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Differential effects of dorsal and ventral medial prefrontal cortex inactivation during natural reward seeking, extinction, and cue-induced reinstatement

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ABSTRACT

Rodent dorsal medial prefrontal cortex (mPFC), typically prelimbic cortex, is often described as promoting actions such as reward seeking, whereas ventral mPFC, typically infralimbic cortex, is thought to promote response inhibition. However, both dorsal and ventral mPFC are necessary for both expression and suppression of different behaviors, and each region may contribute to different functions depending on the specifics of the behavior tested. To better understand the roles of dorsal and ventral mPFC in motivated behavior we pharmacologically inactivated each area during operant fixed ratio 1 (FR1) seeking for a natural reward (sucrose), extinction, cue-induced reinstatement, and progressive ratio sucrose seeking in male Long-Evans rats. Bilateral inactivation of dorsal mPFC, but not ventral mPFC increased reward seeking during FR1. Inactivation of both dorsal and ventral mPFC decreased seeking during extinction. Bilateral inactivation of ventral mPFC, but not dorsal mPFC decreased reward seeking during cue-induced reinstatement. No effect of inactivation was found during progressive ratio. Our data contrast sharply with observations seen during drug seeking and fear conditioning, indicating that previously established roles of dorsal mPFC = going vs. ventral mPFC = stopping are not applicable to all motivated behaviors and/or outcomes. Our results indicate that dichotomous functions of dorsal vs. ventral mPFC, if they exist, may align better with other models, or may require the development of a new framework in which these multifaceted brain areas play different roles in action control depending on the behavioral context in which they are engaged.

SIGNIFICANCE STATEMENT

Dorsal and ventral medial prefrontal cortex have been proposed to control response execution and inhibition, respectively, in contexts such as drug seeking and fear learning. It is unclear, however, whether these roles are generalizable to all behaviors. We found that these opposing roles were not present during natural reward (sucrose) seeking, in contrast with previous drug seeking and fear conditioning literature. Dorsal and ventral mPFC inactivation did impact multiple aspects of seeking, but not in the bidirectional fashion predicted by a generalized go stop model. We conclude that, although these brain areas are clearly important in reward seeking, the dichotomous roles proposed previously are not broadly applicable, and mPFC contributions to these and related behaviors should be reconsidered.

INTRODUCTION

The rodent medial prefrontal cortex (mPFC) plays a key role in numerous behaviors and cognitive functions, including action control, emotional regulation, attention, memory, and decision-making, among others (Dalley et al., 2004; Vertes, 2006; Euston et al., 2012; Barker et al., 2014; Cassaday et al., 2014; Moorman et al., 2015; Eichenbaum, 2017; Ko, 2017). Multiple studies have demonstrated that dorsal mPFC (typically prelimbic cortex) and ventral mPFC (typically infralimbic cortex) have opposing roles that facilitate the execution and inhibition, respectively, of behaviors (Peters et al., 2009; Gass and Chandler, 2013; Gourley and Taylor, 2016). These differences have been observed during drug seeking, fear-associated behaviors, and certain studies of natural reward seeking. For example, dorsal mPFC inactivation reduces reinstatement of drugs of abuse such as cocaine or heroin (McFarland and Kalivas, 2001; McLaughlin and See, 2003; Fuchs et al., 2005; LaLumiere and Kalivas, 2008). In contrast,

ventral mPFC inactivation increases cocaine seeking during extinction, and activation of ventral mPFC decreases reinstatement of cocaine and other drugs of abuse (LaLumiere and Kalivas, 2008; Peters et al., 2008; Muller Ewald and LaLumiere, 2017). In studies of auditory fear conditioning and extinction, dorsal mPFC inactivation decreases fear expression and ventral mPFC inactivation impairs extinction learning and recall (Maren and Quirk, 2004; Peters et al., 2009; Sierra-Mercado et al., 2011). Dorsal and ventral mPFC may also have opposing roles with respect to natural reward seeking: inactivation of dorsal and ventral mPFC decreases and increases in reward seeking, respectively, in certain behavioral paradigms (Rhodes and Killcross, 2004; Rhodes and Killcross, 2007; Ishikawa et al., 2008a, b; Sangha et al., 2014; Eddy et al., 2016; Trask et al., 2017).

However, these dorsal vs. ventral dichotomies are not always observed, and in some cases opposing functions have been described (Moorman et al., 2015). For example, inhibition of dorsal mPFC in models of cocaine dependence result in increased punishment-resistant drug seeking (Chen et al., 2013). Some studies have found an effect of mPFC manipulation on cocaine, but not natural reward seeking (McFarland and Kalivas, 2001; McGlinchey et al., 2016; Gutman et al., 2017). In a discriminative stimulus-driven reward seeking task, both dorsal and ventral mPFC neurons fired during reward seeking and extinction, and inactivation of dorsal or ventral mPFC did not result in specific deficits in execution and extinction of reward seeking (Moorman and Aston-Jones, 2015). In a variable interval sucrose seeking task, dorsal mPFC neurons fired during reward delivery and inactivating this region did not alter reward seeking, whereas ventral mPFC neurons fired during reward collection and inactivating the ventral mPFC delayed the collection of reward (Burgos-Robles et al., 2013). Dorsal mPFC has also been

70 associated with goal directed behaviors, attention, or spatial location representation, and ventral
71 mPFC has been associated with habitual behaviors and emotional regulation, among multiple
72 other functions (Killcross and Coutureau, 2003; Dalley et al., 2004; Euston et al., 2012; Smith et
73 al., 2012; Smith and Graybiel, 2013; Cassaday et al., 2014; Gourley and Taylor, 2016).
74
75 This diversity of results indicates not only that these areas play complex roles in shaping
76 behavior, but also that there may be differences depending on the tasks used to probe mPFC
77 function. Surprisingly, there has been limited characterization of dorsal vs. ventral mPFC
78 contributions to self-initiated instrumental reward seeking and, analogous to described models of
79 drug seeking, extinction and reinstatement. Here we used pharmacological inactivation to
80 characterize the roles of mPFC subregions during these tasks and during a progressive ratio task
81 to assess motivation. We also performed a preliminary assessment of whether or not individual
82 mPFC hemispheres differentially regulate reward seeking, as seen in other behaviors (Sullivan
83 and Gratton, 2002a; Sullivan and Gratton, 2002b), and we performed physiological and
84 behavioral controls to verify the effects of our pharmacological manipulations. Despite observing
85 differential effects of dorsal vs. ventral mPFC inactivation on reward seeking, our findings do
86 not align with previous observations of go/stop dichotomies. Instead they indicate that these
87 brain areas likely perform multiple functions, befitting their complex integrative nature, and that
88 behavioral context, such as the task employed, dictates the contributions of these regions to the
89 behaviors studied.
90
91 **MATERIALS AND METHODS**
92

Animals

Male Long-Evans rats (~9 weeks old and 275-300g upon arrival; Charles River; N = 80) were used in behavioral studies (sucrose self-administration N = 40; extinction N = 16; cue-induced reinstatement progressive ratio N = 16; spontaneous locomotion, N = 8). Two additional male Long Evans rats were used for in vitro electrophysiology studies (see below for details). All rats were single-housed on a reversed light cycle (7:00am on and 7:00pm off) and allowed free access to food and water. Experiments were conducted during active cycle (lights off). All experiments were conducted in accordance with the National Institute of Health guidelines and the standards of the University of Massachusetts Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized with isoflurane in a closed container (5%) and transferred to a stereotaxic frame where they received isoflurane through a nosecone (1.5%-2%). Rats were given systemic antibiotic (0.1 mL cefazolin) and analgesic (1mg/kg meloxicam), and incisions were treated with local anesthetic (0.3mL, 2% lidocane). Bilateral craniotomies were made above the mPFC, and double guide cannulae (26 gauge, Plastics One, Roanoke, VA) were implanted in either dorsal mPFC (+3.0 mm AP; +/- 0.6 mm ML; -2.5 mm DV) or ventral mPFC (+3.0 mm AP; +/-0.6 mm ML; -4.0 mm DV). Three screws were implanted to secure cannulae with dental cement. Rats were allowed 1 week to recover following surgery. Rats tested in the spontaneous locomotor assay (see below) received comparable surgeries, but bilateral guide cannulae were implanted in the shell/core border of the nucleus accumbens (NAc; +1.5 mm AP; +/-1.2 mm ML; -5.4 mm DV).

