Extinction of Cocaine Memory Depends on a Feed-Forward Inhibition Circuit Within the Medial Prefrontal Cortex

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ABSTRACT

BACKGROUND: Cocaine-associated environments (i.e., contexts) evoke persistent memories of cocaine reward and thereby contribute to the maintenance of addictive behavior in cocaine users. From a therapeutic perspective, enhancing inhibitory control over cocaine-conditioned responses is of pivotal importance but requires a more detailed understanding of the neural circuitry that can suppress context-evoked cocaine memories, e.g., through extinction learning. The ventral medial prefrontal cortex (vmPFC) and dorsal medial prefrontal cortex (dmPFC) are thought to bidirectionally regulate responding to cocaine cues through their projections to other brain regions. However, whether these mPFC subregions interact to enable adaptive responding to cocaine-associated contextual stimuli has remained elusive.

METHODS: We used antero- and retrograde tracing combined with chemogenetic intervention to examine the role of vmPFC-to-dmPFC projections in extinction of cocaine-induced place preference in mice. In addition, electrophysiological recordings and optogenetics were used to determine whether parvalbumin-expressing inhibitory interneurons and pyramidal neurons in the dmPFC are innervated by vmPFC projections.

RESULTS: We found that vmPFC-to-dmPFC projecting neurons are activated during unreinforced re-exposure to a cocaine-associated context, and selective suppression of these cells impairs extinction learning. Parvalbumin-expressing inhibitory interneurons in the dmPFC receive stronger monosynaptic excitatory input from vmPFC projections than local dmPFC pyramidal neurons, consequently resulting in disynaptic inhibition of pyramidal neurons. In line with this, we show that chemogenetic suppression of dmPFC parvalbumin-expressing inhibitory interneurons impairs extinction learning.

CONCLUSIONS: Our data reveal that vmPFC projections mediate extinction of a cocaine-associated contextual memory through recruitment of feed-forward inhibition in the dmPFC, thereby providing a novel neuronal substrate that promotes extinction-induced inhibitory control.

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in responding to cocaine but have thus far been considered to function as separate hubs in parallel circuits that regulate this behavior. Recent studies point to reciprocal connectivity between the vmPFC and the dmPFC (14–17). However, how this intrinsic mPFC connectivity contributes to adaptive responding to cocaine-associated stimuli has remained poorly understood.

To this end, we anatomically and physiologically dissected vmPFC-to-dmPFC projections and investigated whether this circuit modulates extinction of responding to a cocaine-associated context using CPP. We found that vmPFC projections innervate parvalbumin-expressing interneurons (PV-INs) in the dmPFC, resulting in disynaptic inhibition of dmPFC pyramidal neurons (PNEs). Furthermore, vmPFC-to-dmPFC neurons were preferentially activated during unreinforced re-exposure to a cocaine-associated context, and selective suppression of vmPFC-to-dmPFC projecting neurons or direct suppression of dmPFC PV-INs impaired extinction of cocaine place preference memory, confirming the critical contribution of this circuit to extinction learning.

METHODS AND MATERIALS

Animals

Male wild-type C57BL/6J and transgenic PV::Cre mice (The Jackson Laboratory, stock no. 017320, maintained on a C57BL/6J background) aged 6–8 weeks at the start of experiments were individually housed. Mice were kept on a 12-hour light/dark cycle with regular laboratory chow food and water available ad libitum. Behavioral experiments were conducted during the animals’ light phase. All experimental procedures were approved by the Central Committee for Animal Experiments (Centrale Commissie Dierproeven) of The Netherlands and the Animal Ethical Care Committee (instantie voor Dierwelzijn) of the Vrije Universiteit Amsterdam.

