present study, all three experiments were run under (as far as possible) identical conditions, and in every case, the inhibitory feature-negative (A+/AX−) discrimination was statistically robust. Across the three experiments, conditioned inhibition was confirmed by either the summation test (CX < C RPM) or the retardation test (X < Y RPM), just not simultaneously within all experiments, and never on the strongest control for summation (CX < CY RPM). We therefore suggest that, while it was not conclusively confirmed, the criteria for conditioned inhibition were partially met. This level of confirmation is consistent with the majority of studies in behavioral neuroscience (Cassaday et al., 2023). With some notable exceptions (appetitively motivated; Rhodes & Killcross, 2007), both tests are not routinely reported. Moreover, in our earlier work using the same appetitive within-subjects inhibitory learning procedure, the retardation, but not the summation, test was passed (Waite et al., 2021). This design, which was used in the present study, is powerful statistically, and, as discussed above, rats act as their own controls for shifts in baseline responding. However, we use matched stimuli from the same modality for X versus Y, which makes the discriminations more challenging, particularly for the rats to differentiate CX− and CY− in the 1-day summation test.

Feature-Negative Discrimination Is Unaffected by Inactivation in mPFC Subregions

Some of the IL placements included in the present study were anterior, encroaching on orbitofrontal and dorsal peduncular cortices. Some of the PL placements were also dorsal, encroaching on anterior cingulate cortex. However, these placements were judged sufficiently borderline for the target mPFC subregion to be reached by diffusion, albeit with a reduced effective dose. Conversely, some infusions well-positioned within IL and PL would have reached beyond these targeted subregions by diffusion. Injection volumes of 0.5 μl/side as used in the present study have been estimated to diffuse 0.5–1 mm from the point of injection and in all directions if there are no physical barriers such as fiber tracts (Allen et al., 2008). Moreover, the minimally pharmacologically active concentrations (at the outer limits of the diffusion radius) are unknown, so the best criterion to determine spread has to be functional (Edeline et al., 2002). Despite this inevitable limitation of microinfusion studies, differential (dose-related) effects have been demonstrated using injection volumes of 0.5 μl/side in PL and IL (e.g., on locomotor activity; Pezze et al., 2017). It should also be noted that some of the anterior placements included in the present study were in the saline group allocation, for example, three of the animals retained in Experiment 3.

A role for IL rather than PL in appetitive inhibitory learning would have been broadly consistent with some (Harrewijn et al., 2021; Ng & Sangha, 2023; Rhodes & Killcross, 2007; Sangha et al., 2014) but not all earlier findings (MacLeod & Bucci, 2010; Meyer & Bucci, 2014). In the present study, we found no evidence that reversible inactivation in either IL or PL impaired acquisition of the feature-negative (A+/AX−) discrimination or the summation test expression (C/CX) of conditioned inhibition.

The most relevant prior studies are the appetitively motivated feature-negative procedures (MacLeod & Bucci, 2010; Meyer & Bucci, 2014; Rhodes & Killcross, 2007), of which one confirmed conditioned inhibition by summation and retardation test (Rhodes & Killcross, 2007). However, this was an excitotoxic lesion study and as such not directly comparable, particularly as retardation testing (where the selective effect of the IL lesion was found) was conducted without any further infusion in the present study. The retardation tests used here were designed to assess drug-free test expression of conditioned inhibition, uncontaminated by any nonspecific effects of drug infusions. Unfortunately, the effect of prior inactivation on the

retardation test expression of conditioned inhibition (conducted drug-free without any further infusions) could not be assessed because retardation was not demonstrated in Experiment 1.

Conceivably, the lack of effects with manipulations in either mPFC subregion alone might suggest that both are important. We did not test for the possibility of additive effects in the present study. Previous studies examining the individual roles of PL and IL in fear discrimination have found mixed results. While some studies have demonstrated distinct contributions of PL and IL to fear discrimination (Pollack et al., 2018; Sangha et al., 2014), others suggest that both PL and IL are involved and may act in concert (Corches et al., 2019; Giustino & Maren, 2015).

Feature-Negative Discrimination Is Unaffected by Dopamine D1 Receptor Agents in Medial Prefrontal Cortex Subregions

Other studies of appetitive conditioning using the same procedures have shown effects of either PL or IL microinfusions of drugs, including DA D1 receptor agents. Appetitive trace conditioning was impaired by SKF-81297 (0.05 μg in 0.5 μl/side) microinjected into either PL or IL mPFC (Pezze et al., 2015), as well as after infusion of scopolamine at either PL or IL coordinates and using the same 0.5-μl injection volume (Pezze et al., 2017). In the latter study, dissociation was nonetheless demonstrated, in that locomotor activity was increased after infusion in IL only; it was just the associative learning that was impaired irrespective of mPFC subregion and without the need for the additive effects that would result from treating both subregions.