Baclofen/Muscimol Infusions

Rats were bilaterally injected with 0.3 µL of either artificial cerebrospinal fluid (aCSF) or a 1.0 nmol/0.1 nmol mixture of the GABA-A and -B receptor agonists baclofen and muscimol (BM; Tocris Bioscience, Avonmouth, Bristol, UK) dissolved in aCSF. Injection cannulae (33 gauge, Plastics One) were inserted bilaterally and protruded 1mm below the guide cannulae. Solutions were delivered over the course of 1 minute using a microinfusion pump (UMP3/Micro 4, World Precision Instruments, Sarasota, FL), and the injection cannulae were maintained in place for an extra minute to allow diffusion of the fluid. For the NAc locomotion task, injection cannulae extended 2mm beyond guide cannulae. Rats were tested at least 5 minutes after the injection cannulae were removed.

Apparatus

All operant testing was conducted in Med Associates chambers housed in sound attenuation cubicles (Med Associates, Fairfax, VT). Nose pokes were located on the left and right walls of the operant chambers. Beneath the right nose poke was a well where reward (0.1 ml of 15% sucrose solution) was dispensed. Each chamber was illuminated by a house light, and a fan provided approximately 60 dBA background noise. The same boxes were used for extinction, cue-induced reinstatement, and PR experiments, although the inactive nose poke was inaccessible during extinction sessions. For the NAc locomotion experiments, rats were placed in a plexiglass chamber (39.4x 39.4 x 52.1 cm) with black colored walls and a stainless-steel grid floor. A digital camcorder (Canon VIXIA HF R52) was mounted above the box to record locomotor activity.

Behavioral test groups

Three operant test groups were used in these studies. The first group received inactivation during FR1 sucrose seeking. The second group received inactivation during early and late extinction. The third group received inactivation during cue-induced reinstatement and progressive ratio sessions. The FR1 group received bilateral and unilateral inactivation. Because no major effects were found with unilateral inactivation, the extinction and cue-induced reinstatement/progressive ratio groups received only bilateral inactivation. The FR1 group also received inactivation during extinction, cue-induced reinstatement, and progressive ratio. In this group, we observed no significant effects of manipulation in any of these tests, leading us to consider the possibility that multiple infusions during self-administration resulted in long-lasting damage occluding any potential effects of regional inactivation. Thus, separate groups were run for extinction and cue-induced reinstatement/progressive ratio sessions. Details on testing are below.

Sucrose self-administration

Before surgery, rats were trained to self-administer sucrose on a fixed-ratio 1 (FR1) schedule. A 10-15 sec house light illumination signaled the time-out, during which nose poking in the left (inactive) and right (active) nose pokes were recorded but did not elicit any consequences. Upon house light offset, nose poking in the right nose poke elicited a tone (15 kHz, 74 dBA, 1 sec) and delivery of 0.1 ml 15% sucrose in the well beneath the nose poke. The first active poke after the time-out was counted as a "trial initiation" to distinguish these pokes from other (e.g., time-out) active nose pokes. Trials in which the rat exited the nose poke and entered the well in less than 1 sec after sucrose was dispensed were counted as "rewarded well-entries". Surgeries were

performed after rats reached at least 100 rewarded well-entries and met criteria of 80% rewards collected within 1 sec of delivery. After recovery, rats were retrained for 3 to 10 days (**Figure 1C**). After re-training, rats received a sham infusion in which the injector cannula was inserted and left in place for one minute, but nothing was infused. Testing started the following day. Rats were tested on an FR1 schedule for eight days in total after sham infusion test day. Sessions lasted one hour or until the rat performed 160 trials. During testing, each rat received four separate infusions in counterbalanced order across days: 1) bilateral BM, 2) bilateral aCSF, 3) BM in left hemisphere and 4) aCSF in the right hemisphere, and aCSF in the right hemisphere and BM in the left hemisphere (**Figure 1C**). In between infusion days, rats were run on FR1 with no infusion in order to avoid potential rebound effects and to maintain task performance.

Extinction

A second cohort of rats was trained to reliably respond for sucrose under the FR1 schedule described above. After stable FR1 performance (100 rewarded well-entries and 80% rewards collected within 1 sec), rats received inactivation tests during early and late extinction sessions (**Figure 2B**). Rats received one of two conditions on the first day of early extinction BM or aCSF). They were then retrained on FR1 for two days, and received a second day of early extinction during infusion with the opposite drug or vehicle combination. We included two days of FR1 retraining in between each early extinction day in order to allow paired analysis of early extinction within rats. Rats were then extinguished until they responded with fewer than 20 nose pokes per session for two continuous sessions. On the last two days of extinction (late extinction) rats received counterbalanced BM/aCSF treatments as in early extinction.

Cue-Induced Reinstatement

A third cohort of rats was trained to reliably respond for sucrose under the FR1 schedule described above and then extinguished to the point of responding with fewer than 20 nose pokes per session for two continuous sessions **(Figure 3B**). Rats were then tested in cue-induced reinstatement sessions. During reinstatement, nose pokes on an FR1 schedule elicited a tone but no sucrose delivery. Rats were bilaterally infused with either BM or aCSF on two separate reinstatement days in a counterbalanced fashion. Reinstatement tests were separated by extinction sessions until rats reached criteria of two sessions with fewer than 20 nose pokes.

Progressive ratio

After cue-induced reinstatement, the same rats that were tested on reinstatement were tested on a progressive ratio (PR) sucrose seeking task. The PR test environment was the same as for FR1, but the number of nose pokes required to receive reward increased on each trial based on the equation: Response ratio (rounded to the nearest integer) = $[5e^{(\text{injection number} \times 0.2)}] - 5$ (Richardson and Roberts, 1996). The highest reward number acquired was considered the breakpoint and was analyzed, along with nose pokes and well entries, as a measure of motivation. Rats were bilaterally infused with BM and aCSF prior to testing on separate PR testing days. PR testing lasted either six hours or until 60 minutes of no nose pokes occurred. PR test days were separated by two consecutive days of FR1 training.

Spontaneous Locomotion

In order to verify the behavioral effects of BM, we tested the effect of NAc inactivation during a spontaneous locomotor assay. Methods were based on those described previously (Fuchs et al.,

208 2004). A new cohort of rats was infused with either BM or aCSF in NAc and placed into a novel
209 box 10 minutes after the infusion. Behavior was video recorded for one hour and later analyzed
210 using ANY-maze software (ANY-maze, Wood Dale, IL), in which we divided the chamber in 8
211 zones and counted numbers of line crosses into each zone.
212
213 ***Whole-Cell Patch-Clamp***
214 To verify the physiological effects of BM, we recorded the activity of mPFC neurons in vitro
215 during bath application of BM. Seven neurons from two male Long-Evans rats, approximately
216 25 days old, were included in this analysis. Rats were deeply anesthetized with isoflurane and
217 sacrificed using rapid decapitation, and brains were removed and immersed in ice-cold cutting
218 solution (in mM: 250 Glycerol, 26 $NaHCO_3$, 2.5 KCl, 1.2 NaH_2PO_4, 11 Glucose, 2.4 $CaCl_2$,
219 and 1.2 $MgCl_2$; 310 mOsms; pH = 7.4 when saturated with 95% O_2/5% CO_2). 300 μm coronal
220 sections were obtained using a vibrating blade microtome (VT1000S, Leica Biosystems Inc.,
221 Buffalo Grove, IL), and were immediately transferred to artificial cerebral spinal fluid (aCSF;
222 37°C; in mM: 250 Glycerol, 26 $NaHCO_3$, 2.5 KCl, 1.2 NaH_2PO_4, 11 Glucose, 2.4 $CaCl_2$, and
223 1.2 $MgCl_2$; 310 mOsms; pH = 7.4 when saturated with 95% O_2/5% CO_2). After 30 minutes
224 under these conditions, slices were kept in bubbled aCSF at room temperature for the remainder
225 of the experiment. Glass pipettes were pulled from borosilicate glass tubes (1B150F-4, World
226 Precision Instruments, Sarasota, FL) using a two-stage, vertical puller (PC-10, Narishige
227 International USA, East Meadow, NY), and were backfilled with internal solution (in mM: 110
228 K-Gluconate, 8 NaCl, 30 KCl, 1 $MgCl_2$, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.5 GTP; 298
229 mOsms; pH = 7.4). When filled, pipettes had a tip resistance of 5-8 MΩ. Once whole-cell
230 configuration was achieved, cells were allowed to stabilize for at least 5 minutes before

recordings proceeded. Spontaneous post-synaptic currents (sPSCs) were recorded in voltage clamp mode from pyramidal neurons held at -70 mV in the medial wall of the prefrontal cortex. Recordings were taken before (range: 3-11 min), during (range: 3-13 min), and after (range: 4-30 min) application of BM. Series resistance was monitored throughout the recordings. Recordings were concatenated offline in Igor Pro (Wavemetrics, Lake Oswego, OR) to create one contiguous file, which was then exported to Spike2 (Cambridge Electronic Design Limited, Science Park, Cambridge, UK) where it was low-pass filtered above 100 Hz. Timestamps were obtained in Spike2 through waveform-based template matching. For both the pre-treatment and treatment segments, the length of each recording was standardized to that of the shortest recording by exclusively including the last 3 minutes, and PSC frequency was tabulated for three minute periods before, during, and after BM treatment.