Adeno-associated Virus Vectors and Stereotactic Microinjections

AAV-CaMKII:Chr2R(H134R)-EYFP (titer: 4.0 × 10^13), AAV-hSyn::DIO-mCherry and AAV-hSyn::DIO-hM4Di-mCherry (titers: 5.0–6.0 × 10^13), AAV-CaMKII:·mCherry (titer: 3.3 × 10^13) (UNC Vector Core), and AAV-CaMKII:·hM3Dq-mCherry (titer: 4.7 × 10^12) (version 96; Zürich Viral Vector Facility) were packaged as serotype 2/5. For retrograde tracing, we used retroAAV2-hSyn::EGFP-iCre (titer: 2 × 10^15) (version 146; Zürich Viral Vector Facility) and retro-AAV2-hSyn::Cre (titer: 1.3 × 10^15) [Janelia Research Campus (18)]. For stereotaxic microinjection of adeno-associated viruses, mice received 0.05 mg/kg Temgesic (RB Pharmaceuticals) 30 minutes before the start of surgery and were then anesthetized with isoflurane and mounted onto a stereotaxic frame. Lidocaine (2%) (Sigma-Aldrich Chemie N.V.) was topically applied to the skull before incision to provide local analgesia. Microinjection needles were used to infuse virus in the dmPFC (+1.8 mm anterior-posterior [AP]; +0.45 mm medial-lateral [ML]; −2.1 mm dorsal-ventral [DV]; relative to bregma) or vmPFC (+1.9 mm AP; −0.5 mm ML; −3.0 mm DV; relative to bregma, unless otherwise indicated) at a flow rate of 0.1 μL/min followed by an additional 5 minutes to allow diffusion, followed by stepwise retraction of the needle. For visualization of vmPFC-to-dmPFC projecting neurons, retroAAV-hSyn::EGFP-iCre (0.4 μL) was unilaterally injected in the dmPFC and AAV-hSyn::DIO-mCherry (0.4 μL) in the contralateral vmPFC. For chemogenetic suppression of vmPFC-to-dmPFC projecting neurons, retroAAV-hSyn::EGFP-iCre (0.3 μL/hemisphere) was bilaterally injected in the dmPFC and Cre-dependent AAV-hSyn::DIO-hM4Di-mCherry virus (0.3 μL/hemisphere) was bilaterally injected in the vmPFC (ML–DV angle 25°; +1.9 mm AP; ±1.37 mm ML; −3.0 mm DV; relative to bregma). For chemogenetic suppression of PV-INs, PV::Cre mice received AAV-hSyn::DIO-hM4Di-mCherry or AAV-hSyn::DIO-mCherry bilaterally in the dmPFC (0.5 μL/hemisphere). For electrophysiology recordings, PV::Cre mice received AAV-CaMKII:Chr2R(H134R)-EYFP in the vmPFC (0.4 μL/unilateral) and AAV-hSyn::DIO-hM4Di-mCherry or AAV-hSyn::DIO-mCherry in the contralateral dmPFC (0.5 μL/unilateral). Analgesia was provided 24 and 48 hours after surgery (carprofen, 5 mg/kg). Animals remained in their home cage for 3 weeks until the start of behavioral experiments or for at least 5 weeks until electrophysiological recordings were performed. Mice with virus misplacements were excluded from analysis (n = 1 cholera toxin B [CTB] tracing [Figure 1F]) (n = 1 Ext group and n = 1 No Ext group [Figure 2B–D]) (n = 2 hM4Di-mCherry group [Figure 2E, F]) (n = 3 hM3Dq-mCherry clozapine N-oxide [CNO] group [Figure S5B]).

CTB Tracing

CTB conjugated to Alexa Fluor 555 (CTB-555) (C34776) (Thermo Fisher) or CTB conjugated to Alexa Fluor 488 (CTB-488) (C34775) (Thermo Fisher) was dissolved in sterile phosphate-buffered saline (PBS) (0.33 or 1% wt/vol; because no differences were observed between the 2 concentrations, animals were pooled) and injected into the dmPFC (+1.8 mm AP; +0.45 mm ML; −2.1 mm DV) and NAc shell (+1.25 mm AP; +0.6 mm ML; −4.7 mm DV). One week later, animals were sacrificed by transcardial perfusion.

Cocaine CPP

The conditioning apparatus consisted of 2 main compartments that differed in tactile and visual cues, connected by a small center compartment (19). On day 0, baseline preference for the main compartments was assessed by allowing animals to freely explore all compartments (pretest; 10 min). The cocaine-paired and saline-paired compartment were counterbalanced within all groups, such that on average, the groups did not have a baseline preference for one of the two main compartments, thereby allowing an unbiased procedure. Conditioning sessions (15 min) were conducted twice daily over 3 consecutive days. For this, mice received saline (intraperitoneal [i.p.]; morning) or cocaine (15 mg/kg in saline; i.p.; afternoon) before being confined to one of the main compartments. After 3 weeks of forced abstinence in the home cage, animals were subjected to extinction training and/or a postconditioning test (post-test) (19). For extinction training, mice were confined to the cocaine- and saline-paired compartment (15 min each) in
the absence of cocaine or saline treatment. In a post-test, we
determined preference scores by allowing animals to freely
explore all compartments for 5 minutes (to avoid within-
session extinction) under drug-free conditions. Time spent in
each compartment was measured using a video camera and
Ethovision video-tracking software (Noldus). A preference
score for each animal was calculated as time spent in cocaine-
paired compartment minus saline-paired compartment.