In Experiments 2 and 3, microinfusions of either SKF-81297 or SCH-23390 were without effect on the acquisition of the feature-negative discrimination or on the retardation test expression of conditioned inhibition (with retardation tested drug-free). Previous studies have identified the role of DA in appetitive conditioned inhibition. Seven days of systemic pretreatment with the indirect DA agonist amphetamine (prior to any conditioning) were found to enhance conditioned inhibition of reward (Harmer & Phillips, 1999). Moreover, the role of midbrain (primate A8, A9, A10; rat VTA) DA has been confirmed in electrophysiological (Tobler et al., 2003; Yan et al., 2019) and optogenetic studies of appetitively motivated conditioned inhibition (Chang et al., 2016, 2018).

Giving the drug infusions before learning in principle affects both acquisition and early consolidation because the drug remains active (at a less effective dose, consistent with the half-life) for a while after the learning sessions have terminated. Immediate posttraining infusion would have been a better method to isolate effects on consolidation (Simon & Setlow, 2006) and in the absence of direct effects on performance of the kind seen in Experiment 3 (and Experiment 1). Based on the effects of amphetamine, infusions with a DA D1 agonist and antagonist might be expected to enhance and impair consolidation of the feature-negative discrimination, respectively. However, while it is possible that we would have seen effects with immediate posttraining infusions, we would still have expected drug infusions prior to training to affect early consolidation if (DA transmission in) PL and/or IL was involved.

Conclusions and Implications

A role for DA within mPFC in appetitive conditioned inhibition has not been previously reported and was not found here. The results obtained with both the DA D1 agonist and antagonist suggest that the mesocorticolimbic projection to mPFC is inessential for appetitive feature-negative discrimination learning (Cassaday et al., 2023). These negative findings apparently contradict earlier evidence showing a role for DA in appetitively motivated conditioned inhibition (Chang et al., 2016, 2018; Harmer & Phillips, 1999; Tobler et al., 2003; Yan et al., 2019). This inconsistency may be because we targeted the wrong receptor subtype in the present study, if DA modulation of inhibitory learning in mPFC is mediated by the DA D2-like receptor family (Jenni et al., 2017; Mishra et al., 2018). The role of DA D2-like receptors within mPFC in appetitive conditioned inhibition remains to be tested.

It is also possible that activation at more than one receptor subtype is key, which could be why manipulating signaling at just one receptor subtype in isolation had no effect and why optogenetic DA manipulations and treatment with systemic amphetamine, which would affect all receptor subtypes, are effective (Chang et al., 2016, 2018; Harmer & Phillips, 1999; both appetitively motivated). Alternatively, the focus on DA, although with some justification based on previous studies, may be too simplistic. For example, DA neurons in the VTA show cellular heterogeneity (e.g., coreleasing glutamate) and with different populations of cells implicated in different aspects of motivated behavior (Morales & Margolis, 2017). Molecular and anatomical heterogeneity have similarly been identified in nucleus accumbens DA projections (Verharen et al., 2020). In addition to the DA pathways, 5-hydroxytryptamine has been implicated in conditioned inhibition (Desrochers & Nautiyal, 2022; Lister et al., 1996).

Moreover, in the present study, the microinfusions were used at the feature-negative discrimination phase in the first instance; because effects on feature-negative discrimination learning are of interest in their own right, successful feature-negative discrimination is fundamental to conditioned inhibition, and this discrimination is reliably learned. Only the summation test performance was examined under drug following microinfusion; retardation testing was conducted drug-free (without any further microinfusion). As the effects of excitotoxic mPFC lesions on conditioned inhibition were mediated at the retardation test stage (Rhodes & Killcross, 2007), follow-up microinfusion studies in IL and PL should further examine stage of procedure effects and ideally with more robust conditioned inhibition procedures (as retardation test performance was inconsistent in the present study).

The focus on mPFC may also be too narrow; the hippocampus (Chan et al., 2003) and retrosplenial cortex (Nelson et al., 2018) have been identified as regions implicated in appetitive conditioned inhibition as confirmed by summation and/or retardation test. Although sparse in the hippocampus, DA fibers are observed in retrosplenial cortex (Van Eden et al., 1987), and DA receptors in retrosplenial cortex have been shown to modulate cognitive function (de Landeta et al., 2022).

Finally, it must be acknowledged that the present study used only male rats. This was a resource limitation, and female rats should be included in future studies, and with sufficient statistical power to detect sex differences.

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