Histology

After final test sessions, rats were deeply anesthetized with Ketamine/Xylazine (80 mg/kg: 10 mg/kg i.p.), and brains were extracted, stored in 4% paraformaldehyde overnight, and transferred to 20% (wt/vol) solution of sucrose/0.1% sodium azide in phosphate buffer at 4 °C. Coronal sections 40 μm thick were cut using a cryostat, mounted on slides, stained with neutral red and cover slipped. Cannula placements were verified by comparing cannula damage to a rat brain atlas (Paxinos and Watson, 2007). Two ventral mPFC rats in the FR1 group, one ventral mPFC rat in the extinction group, and one dorsal and one ventral mPFC rat in the reinstatement group were excluded from analysis due to blocked cannulae or excessive tissue damage. Two rats were excluded from the locomotion task because of cannula misplacements. Cannula placements are

shown in **Figures 1-3** for rats in operant testing groups and in **Figure 6** for rats in spontaneous locomotor tests.

Analysis

Data were analyzed using Prism (GraphPad Software, La Jolla, CA). Total numbers and rates (total number divided by the time taken to complete the task) of active and inactive nose pokes and well entries for the FR1 task were calculated and differences were assessed using one-way repeated measure (RM) ANOVA followed by planned Dunnett's test for multiple comparisons to compare each treatment to bilateral aCSF. In addition to number of responses, we also measured response rate during FR1 as some rats finished the task before the one hour of duration of the task. Total numbers of nose pokes and well entries for extinction, cue-induced reinstatement, and progressive ratio data were analyzed using one-way ANOVA and paired t-tests. Numbers of nose pokes during FR1, early extinction, late extinction, and cue-induced reinstatement were divided into quartiles and data were analyzed using paired two-way ANOVA (treatment x quartile). Locomotion was analyzed using a two-way ANOVA comparing an interaction between 10-minute bins of time and infusion condition. Simple effects for locomotion data, as well as patch clamp data were analyzed using a one-way RM ANOVA. Means and standard error of the mean were presented as (mean ± SEM).

RESULTS

Dorsal, but not ventral mPFC inactivation increased reward seeking during FR1 sucrose self-administration

All rats were highly motivated to perform the FR1 sucrose seeking task (**Figure 1**). RM ANOVA did not reveal significant differences among groups for number of nose pokes ($F(3,19) = 2.37$, $p=0.08$). However, planned Dunnet's tests vs. aCSF revealed an increase in total number of nose pokes when dorsal mPFC was bilaterally inactivated (**Figure 1D**; $p<0.05$, Dunnett's). Bilateral inactivation also increased overall rate of nose pokes ($F(3,19=2.76$, $p= 0.050$, RM ANOVA across all manipulations; $p<0.05$, Dunnett's for bilateral BM vs bilateral aCSF), and in rate of time out nose pokes ($F(3,19)=2.31$, $p=0.086$, RM ANOVA; $p<0.05$ Dunnett's). Bilateral dorsal mPFC inactivation increased number of rewarded well entries, defined as entering the well in less than 1 second after sucrose was dispensed, compared to aCSF (**Figure 1E**; $F(3,19)=2.40$, $p=0.077$, RM ANOVA; $p<0.05$ Dunnett's). We also observed a significant increase in the number of initiated trials ($F(3,19=3.13$, $p=0.033$), but Dunnett's tests did not reveal any significant differences compared to bilateral aCSF ($p>0.05$). Unilateral inactivation of dorsal mPFC had no significant effect on numbers or rate of nose pokes or well entries (all $p>0.05$, Dunnett's). Ventral mPFC inactivation, bilateral or unilateral, had no significant effects on number or rate of nose pokes or well entries (**Figure 1F, G**; all $p>0.05$, RM ANOVA and Dunnett's). There were also no effects of inactivation on latency to initiate trials or collect reward after dorsal or ventral mPFC inactivation (all $p>0.05$, RM ANOVA and Dunnett's). Inactive nose poke responses were low and there were no effects of manipulation on inactive responses (range means 1.6 to 5.3, all $p>0.05$, RM ANOVA and Dunnett's)

Dorsal and ventral mPFC inactivation decreased reward seeking during extinction

Fifteen rats received bilateral inactivation of dorsal (n = 8) or ventral (n = 7) mPFC during early (days 1 and 2) and late (2 days of <20 nose pokes) extinction sessions (**Figure 2**). There were no

effects of inactivation of dorsal or ventral mPFC during early extinction. However, inactivation of dorsal mPFC significantly reduced both nose pokes ($t(7) = 4.00$, $p=0.005$) and well entries ($t(7) = 2.38$, $p=0.049$) (**Figure 2E, F**). Inactivation of ventral mPFC significantly decreased well entries ($t(6) = 2.86$, $p=0.029$) (**Figure 2J**) and, although it appeared that nose pokes were reduced (**Figure 2I**), this effect was not significant ($t(6) = 1.01$, $p=0.35$).

Ventral, but not dorsal mPFC inactivation decreased reward seeking during cue-induced reinstatement

After aCSF treatment on cue-induced reinstatement tests, rats exhibited a significantly increased number of nose pokes compared to the last day of extinction (dorsal mPFC: **Figure 3D**; $t(6)=3.44$, $p=0.014$; ventral mPFC: **Figure 3I**; $t(6)=3.88$, $p=0.008$, paired t-test). Bilateral inactivation of ventral mPFC significantly decreased total number of reinstatement nose pokes (**Figure 3I**; $t(6)=3.05$, $p=0.023$, paired t-test) relative to aCSF treatment. There was also a decrease in number of time-out nose pokes (**Figure 3J**; $t(6)=2.57$, $p=0.042$; paired t-test) and number of initiated trials (**Figure 3K**; $t(6)=3.71$, $p=0.010$). There were no significant effects of bilateral inactivation of dorsal mPFC on nose pokes or well entries (**Figure 3D-G**; all $p>0.05$, paired t-test). There were also no significant effects of either dorsal or ventral mPFC inactivation on inactive nose pokes (all $p>0.05$, paired t-test). Of note the effects on ventral mPFC inactivation observed here were directionally consistent with those observed during reinstatement in our first test group (see Methods). Although the effects in that group were milder and not significant (likely due to 8 prior cannula infusions), the directional consistency across study groups combined with the significant effects observed here strongly supports the reliability of these findings.

Neither dorsal or ventral mPFC inactivation affected reward seeking during progressive ratio sucrose self-administration

Rats demonstrated reliably high levels of sucrose seeking during progressive ratio as measured by nose pokes, well entries, and breakpoints (**Figure 4**). There was no effect of either dorsal or ventral mPFC inactivation on numbers of total active nose pokes, initiated trials, time-out nose pokes, well entries, breakpoint values, or inactive nose pokes (all $p>0.05$, paired t-tests).

Within-session analysis of inactivation effects

One possible outcome of inactivation may have been a transient effect during part of the session that was not overall apparent by comparing total numbers of nose pokes (e.g., effects only early or late during a session). To address this, we divided sessions into four quartiles and compared nose poking during BM vs. aCSF sessions using a repeated measures two-factor ANOVA (treatment x quartile). The results of these analyses are shown in **Figure 5** for FR1, early and late extinction, and cue-induced reinstatement. Analyses were performed for progressive ratio as well, but there were no significant effects either overall or within sessions. As expected there were overall significant main effects of treatment for dorsal mPFC inactivation during FR1 ($F(1, 76)=7.71$, $p=0.007$) and late extinction ($F(1, 28)=9.27$, $p=0.005$). Post-hoc multiple comparisons (Sidak's MCT) revealed significant differences during the second quartile during FR1 ($t=3.11$, $p=0.011$) and during the first quartile during late extinction ($t=2.97$, $p=0.024$). Despite a significant main effect of treatment after ventral mPFC inactivation during cue-induced reinstatement ($F(1, 24)=5.22$, $p=0.030$), there were no significant treatment effects in any quartile, indicating consistent small reductions throughout the entire session. There were no

effects of treatment on nose poking behavior in any of the other analyzed sessions and no interaction effects.