Chemogenetic Intervention

CNO (HB6149 HelloBio) was dissolved in sterile saline. Mice
received an injection of 5 mg/kg (i.p.; hM4Di-mCherry
experiments) or 3 mg/kg (i.p.; hM3Dq-mCherry experiment)
CNO 30 minutes before an extinction session.

Immunohistochemistry

Mice were transcardially perfused using ice-cold PBS pH 7.4,
followed by ice-cold 4% paraformaldehyde in PBS pH 7.4.
Brains were removed, postfixed overnight in 4% parafor-
maldehyde solution, and then immersed in 30% sucrose in
PBS with 0.02% NaN3. Brains were then sliced in 35-μm
coronal sections using a cryostat and stored in PBS with
0.02% NaN3 at 4 °C until further use. Immunohistochemical
stainings were performed using standard procedures (19) with
the following antibodies: rabbit anti-Fos (1:500, sc52), mouse

Figure 1. Identification of vmPFC-to-dmPFC projecting neurons. (A) AAV-CaMKIIα::ChR2-EYFP was injected into the vmPFC. EYFP+ vmPFC terminals were present in the ipsi- and contralateral dmPFC. Scale bar = 250 μm. (B) After retroAAV-hSyn::Cre injection into the dmPFC and Cre-dependent AAV-
hSyn::Dio-mCherry into the contralateral vmPFC, retrogradely labeled mCherry+ cells were observed in the vmPFC. Scale bars = 100 μm. (C) Trajectory of retrogradely labeled contralateral projections (mCherry+). Axonal fibers (arrows) were present in the fmi (right top panel) and the cc (right bottom panel). Scale bars = 100 μm. (D) Example of mCherry+ axonal fibers of vmPFC neurons in the dmPFC and NAC shell. Boxed areas (top) indicate region imaged at higher magnification (bottom). Contralateral retrograde labeling n = 3; bilateral retrograde labeling n = 3. Scale bar top = 100 μm; bottom = 50 μm. (E) CTB-488 was injected into NAc shell and CTB-555 in the dmPFC (n = 5 mice). CTB-488+ and CTB-555+ neurons were observed and quantified in the vmPFC. Arrowheads indicate colocalization. Scale bar = 50 μm. (F) Colocalization of NAc shell projecting neurons within the dmPFC-projecting population (left) and vice versa (right). All graphs show mean ± SEM. ac, anterior commissure; cc, corpus callosum; CTB-488, cholera toxin subunit B conjugated to Alexa Fluor 488; CTB-
555, CTB conjugated to Alexa Fluor 555; dmPFC, dorsal medial prefrontal cortex; fmi, forceps minor; LV, lateral ventricle; ml, midline; NAC, nucleus
accumbens; vmPFC, ventral medial PFC.