Baclofen/muscimol infusions into the NAc disrupted spontaneous locomotion

Because mPFC inactivation results were unexpected compared to previous studies, we verified the effect of our BM infusions by inactivating NAc during spontaneous locomotion - a reliable behavioral assay that is sensitive to BM inactivation of NAc (Fuchs et al., 2004; Stopper and Floresco, 2011). We infused BM or aCSF bilaterally in NAc (**Figure 6A**) and measured locomotor activity in 10 minute bins (**Figure 6B**). As expected, there was a statistically significant interaction between the effects of drug and time on locomotion, (**Figure 6B**; $F(5, 24) = 3.35$, $p = 0.020$; two-way ANOVA). Locomotion was initially elevated and decreased over time in aCSF-infused rats ($F(5,2)=6.99$, $p=0.005$; one-way ANOVA). BM-infused rats showed decreased locomotion during the initial stages of testing relative to aCSF and did not show a significant difference in locomotion over time ($F(5,2)=0.22$, $p=0.947$; one-way ANOVA). These results are consistent with previous findings (Fuchs et al., 2004; Stopper and Floresco, 2011), and confirmed that differences observed between our mPFC inactivation effects and those described in previous studies were not due to lack of efficacy of our BM infusions.

Baclofen/Muscimol decreased sPSCs in rat prefrontal neurons

To further validate the inhibitory influence of our BM infusions at the specific concentrations given, we measured the effects of BM application on mPFC neuronal activity *in vitro*. BM bath application significantly decreased spontaneous activity in prefrontal neurons (**Figure 6C**, n = 7 neurons from 2 rats), as demonstrated by a statistically significant suppressive effect of BM on

sPSCs (5b; $F(2,6)=5.6$, $p=0.019$; one-way ANOVA). Post hoc analyses revealed a significant decrease in number of sPSCs during BM and during washout (**Figure 6D**; $p<0.05$; Tukey's Multiple Comparison Test). These results confirm the reliably inhibitory effect on mPFC neurons of the BM cocktail concentration used in our behavioral studies.

DISCUSSION

Previous work has led to the hypothesis that dorsal and ventral mPFC play opposing roles in driving behavior, particularly in the context of action execution vs. suppression (Peters et al., 2009; Gass and Chandler, 2013; Barker et al., 2014; Gourley and Taylor, 2016; Muller Ewald and LaLumiere, 2017). The reasons for this distinction are relatively clear, as described in multiple studies referenced in detail in (Peters et al., 2009; Moorman et al., 2015; Gourley and Taylor, 2016; Muller Ewald and LaLumiere, 2017). For example, manipulation of dorsal mPFC frequently disrupts behavioral execution such as drug/reward seeking or expression of conditioned fear (McFarland et al., 2004; Ishikawa et al., 2008b; Sierra-Mercado et al., 2011; Eddy et al., 2016; Trask et al., 2017). In contrast, ventral mPFC manipulation has been shown to regulate behavioral inhibition in certain circumstances, such as during extinction (Ishikawa et al., 2008b; Peters et al., 2008; Sierra-Mercado et al., 2011; Peters and De Vries, 2013; Augur et al., 2016; Muller Ewald and LaLumiere, 2017). However, a number of studies have called the ubiquity of this dichotomy into question (McFarland et al., 2003; Jonkman et al., 2009; Bossert et al., 2011; Chen et al., 2013; Willcocks and McNally, 2013; Martin-Garcia et al., 2014; Moorman and Aston-Jones, 2015; Moorman et al., 2015; Gutman et al., 2016; McGlinchey et al., 2016), prompting us to perform the experiments described here.

Our results do not support a clear dichotomy for dorsal vs. ventral mPFC during natural reward seeking. Based on the studies described above, we expected that inactivation of dorsal mPFC would decrease sucrose seeking and have no effect on extinction, and that ventral mPFC inactivation would increase sucrose seeking and induce cue-induced reinstatement during extinction. Instead, dorsal mPFC inactivation increased sucrose seeking during FR1 self-administration and had no effect during cue-induced reinstatement. Ventral mPFC inactivation decreased sucrose seeking during cue-induced reinstatement and had no effect during FR1. Inactivation of both subregions decreased responding during late extinction, as shown by significantly reduced nose pokes and well-entries after dorsal mPFC inactivation and significantly reduced well entries after ventral mPFC inactivation. Inhibition of neither region influenced reward seeking under a progressive ratio schedule, again in line with a lack of general regulation of action execution or suppression. Together these results make a strong case against a universal dichotomous role for dorsal vs. ventral mPFC in action execution vs. inhibition.

Because our results were somewhat surprising, we performed controls to verify that our inactivations were effective. NAc inactivation with BM decreased spontaneous locomotion, in line with previous work (Fuchs et al., 2004; Stopper and Floresco, 2011), and bath application of BM inhibited spontaneous activity in rat mPFC neurons. Both findings support the efficacy of our BM treatments. We conclude that the effects observed did in fact result from mPFC inactivation during behavior.

413 The absence of absolute differences is in line with some previous work examining dorsal vs.
414 ventral mPFC in execution vs. suppression of reward seeking, as described above. However, in
415 many of these studies, the tasks employed used slightly more complex rules to guide behavior
416 such as the use of a discriminative stimulus (Ishikawa et al., 2008b; Moorman and Aston-Jones,
417 2015; Gutman et al., 2016). The goal of this study was to attempt to isolate self-initiated action
418 execution or inhibition to identify mPFC subregion contributions, in line with those seen in
419 studies of drug seeking. If, in fact, dorsal and ventral mPFC play opposing roles in the
420 regulation of action execution and inhibition, this should have been clearly demonstrable under
421 the behavioral conditions in the current study. Instead, our data argue for an influence of
422 context, in this case the behavioral task performed, on mPFC regulation of behavior, as reported
423 previously (Moorman and Aston-Jones, 2015; McGlinchey et al., 2016). Similarly complex
424 results have been observed in Pavlovian contexts (Sangha et al., 2014; Mendoza et al., 2015).
425
426 An additional finding was an overall lack of effect of unilateral inactivation on sucrose seeking.
427 Previous studies have shown differential contributions of left vs. right mPFC in stress-related
428 paradigms (Sullivan and Gratton, 2002a), leading us to consider the possibility that left or right
429 mPFC may play a disproportionate role in reward seeking. Although the only significant effect
430 during FR1 was seen after bilateral dorsal mPFC inactivation, right hemisphere dorsal mPFC
431 inhibition produced qualitatively similar results in some cases, though the effects were not
432 significant in planned comparisons. Accordingly, we did not pursue unilateral inactivations in
433 cue-induced reinstatement or progressive ratio. Despite our overall lack of lateralization
434 findings, a study more directly designed to explore this question may be worth undertaking in
435 future work.

436

437 One possible distinction between our results and some previous studies is the type of behavior
438 used to evaluate mPFC control. It might not be surprising that studies using different behaviors
439 may result in different effects of mPFC inactivation. This is most obvious for fear conditioning
440 studies, where the behavioral readout is actually freezing – a combination of both an emitted
441 behavior (based on a decision to freeze) and a lack of action (freezing), in some cases combined
442 with a suppression of food self-administration (Sierra-Mercado et al., 2011; Giustino and Maren,
443 2015). A more subtle distinction is between the use of nose poke operanda, as employed here
444 and in some studies (Willcocks and McNally, 2013), and the use of lever presses in other
445 previous studies (Ishikawa et al., 2008b; Peters et al., 2008). Although this may not be a critical
446 determinant, there are differential learning rates between nose poke and lever presses (Schindler
447 et al., 1993), and different neural substrates underlying the two behaviors (Bassareo et al., 2015).
448 This influence of action type on mPFC contributions to behavior is currently under investigation
449 in our laboratory.

450

451 The most salient differences exist between our findings and previous studies of cocaine self-
452 administration, extinction, and reinstatement. Multiple studies have shown a prominent role for
453 dorsal mPFC in driving cue-induced reinstatement of cocaine seeking as well as a critical role for
454 ventral mPFC suppressing cocaine seeking after extinction learning (McFarland and Kalivas,
455 2001; McLaughlin and See, 2003; Fuchs et al., 2005; LaLumiere and Kalivas, 2008; Peters et al.,
456 2008; Peters et al., 2009; Gass and Chandler, 2013; Moorman et al., 2015; Gourley and Taylor,
457 2016; Muller Ewald and LaLumiere, 2017), though see counterexamples such as (Chen et al.,
458 2013) and others described in (Moorman et al., 2015). A fundamental and yet-unanswered

question is why these reliable roles for dorsal and ventral mPFC in regulation of cocaine-associated actions are not observed in sucrose seeking, as described here, or in other types of reward seeking (McFarland and Kalivas, 2001; McGlinchey et al., 2016; Gutman et al., 2017). One possibility might be the nature of the reinforcer. Cocaine may be a more salient reinforcer than sucrose, thereby differentially engaging mPFC subregions based on some motivational intensity gradient, though see (Lenoir et al., 2007). Another possible explanation is that repeated cocaine induces neuroplastic changes in the mPFC that results in differential regulation of seeking behavior relative to natural rewards (Robinson and Kolb, 1999; Robinson et al., 2001; McFarland et al., 2003; Munoz-Cuevas et al., 2013; Radley et al., 2015; Siemsen et al., 2019). Cocaine also induces both appetitive and aversive behaviors (Ettenberg, 2004), whereas there are fewer aversive components to sucrose. mPFC subregions may regulate behaviors associated with a mixed-valence pharmacological stimulus differently than a purely appetitive reinforcer. Another potential explanation may be the way that reward is delivered: cocaine is typically self-administered intravenously whereas sucrose must be collected following a correct operant response. These and other potential explanations are currently under investigation in our laboratory, motivated by the very clear differences in mPFC contributions to ostensibly the same behavior related to different outcomes.

Rodent mPFC subregions play a host of functions instead of or in addition to action expression vs. inhibition (Dalley et al., 2004; Kesner and Churchwell, 2011; Euston et al., 2012; Cassaday et al., 2014). In some cases, dorsal and ventral mPFC functions have been shown to be dichotomous. For example, when comparing goal-directed (outcome sensitive) vs. habitual (outcome insensitive) reward seeking, there appear to be differences whereby dorsal mPFC

482 preferentially regulates goal-directed and ventral mPFC controls habitual behaviors (Killcross
483 and Coutureau, 2003; Smith et al., 2012; Barker et al., 2014; Barker et al., 2015; Smith and
484 Laiks, 2018). Because we did not explicitly test goal-directed vs. habitual behavior using, e.g.,
485 reward devaluation, we cannot make strong claims about our effects in this framework, though
486 this might be a useful avenue for future studies integrating mPFC functions across behavioral
487 paradigms.
488
489 Despite not observing clear dichotomous dorsal and ventral mPFC functions, we did see
490 selective effects of inactivation. Bilateral dorsal mPFC inactivation increased FR1 sucrose
491 seeking. This finding is aligned with those demonstrating a response-suppression role for dorsal
492 mPFC, such as is observed during punishment-associated cocaine seeking (Chen et al., 2013). It
493 is also in line with previous work demonstrating increased operant behavior following dorsal
494 mPFC inactivation (Jonkman et al., 2009) and other studies showing dorsal mPFC involvement
495 in response inhibition in other tasks (Narayanan et al., 2006; Ragozzino, 2007; MacLeod and
496 Bucci, 2010; Bari and Robbins, 2013; Meyer and Bucci, 2014; Hardung et al., 2017). Although
497 in our study there was no need for dorsal mPFC to suppress behavior, reward-associated
498 decisions, even without challenges such as punishment, may require balance between response
499 inhibition driven by the effort associated with reward seeking vs. the excitatory drive to acquire a
500 reward. Here dorsal mPFC inactivation increased both rewarded and non-rewarded nose pokes.
501 On the one hand, this suggests that dorsal mPFC inactivation resulted in a general release on any
502 inhibition of behavior, or "taking the brakes off." However, it is worth noting that these
503 increases were not seen for inactive nose pokes, during other non-rewarded tasks (extinction,
504 reinstatement), or even during progressive ratio testing, in which rewards were available. In fact,

505 dorsal mPFC inactivation decreased nose pokes in late extinction, when reward was not
506 available. These results underscore the fact that behavioral context and task details influence
507 contributions of mPFC to behavior – in some cases dorsal mPFC plays a response-invigorating
508 role whereas in others it is suppressive.
509
510 Similarly, ventral mPFC is frequently associated with behavior suppression, particularly during
511 extinction (Maren and Quirk, 2004; Peters et al., 2009; Sierra-Mercado et al., 2011; Gourley and
512 Taylor, 2016; Muller Ewald and LaLumiere, 2017). In our study, ventral mPFC inactivation
513 decreased cue-induced reinstatement, in line with previous studies of reinstatement for heroin
514 (Rogers et al., 2008; Bossert et al., 2011; Bossert et al., 2012) and methamphetamine (Rocha and
515 Kalivas, 2010) seeking, but in contrast with previous studies of cocaine seeking and fear
516 conditioning (LaLumiere and Kalivas, 2008; Peters et al., 2008; Muller Ewald and LaLumiere,
517 2017). Ventral mPFC inactivation also had little inhibitory effect on alcohol seeking and did not
518 counteract extinction (Willcocks and McNally, 2013). It is unclear what differentiates ventral
519 mPFC contributions to sucrose, alcohol, methamphetamine, and heroin reinstatement vs.
520 extinction of cocaine and fear conditioning, though there are obviously substantial differences in
521 neural encoding of different drugs/rewards/punishment, type of reinstatement (e.g., cue vs.
522 context), or other as-yet undefined factors (Badiani et al., 2011; Peters et al., 2013).
523
524 In summary, our results make it clear that dorsal and ventral mPFC do not universally exhibit
525 opposing control over behavior. Instead our findings should be integrated with previous work in
526 which dichotomies were observed, along with other studies involving, e.g., response inhibition,
527 in order to identify how different behavioral tasks differentially engage mPFC subregions. We

also note that an emphasis on neuronal ensembles and networks should be emphasized in future work (Gabbott et al., 2005; Bossert et al., 2011; Moorman et al., 2015; Pfarr et al., 2015; Warren et al., 2016; George and Hope, 2017; Kim et al., 2017). It is possible that different findings across studies may result from differentially targeting subregional circuits (e.g., mPFC projections to NAc core vs. shell, or amygdala). The use of circuit specific techniques and other precision-enhancing technologies, combined with a rigorous assessment of behavioral details, has the potential to significantly advance our understanding of mPFC function, including its contributions to complex behavior and mental diseases.

REFERENCES

Augur IF, Wyckoff AR, Aston-Jones G, Kalivas PW, Peters J (2016) Chemogenetic Activation of an Extinction Neural Circuit Reduces Cue-Induced Reinstatement of Cocaine Seeking. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:10174-10180.

Badiani A, Belin D, Epstein D, Calu D, Shaham Y (2011) Opiate versus psychostimulant addiction: the differences do matter. Nature reviews Neuroscience 12:685-700.

Bari A, Robbins TW (2013) Inhibition and impulsivity: behavioral and neural basis of response control. Progress in neurobiology 108:44-79.

Barker JM, Taylor JR, Chandler LJ (2014) A unifying model of the role of the infralimbic cortex in extinction and habits. Learning & memory (Cold Spring Harbor, NY) 21:441-448.

Barker JM, Corbit LH, Robinson DL, Gremel CM, Gonzales RA, Chandler LJ (2015) Corticostriatal circuitry and habitual ethanol seeking. Alcohol 49:817-824.

Bassareo V, Cucca F, Frau R, Di Chiara G (2015) Differential activation of accumbens shell and core dopamine by sucrose reinforcement with nose poking and with lever pressing. Behavioural brain research 294:215-223.

Bossert JM, Stern AL, Theberge FR, Cifani C, Koya E, Hope BT, Shaham Y (2011) Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nature neuroscience 14:420-422.

Bossert JM, Stern AL, Theberge FR, Marchant NJ, Wang HL, Morales M, Shaham Y (2012) Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:4982-4991.

Burgos-Robles A, Bravo-Rivera H, Quirk GJ (2013) Prelimbic and Infralimbic Neurons Signal Distinct Aspects of Appetitive Instrumental Behavior. PloS one 8:1-7.

Cassaday HJ, Nelson AJ, Pezze MA (2014) From attention to memory along the dorsal-ventral axis of the medial prefrontal cortex: some methodological considerations. Frontiers in systems neuroscience 8:160.

Chen BT, Yau H-J, Hatch C, Kusumoto-Yoshida I, Cho SL, Hopf FW, Bonci A (2013) Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. Nature 496:359-362.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neuroscience and biobehavioral reviews 28:771-784.

574 Eddy MC, Todd TP, Bouton ME, Green JT (2016) Medial prefrontal cortex involvement in the
575 expression of extinction and ABA renewal of instrumental behavior for a food reinforcer.
576 Neurobiology of learning and memory 128:33-39.

577 Eichenbaum H (2017) Prefrontal–hippocampal interactions in episodic memory. Nature Reviews
578 Neuroscience 18:547-558.

579 Ettenberg A (2004) Opponent process properties of self-administered cocaine. Neuroscience
580 and biobehavioral reviews 27:721-728.

581 Euston DR, Gruber AJ, McNaughton BL (2012) The role of medial prefrontal cortex in memory
582 and decision making. Neuron 76:1057-1070.

583 Fuchs RA, Evans KA, Parker MC, See RE (2004) Differential involvement of the core and shell
584 subregions of the nucleus accumbens in conditioned cue-induced reinstatement of
585 cocaine seeking in rats. Psychopharmacology 176:459-465.

586 Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the
587 dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in
588 contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology : official
589 publication of the American College of Neuropsychopharmacology 30:296-309.

590 Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ (2005) Prefrontal cortex in the rat:
591 projections to subcortical autonomic, motor, and limbic centers. The Journal of
592 comparative neurology 492:145-177.