A

B

C

D

E

F
vmPFC-to-dmPFC projecting neurons mediate extinction of cocaine conditioned place preference memory. (A) Experimental design of the conditioning paradigm. Red lines depict fictional trajectories of mice during tests. On day 24, animals remained in their home cage (No Ext; n = 7) or underwent extinction training (Ext; n = 7) through unreinforced re-exposure to the saline and cocaine context. On day 25, preference for the cocaine context was reduced in the Ext compared with the No Ext group (t_{12} = 2.417, p = .033). (B) Mice with retrogradely labeled vmPFC-to-dmPFC projecting neurons underwent conditioning. On day 24, animals were re-exposed to the cocaine context for 15 min in the presence (No Ext; n = 5) or absence (Ext; n = 5) of cocaine reinforcement. (C) Representative images of DAPI, Fos, and mCherry expression in the vmPFC. Scale bar = 50 μm. (D) Percentage of Fos+/mCherry+ neurons did not differ between groups. In the Ext group, Fos colocalized more with the mCherry+ than mCherry− population (population × group interaction F_{1,8} = 8.86, p = .018; post hoc Fos+/mCherry+ vs. Fos−/mCherry− Ext group t_8 = 3.12, p = .028). (E) Top: hM4Di-mCherry (n = 6) or mCherry (n = 8) was bilaterally expressed in vmPFC-to-dmPFC projecting neurons. Bottom: representative example of hM4Di-mCherry expression in vmPFC. (F) Top: experimental design. Mice received CNO before extinction training. Test: preference for the cocaine context was higher in hM4Di-mCherry mice than control mice (U = 44, p = .008). All graphs show mean ± SEM. CNO, clozapine N-oxide; coc, cocaine; CPP, conditioned place preference; dmPFC, dorsal medial prefrontal cortex; Ext, extinction training; fmi, forceps minor; sal, saline; vmPFC, ventral medial prefrontal cortex.
anti-PV (1:1000, MAB1572), mouse anti-GAD67 (1:1000, MAB5406), and NeuroTrace 500/525 green fluorescent Nissl stain or DAPI (Vectashield). For quantification experiments, 4–6 z-stacks per animal were generated using a confocal microscope (Zeiss LSM510 or Nikon Ti) with the experimenter blinded to the treatment conditions. ImageJ software was used to extract the regions of interest of the cells stained with Nissl or DAPI (Gaussian filter, Li threshold). Only regions of interest within a predefined range for size (70–2000 square units; to exclude glial cells and nonspecific staining) and circularity (0.5–1.0) were included. Because cells were frequently present in 2 or 3 subsequent images of a z-stack, MATLAB (version 8.6; The MathWorks, Inc.) was used to group the regions of interest that belonged to the same Nissl or DAPI cell and to count the total number of Nissl+ or DAPI+ cells in a stack. Cells expressing hM4Di-mCherry, mCherry, PV, GAD67, Fos, or CTB-488/555 were counted manually.

**Electrophysiological Recordings**

See the Supplement.

**Quantification and Statistical Analysis**

Statistical details are presented in the figure legends. Number of animals is shown as n and number of cells as n_{cells}. All graphs show means ± SEM. SPSS software (version 25; IBM Corp.) and Prism (GraphPad Software, Inc.) were used for statistical analysis. Comparisons between groups were made using two-tailed unpaired t tests or, in case of paired data, a two-tailed paired t test. When the data was not modeled by a normal distribution, analysis was subjected to nonparametric Mann-Whitney U test for between-group comparisons and Wilcoxon signed-rank test for within-subject comparisons. To investigate differences in activation of labeled cells between groups (group × population [Fos’/mCherry’ vs. Fos’/ mCherry’]), a repeated measures analysis of variance was conducted, followed by post hoc Bonferroni tests. Significance was set at p < .05.

**RESULTS**

**Identification of vmPFC-to-dmPFC Projecting Neurons**

We previously found that optogenetic stimulation of vmPFC PNs has no effect on expression of cocaine CPP memory during the first 2 days after conditioning but facilitates extinction 3 weeks after conditioning (19). We now aimed to identify the neuronal target of vmPFC PN projections that promote extinction learning. To anatomically trace projections of vmPFC PNs, we unilaterally expressed CaMKIIα promoter-driven ChR2 (channelrhodopsin-2) fused to EYFP in the vmPFC of mice and observed EYFP+ axonal fibers in both the ipsi- and contralateral dmPFC (Figure 1A). Layer 5/6 of the dmPFC exhibited the highest density of EYFP+ fibers. We then applied retrograde viral tracing by injecting retroAAV-hSyn::Cre in the dmPFC and Cre-dependent AAV-hSyn::DIO-mCherry in the contralateral vmPFC. This revealed a population of vmPFC-to-dmPFC projecting neurons (Figure 1B). Axonal fibers were observed in the corpus collosum, indicating that they ran via the ipsilateral forceps minor of the corpus collosum through the corpus collosum and then back through the contralateral forceps minor of the corpus collosum to terminate in the dmPFC (Figure 1C). The vmPFC heavily innervates the NAc shell (19) and projections to this region have been implicated in extinction of cocaine seeking (12,13). Therefore, we questioned whether vmPFC neurons that project to the dmPFC have collateral projections to the NAc shell. Whereas dense mCherry+ axonal fibers were observed in the dmPFC after retrograde labeling, very sparse mCherry+ fibers were observed in the NAc shell (Figure 1D), suggesting that vmPFC neurons that project to the dmPFC and NAc shell overlap to a small extent only. To confirm this, we retrogradely labeled vmPFC neurons by injection of CTB-488 or CTB-555 in the NAc shell and dmPFC, respectively (Figure 1E). We examined colocalization in the contralateral vmPFC to exclude the possibility that neurons in the ipsilateral vmPFC were labeled as a result of CTB injection in the adjacent dmPFC and/or NAc shell. Of the vmPFC-to-dmPFC projecting neurons (CTB-555+), 9.96 ± 4.8% (mean ± SEM) were also CTB-488+ (Figure 1F). Inversely, only 5.54 ± 2.1% of the vmPFC-to-NAc shell projecting neurons (CTB-488+) were CTB-555+. Hence, vmPFC neurons that project to the dmPFC and NAc shell represent largely distinct populations.