593 Gass JT, Chandler LJ (2013) The Plasticity of Extinction: Contribution of the Prefrontal Cortex in
594 Treating Addiction through Inhibitory Learning. Front Psychiatry 4:46.

595 George O, Hope BT (2017) Cortical and amygdalar neuronal ensembles in alcohol seeking,
596 drinking and withdrawal. Neuropharmacology 122:107-114.

597 Giustino TF, Maren S (2015) The Role of the Medial Prefrontal Cortex in the Conditioning and
598 Extinction of Fear. Frontiers in behavioral neuroscience 9:298.

599 Gourley SLSL, Taylor JRJR (2016) Going and stopping: dichotomies in behavioral control by the
600 prefrontal cortex. Nature neuroscience 19:656-664.

601 Gutman AL, Ewald VA, Cosme CV, Worth WR, LaLumiere RT (2016) The infralimbic and prelimbic
602 cortices contribute to the inhibitory control of cocaine-seeking behavior during a
603 discriminative stimulus task in rats. Addiction biology.

604 Gutman AL, Nett KE, Cosme CV, Worth WR, Gupta SC, Wemmie JA, LaLumiere RT (2017)
605 Extinction of Cocaine Seeking Requires a Window of Infralimbic Pyramidal Neuron
606 Activity after Unreinforced Lever Presses. The Journal of neuroscience : the official
607 journal of the Society for Neuroscience 37:6075-6086.

608 Hardung S, Epple R, Jackel Z, Eriksson D, Uran C, Senn V, Gibor L, Yizhar O, Diester I (2017) A
609 Functional Gradient in the Rodent Prefrontal Cortex Supports Behavioral Inhibition.
610 Current biology : CB 27:549-555.

611 Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008a) Dorsomedial prefrontal cortex
612 contribution to behavioral and nucleus accumbens neuronal responses to incentive
613 cues. The Journal of neuroscience : the official journal of the Society for Neuroscience
614 28:5088-5098.

615 Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008b) Contributions of the amygdala and medial
616 prefrontal cortex to incentive cue responding. Neuroscience 155:573-584.

617 Jonkman S, Mar AC, Dickinson A, Robbins TW, Everitt BJ (2009) The rat prelimbic cortex
618 mediates inhibitory response control but not the consolidation of instrumental learning.
619 Behavioral neuroscience 123:875-885.

620 Kesner RP, Churchwell JC (2011) An analysis of rat prefrontal cortex in mediating executive
621 function. Neurobiology of learning and memory 96:417-431.

622 Killcross S, Coutureau E (2003) Coordination of actions and habits in the medial prefrontal
623 cortex of rats. Cerebral cortex 13:400-408.

624 Kim CK, Ye L, Jennings JH, Pichamoorthy N, Tang DD, Yoo AW, Ramakrishnan C, Deisseroth K
625 (2017) Molecular and Circuit-Dynamical Identification of Top-Down Neural Mechanisms
626 for Restraint of Reward Seeking. Cell 170:1013-1027 e1014.

627 Ko J (2017) Neuroanatomical Substrates of Rodent Social Behavior: The Medial Prefrontal
628 Cortex and Its Projection Patterns. Frontiers in Neural Circuits 11:1-16.

629 LaLumiere RT, Kalivas PW (2008) Glutamate release in the nucleus accumbens core is necessary
630 for heroin seeking. The Journal of neuroscience : the official journal of the Society for
631 Neuroscience 28:3170-3177.

632 Lenoir M, Serre F, Cantin L, Ahmed SH (2007) Intense sweetness surpasses cocaine reward. PloS
633 one 2:e698.

634 MacLeod JE, Bucci DJ (2010) Contributions of the subregions of the medial prefrontal cortex to
635 negative occasion setting. Behavioral neuroscience 124:321-328.

636 Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. Nature reviews Neuroscience
637 5:844-852.

638 Martin-Garcia E, Courtin J, Renault P, Fiancette JF, Wurtz H, Simonnet A, Levet F, Herry C,
639 Deroche-Gamonet V (2014) Frequency of cocaine self-administration influences drug
640 seeking in the rat: optogenetic evidence for a role of the prelimbic cortex.

Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 39:2317-2330.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:8655-8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:3531-3537.

McFarland K, Davidge SB, Lapish CC, Kalivas PW (2004) Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:1551-1560.

McGlinchey EM, James MH, Mahler SV, Pantazis C, Aston-Jones G (2016) Prelimbic to Accumbens Core Pathway Is Recruited in a Dopamine-Dependent Manner to Drive Cued Reinstatement of Cocaine Seeking. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:8700-8711.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 168:57-65.

Mendoza J, Sanio C, Chaudhri N (2015) Inactivating the infralimbic but not prelimbic medial prefrontal cortex facilitates the extinction of appetitive Pavlovian conditioning in Long-Evans rats. Neurobiology of learning and memory 118:198-208.

Meyer HC, Bucci DJ (2014) The contribution of medial prefrontal cortical regions to conditioned inhibition. Behavioral neuroscience 128:644-653.

Moorman DE, Aston-Jones G (2015) Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. Proc Natl Acad Sci U S A 112:9472-9477.

Moorman DE, James MH, McGlinchey EM, Aston-Jones G (2015) Differential roles of medial prefrontal subregions in the regulation of drug seeking. Brain research 1628:130-146.

Muller Ewald VA, LaLumiere RT (2017) Neural systems mediating the inhibition of cocaine-seeking behaviors. Pharmacol Biochem Behav.

Munoz-Cuevas FJ, Athilingam J, Piscopo D, Wilbrecht L (2013) Cocaine-induced structural plasticity in frontal cortex correlates with conditioned place preference. Nature neuroscience 16:1367-1369.

675 Narayanan NS, Horst NK, Laubach M (2006) Reversible inactivations of rat medial prefrontal
676 cortex impair the ability to wait for a stimulus. Neuroscience 139:865-876.

677 Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates, 6th Edition. Amsterdam ;
678 Boston ;: Academic Press/Elsevier.

679 Peters J, De Vries TJ (2013) D-cycloserine administered directly to infralimbic medial prefrontal
680 cortex enhances extinction memory in sucrose-seeking animals. Neuroscience 230:24-
681 30.

682 Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for
683 inhibiting cocaine seeking in extinguished rats. The Journal of neuroscience : the official
684 journal of the Society for Neuroscience 28:6046-6053.

685 Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in
686 prefrontal cortex. Learn Mem 16:279-288.

687 Peters J, Pattij T, De Vries TJ (2013) Targeting cocaine versus heroin memories: divergent roles
688 within ventromedial prefrontal cortex. Trends Pharmacol Sci 34:689-695.

689 Pfarr S, Meinhardt MW, Klee ML, Hansson AC, Vengeliene V, Schonig K, Bartsch D, Hope BT,
690 Spanagel R, Sommer WH (2015) Losing Control: Excessive Alcohol Seeking after Selective
691 Inactivation of Cue-Responsive Neurons in the Infralimbic Cortex. The Journal of
692 neuroscience : the official journal of the Society for Neuroscience 35:10750-10761.

693 Radley JJ, Anderson RM, Cosme CV, Glanz RM, Miller MC, Romig-Martin SA, LaLumiere RT
694 (2015) The Contingency of Cocaine Administration Accounts for Structural and
695 Functional Medial Prefrontal Deficits and Increased Adrenocortical Activation. The
696 Journal of neuroscience : the official journal of the Society for Neuroscience 35:11897-
697 11910.

698 Ragozzino ME (2007) The contribution of the medial prefrontal cortex, orbitofrontal cortex, and
699 dorsomedial striatum to behavioral flexibility. Annals of the New York Academy of
700 Sciences 1121:355-375.

701 Rhodes SE, Killcross S (2004) Lesions of rat infralimbic cortex enhance recovery and
702 reinstatement of an appetitive Pavlovian response. Learn Mem 11:611-616.

703 Rhodes SEV, Killcross AS (2007) Lesions of rat infralimbic cortex enhance renewal of
704 extinguished appetitive Pavlovian responding. European Journal of Neuroscience
705 25:2498-2503.

706 Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration
707 studies in rats: a method to evaluate reinforcing efficacy. Journal of neuroscience
708 methods 66:1-11.

709 Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in
710 the nucleus accumbens and prefrontal cortex following repeated treatment with
711 amphetamine or cocaine. The European journal of neuroscience 11:1598-1604.

712 Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the
713 morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex.
714 Synapse 39:257-266.

715 Rocha A, Kalivas PW (2010) Role of the prefrontal cortex and nucleus accumbens in reinstating
716 methamphetamine seeking. The European journal of neuroscience 31:903-909.

717 Rogers JL, Ghee S, See RE (2008) The neural circuitry underlying reinstatement of heroin-
718 seeking behavior in an animal model of relapse. Neuroscience 151:579-588.

719 Sangha S, Robinson PD, Greba Q, Davies DA, Howland JG (2014) Alterations in reward, fear and
720 safety cue discrimination after inactivation of the rat prelimbic and infralimbic cortices.
721 Neuropsychopharmacology : official publication of the American College of
722 Neuropsychopharmacology 39:2405-2413.

723 Schindler CW, Thorndike EB, Goldberg SR (1993) Acquisition of a Nose-Poke Response in Rats as
724 an Operant. B Psychonomic Soc 31:291-294.

725 Siemsen BM, Giannotti G, McFaddin JA, Scofield MD, McGinty JF (2019) Biphasic effect of
726 abstinence duration following cocaine self-administration on spine morphology and
727 plasticity-related proteins in prelimbic cortical neurons projecting to the nucleus
728 accumbens core. Brain structure & function 224:741-758.

729 Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011) Dissociable roles of prelimbic and
730 infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression
731 and extinction of conditioned fear. Neuropsychopharmacology : official publication of
732 the American College of Neuropsychopharmacology 36:529-538.

733 Smith KS, Graybiel AM (2013) A Dual Operator View of Habitual Behavior Reflecting Cortical and
734 Striatal Dynamics. Neuron.

735 Smith KS, Virkud A, Deisseroth K, Graybiel AM (2012) Reversible online control of habitual
736 behavior by optogenetic perturbation of medial prefrontal cortex. Proceedings of the
737 National Academy of Sciences of the United States of America 109:18932-18937.

738 Smith RJ, Laiks LS (2018) Behavioral and neural mechanisms underlying habitual and compulsive
739 drug seeking. Prog Neuropsychopharmacol Biol Psychiatry 87:11-21.

740 Stopper CM, Floresco SB (2011) Contributions of the nucleus accumbens and its subregions to
741 different aspects of risk-based decision making. Cogn Affect Behav Neurosci 11:97-112.

742 Sullivan RM, Gratton A (2002a) Prefrontal cortical regulation of hypothalamic-pituitary-adrenal
743 function in the rat and implications for psychopathology: side matters.
744 Psychoneuroendocrinology 27:99-114.

745 Sullivan RM, Gratton A (2002b) Behavioral effects of excitotoxic lesions of ventral medial
746 prefrontal cortex in the rat are hemisphere-dependent. Brain research 927:69-79.

747 Trask S, Shipman ML, Green JT, Bouton ME (2017) Inactivation of the Prelimbic Cortex
748 Attenuates Context-Dependent Operant Responding. The Journal of neuroscience : the
749 official journal of the Society for Neuroscience 37:2317-2324.

750 Vertes RP (2006) Interactions among the medial prefrontal cortex, hippocampus and midline
751 thalamus in emotional and cognitive processing in the rat. Neuroscience 142:1-20.

752 Warren BL, Mendoza MP, Cruz FC, Leao RM, Caprioli D, Rubio FJ, Whitaker LR, McPherson KB,
753 Bossert JM, Shaham Y, Hope BT (2016) Distinct Fos-Expressing Neuronal Ensembles in
754 the Ventromedial Prefrontal Cortex Mediate Food Reward and Extinction Memories.
755 The Journal of neuroscience : the official journal of the Society for Neuroscience
756 36:6691-6703.

757 Willcocks AL, McNally GP (2013) The role of medial prefrontal cortex in extinction and
758 reinstatement of alcohol-seeking in rats. The European journal of neuroscience 37:259-
759 268.
760

761

762 **FIGURE LEGENDS**

763

764 **Figure 1**. Cannula placements, test design, and FR1 data. (A) Cannula placements for FR1
765 cohort. Dorsal mPFC cannula placements (triangles) and ventral mPFC cannula placements
766 (circles). Numbers are A/P distance from bregma. (B) Histology of coronal slices stained with
767 neutral red showing cannula tracks for dorsal (top) and ventral (bottom) mPFC. (C) Timeline for
768 FR1 testing. Rats were retrained for 3 to 10 days after surgery. They then received sham
769 infusions followed by 8 days of FR1 tests. Rats received one of four infusions every other day of
770 testing: bilateral inactivation, bilateral aCSF, unilateral left, or right inactivation,
771 counterbalanced across rats. All rats received all four conditions. aCSF (stripes) = control
772 infusion, BI (solid) = bilateral inactivation, LI (dots) = inactivation of left hemisphere, RI
773 (checkers) = inactivation of right hemisphere. (**D, F**) total number of nose pokes, time-out nose
774 pokes, and initiated trials. (**E, G**) total number of well entries, non-rewarded well entries, and
775 rewarded well entries. (D, E) There was a significant increase in total number of nose pokes and
776 total number of rewarded well entries when the dorsal mPFC was bilaterally inactivated (*). (F,
777 G) Ventral mPFC inactivation did not affect nose poking or well entries. *$p<0.05$, Dunnett's test
778 for planned multiple comparison.

779

780 **Figure 2**. Cannula placements, test design, and extinction data for extinction cohort. (A) Dorsal
781 mPFC cannula placements (triangles) and ventral mPFC cannula placements (circles). (B)
782 Timeline for extinction task. Extinction rats were trained on FR1 but only received bilateral
783 infusions during early and late extinction. (C, G) There was a significant decrease in number of
784 nose pokes between last day of FR1 and aCSF treatment during extinction (#). (C, D; G, H)

Bilateral inactivation of dorsal or ventral mPFC did not significantly affect nose pokes or well entries during early extinction. (E, F) Inactivation of dorsal mPFC during late extinction decreased nose pokes and well entries (*). (I) There was no effect of ventral mPFC inactivation for number of nose pokes during late extinction. (J) However, there was a decrease in number of well entries during ventral mPFC inactivation during late extinction (*). # and *p<0.05, paired t-test.

Figure 3. Cannula placements, test design, and reinstatement data for reinstatement cohort. (A) Dorsal mPFC cannula placements (triangles) and ventral mPFC cannula placements (circles). (B) Timeline for reinstatement task. Reinstatement rats were trained on FR1 and extinction but only received bilateral infusion during reinstatement. (C, H) Number of nose pokes during FR1 session the day before extinction training. (D, I) There was a significant increase in nose pokes on aCSF reinstatement infusion day compared to last day of extinction (#). (D-G) Bilateral inactivation of dorsal mPFC did not significantly affect nose pokes, time-out nose pokes, initiated trials, or well entries. (I-L) Bilateral ventral mPFC inactivation significantly decreased total number of nose pokes, time out nose pokes, and initiated trials (*), but not rewarded well entries. # and *p<0.05, paired t-test.

Figure 4. Progressive ratio data. No significant effects of dorsal mPFC (A-C) or ventral mPFC (D-F) inactivation on nose pokes, well entries, or break point.

Figure 5. Average number of nose pokes per quartile for FR1, early extinction, late extinction, cue-induced reinstatement, and progressive ratio. Dorsal mPFC inactivation increased FR1 nose

808 pokes, notably in the first half of the session. Dorsal mPFC inactivation decreased late
809 extinction nose pokes, primarily early in the session. Ventral mPFC inactivation decreased cue-
810 induced reinstatement nose pokes, but the effect was distributed across the session. *p<0.05,
811 **p<0.01, two factor ANOVA (treatment x quartile); #=p<0.05, Sidak's MCT.
812
813 **Figure 6**. Behavioral and physiological verification of BM efficacy. BM infusion in NAc
814 disrupted spontaneous locomotion, and in vitro BM infusion decreased sPSCs in mPFC neurons.
815 (A) Cannula placements for locomotion study. (B) aCSF-infused rats decreased locomotion over
816 time, but this effect was not observed for rats receiving BM infusions *p<0.05, RM ANOVA.
817 (C) sPSCs of one representative neuron. (D) Mean sPSC frequency before BM, after BM, and
818 after washout. *p<0.05, Tukey's Multiple Comparison Test. (E) Example recorded rat mPFC
819 neuron stained with Alexa Fluor 488.
820