**vmPFC-to-dmPFC Projecting Neurons Mediate Extinction of Cocaine CPP Memory**

We next assessed whether vmPFC-to-dmPFC projecting cells are activated on re-exposure to a cocaine-associated context. Mice were conditioned to associate one of two distinct contexts with cocaine reward, and after 3 weeks of forced abstinence, they showed a strong preference to explore the previously cocaine-paired context over the neutral (saline-paired) context (Figure 2A). Unreinforced re-exposure to the cocaine and neutral context on the day before the test reduced preference for the cocaine context (Figure 2A) (19), pointing to successful extinction learning. Next, independent groups underwent conditioning and were re-exposed to the cocaine-associated context in the presence (no extinction) or absence (extinction) of cocaine reinforcement (Figure 2B). Ninety minutes later, animals were sacrificed to examine colocalization of the neuronal activity marker Fos and retrogradely labeled mCherry+ vmPFC-to-dmPFC projecting neurons (Figure 2C). Both groups showed a similar percentage of Fos+ and mCherry+ neurons in the vmPFC, but mCherry and Fos preferentially colocalized in mice that did not receive cocaine before the last session (Figure 2D), suggesting that the vmPFC-to-dmPFC projecting neuronal population is activated during extinction learning. Additionally, we found that Fos colocalized more with vmPFC-to-dmPFC projecting neurons after unreinforced exposure to the cocaine context compared with a novel context (Figure S1). To determine whether this projection is necessary for extinction learning, we retrogradely expressed the inhibitory DREADD (designer receptors exclusively activated by designer drug) hM4Di fused to mCherry or mCherry alone (control) in vmPFC-to-dmPFC projecting neurons (Figure 2E). One day after chemogenetic suppression during extinction training, preference for the cocaine context was diminished in control mice, whereas hM4Di mice still showed a robust preference to explore the cocaine context.
(Figure 2F). Note that the CPP score of the mCherry control group is similar to the extinction group in Figure 2A, suggesting that CNO alone did not influence extinction learning. Hence, vmPFC-to-dmPFC projecting neurons are activated on unreinforced re-exposure to a cocaine-associated context, and accordingly, are required for extinction learning.

vmPFC Projections Evoke Strong Monosynaptic Excitation of dmPFC PV-INs and Disynaptic Inhibition of PNs

Global optogenetic stimulation of the vmPFC reduces firing of dmPFC PNs (20), suggesting that vmPFC PNs may target local GABAergic (gamma-aminobutyric acidergic) interneurons. Furthermore, dmPFC PV-INs facilitate extinction of natural reward seeking (21) and conditioned fear (22). We found that PV-INs comprise the majority (~66%) of GABAergic neurons in the dmPFC and are most abundant in dmPFC layers 5/6 (Figure S2), where we also observed the highest density of vmPFC axons (Figure 1A). Based on this, we determined whether vmPFC projections inhibit dmPFC PNs via innervation of PV-INs in this region. Expression of ChR2-EYFP in vmPFC PNs revealed EYFP⁺ puncta on somata and dendrites of PV-INs in the dmPFC (Figure 3A). To determine whether these puncta reflect functional synapses and to compare the strength of innervation of dmPFC PV-INs and PNs, we used PV::Cre mice to label dmPFC PV-INs with mCherry and expressed ChR2-EYFP in vmPFC PNs (Figure 3B). PNs and PV-INs were distinguished based on mCherry expression and electrophysiological profiles (Figure S3A–I). On optic
stimulation of ChR2⁺ vmPFC terminals, we measured evoked excitatory postsynaptic currents (eEPSCs) and evoked inhibitory postsynaptic currents (eIPSCs) in dmPFC PV-INs and PNs using whole-cell electrophysiological recordings (Figure 3C, D; Figure S3J). Evoked excitatory drive was strongest onto dmPFC PV-INs (Figure 3E; Figure S3K), whereas evoked inhibitory drive was strongest onto dmPFC PNs (Figure 3F; Figure S3L). In neurons exhibiting both excitatory and inhibitory responses (12/15 PNs; 8/9 PV-INs), the eEPSC/eIPSC (E/I) ratio robustly favored excitation of dmPFC PV-INs (Figure 3G; Figure S3M). The latency to onset of eEPSCs was shorter than of eIPSCs in PNs and PV-INs (Figure 3H; Figure S3N), suggestive of monosynaptic excitation and disynaptic inhibition. In support of this, the application of the AMPA/kainate receptor antagonist CNQX abolished both eEPSCs and eIPSCs (Figure 3I, J), whereas GABA_A receptor blockade by Gabazine only affected eIPSCs (Figure 3K), demonstrating that glutamate release initiated both evoked responses. Gabazine applied together with CNQX and the NMDA receptor antagonist D-AP5 prevented the residual eEPSC (Figure 3K). In line with this, both responses were also abolished by tetrodotoxin (Figure S3O, P) and coaplication of the potassium channel blocker 4-AP (23) recovered eEPSCs only (Figure S3Q), further confirming the monosynaptic and disynaptic nature of the excitatory and inhibitory response, respectively.

**dmPFC PV-INs Mediate Feed-Forward Inhibition and Extinction of Cocaine CPP Memory**

To determine whether the disynaptic inhibitory response in PNs is evoked by excitation of PV-INs, we expressed hM4Di-mCherry in vmPFC PV-INs and ChR2-EYFP in vmPFC PNs (Figure 4A). PN responses to optic stimulation of ChR2⁺ vmPFC terminals were recorded before and after CNO-mediated suppression of PV-INs (Figure 4B, C). CNO did not alter the eEPSC amplitude (Figure 4D) but reduced the eIPSC amplitude in dmPFC PNs (Figure 4E). Consequently, the E/I ratio shifted toward less inhibition (Figure 4F), confirming that disynaptic inhibition of dmPFC PNs is at least partially mediated by excitation of PV-INs. CNO similarly suppressed disynaptic inhibition of PV-INs (Figure S4). We next determined whether activity of dmPFC PV-INs is necessary to extinguish preference to explore a cocaine-associated context. In PFC::Cre mice, hM4Di-mCherry or mCherry alone (control) was expressed in the majority of dmPFC PV-INs (Figure 4G–I). Following chemogenetic suppression of PV-INs during extinction training, hM4Di animals showed stronger preference for the cocaine context than control mice (Figure 4J), confirming that extinction learning requires activity of these neurons.

Finally, we determined whether chemogenetic stimulation of dmPFC PNs also impairs extinction of cocaine place preference. For this, we bilaterally expressed a CaM1K1/α promoter-driven excitatory DREADD hM3Di fused to mCherry or mCherry alone in dmPFC PNs (Figure S5A). One day after CNO or saline treatment during extinction training, we did not observe a difference in preference scores between groups, and all groups showed a similar low preference for the cocaine context (Figure S5B). Thus, bulk activation of dmPFC PNs does not affect extinction learning, suggesting that vmPFC-mediated recruitment of feed-forward inhibition in the dmPFC does not result in global suppression of local PNs. Moreover, because the saline control groups showed similar preference scores as the CNO-treated groups, this further confirmed that CNO alone did not affect extinction learning.