Figure 1

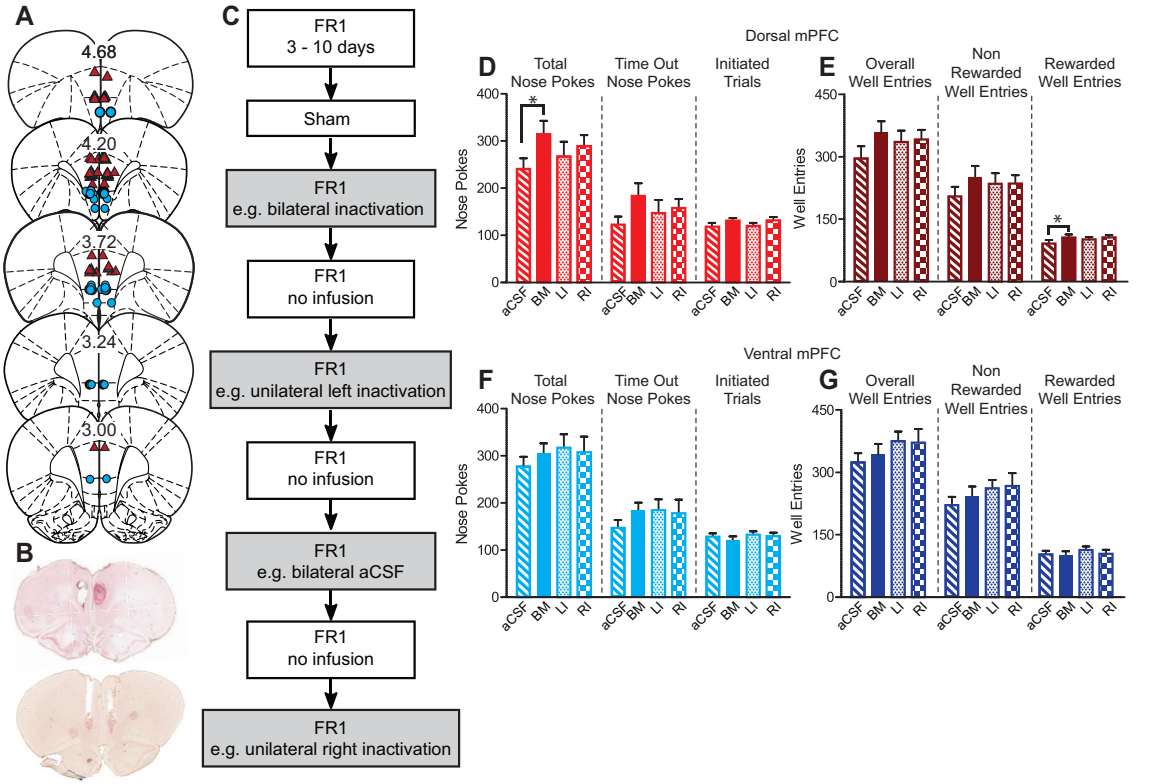
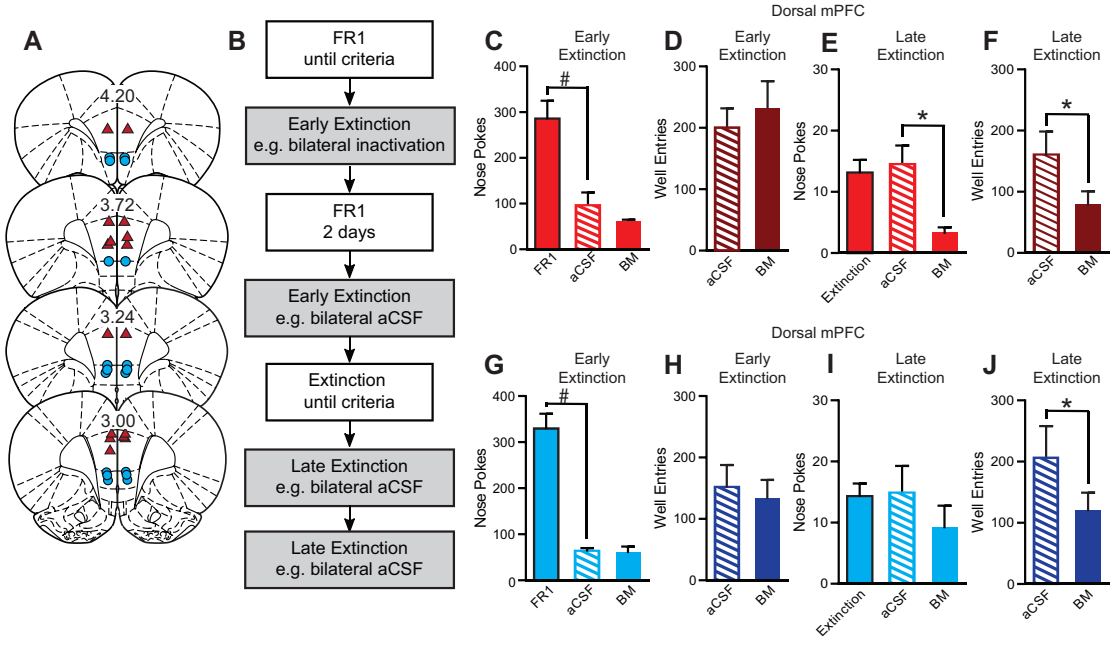


Figure 2



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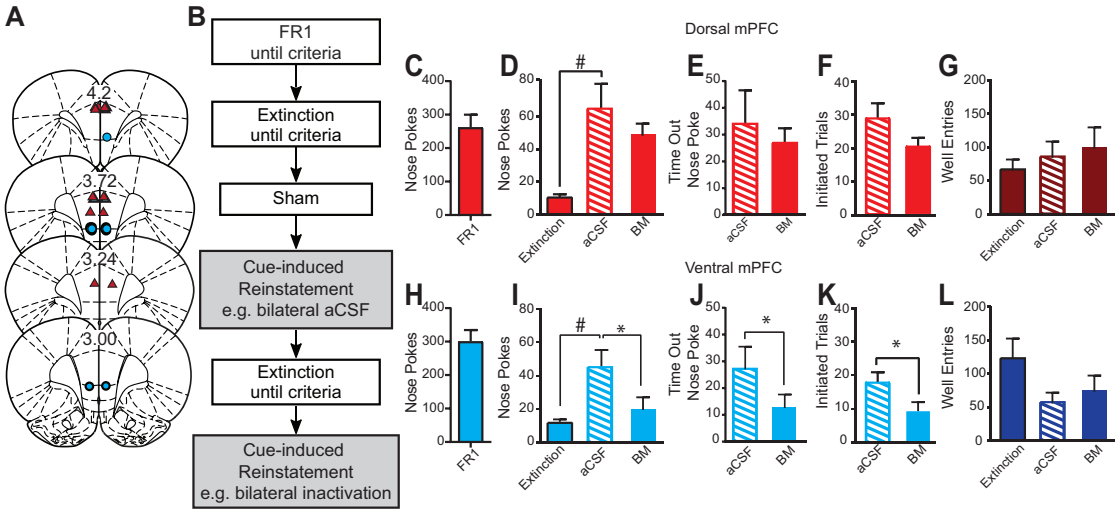

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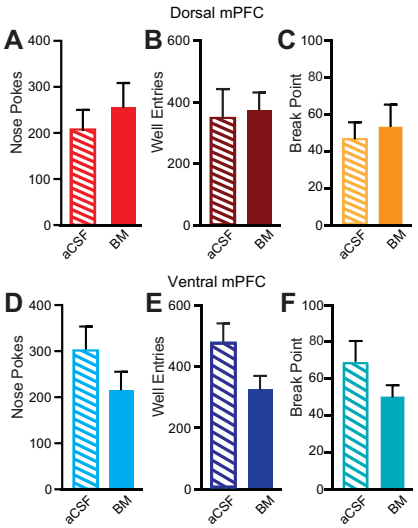

Figure 5

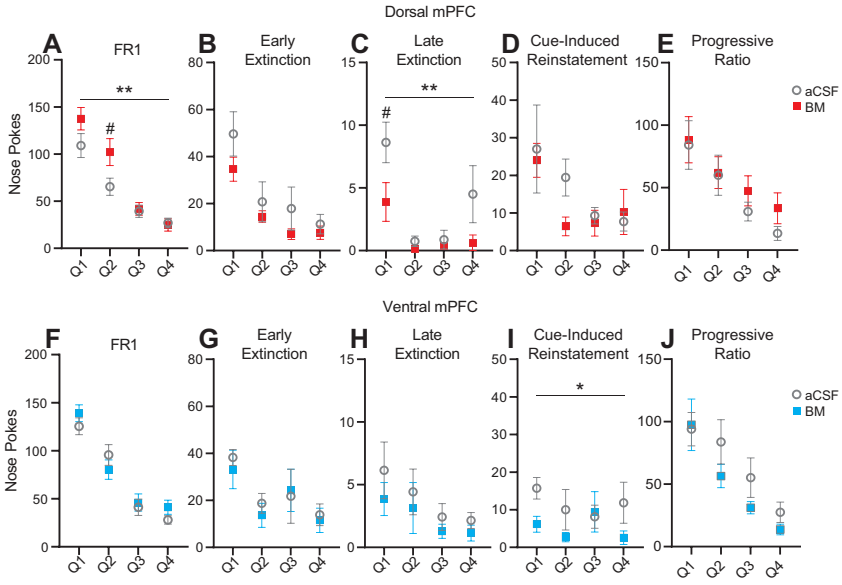

Figure 6