**DISCUSSION**

Our data reveal that extinction of context-evoked cocaine memory depends on activation of an intrinsic mPFC circuit (Figure 4K). We show that vmPFC projections innervate the dmPFC, and vmPFC-to-dmPFC projecting neurons are activated on unreinforced re-exposure to a cocaine-associated context. In line with this, chemogenetic suppression of vmPFC-to-dmPFC projecting neurons prevented extinction of context-evoked cocaine memory. In the dmPFC, PV-INs receive strong monosynaptic excitatory input from vmPFC terminals and subsequently inhibit PNs, typical for feed-forward inhibition (23). Similar to manipulation of vmPFC-to-dmPFC neurons, chemogenetic suppression of dmPFC PV-INs impaired extinction of context-evoked cocaine reward memory. Hence, under extinction conditions, vmPFC projections recruit feed-forward GABAergic inhibition in the dmPFC to attenuate conditioned responding to a cocaine-associated context.

Whereas previous models propose that the dmPFC and vmPFC exert control over conditioned cocaine seeking via divergent projections to other brain regions (2,5,12,13), we now demonstrate that direct connectivity between the vmPFC and dmPFC provides critical adaptive control over responding to cocaine-associated contextual cues. Our findings do not rule out the involvement of vmPFC projections to other regions, such as the NAc shell (12,13), but reveal an additional mechanism for extinction-induced behavioral inhibition. Of relevance is that we found that vmPFC-to-dmPFC projections are required for acquisition of extinction, whereas vmPFC-to-NAc shell connectivity exerts inhibitory control over cocaine seeking after extinction learning (12,13) but not during a first extinction session (24). Global manipulation of vmPFC function, however, affects both the acquisition and expression of extinguished cocaine seeking (25). Because we found that the vmPFC-to-dmPFC projecting neuronal population has little overlap with the vmPFC-to-NAc shell projecting neurons and sends only sparse collateral projections to the NAc shell, this suggests that extinction learning requires vmPFC projections to the dmPFC, whereas vmPFC projections to the NAc shell control the retention of extinguished responding to cocaine-associated cues.

Distinct coexisting neuronal ensembles within the vmPFC exert opposing effects on conditioned behavior (12,26,27). Although the selective suppression of vmPFC-to-dmPFC projecting neurons using our retrograde tracing approach is a more refined intervention method than global vmPFC manipulation, we cannot rule out that the vmPFC population that projects to the dmPFC is composed of distinct neurons that can promote and inhibit cocaine-conditioned responses. However, vmPFC-to-dmPFC neurons were not preferentially activated on reinforced re-exposure to the cocaine-associated context, suggesting that the cocaine-context association is not allocated to this neuronal population. This leaves the possibility that selective suppression of vmPFC-to-dmPFC
neurons that are activated during extinction learning could have a different effect on cocaine conditioned place preference memory. Unfortunately, it is technically not feasible to assess the effect of manipulation of the activated neuronal ensemble on extinction learning in our CPP paradigm, because it would require neuronal activity-dependent labeling and manipulation within the same extinction session.

Recruitment of feed-forward GABAergic inhibition in the dmPFC during extinction learning may result in global suppression of PN firing and a reduction of output from this region. However, we think that the cortical network effect of PV-IN activation might be more complex, potentially enabling a switch between dmPFC PNs that promote or suppress expression of cocaine memory. This hypothesis is supported by our finding that global chemogenetic stimulation of dmPFC PNs does not affect extinction learning. Furthermore, neurons within the prelimbic cortex during the initiation of reward seeking and under extinction conditions, both in a...
context-dependent manner (28). Therefore, distinct neuronal ensembles within the dmPFC may drive and inhibit cocaine-conditioned responses, similar to what has been reported for the vmPFC (12). If this is true, global chemogenetic stimulation of dmPFC PNs may have resulted in simultaneous activation of 2 ensembles with opposing effects on cocaine place preference, which could have canceled out effects on extinction learning. Alternatively, stimulation of dmPFC PNs may not have affected extinction learning, because bulk excitation using chemogenetics does not mimic endogenous firing patterns required to override extinction learning. Whether PV-INs in the dmPFC suppress dmPFC neurons that drive expression of cocaine memory and/or facilitate the recruitment of dmPFC neurons that mediates extinction-induced behavioral inhibition is an important topic for future research.

We implicate the vmPFC-to-dmPFC feed-forward inhibition circuit in extinction of cocaine-conditioned behavior, but this network may be involved in extinction learning in general. Pharmacological inactivation of the vmPFC or inhibition of protein kinase M zeta in this region results in a reemergence of opiate-induced place preference (29,30), and cue-induced heroin seeking is driven by acute AMPA receptor endocytosis and a reduction in synaptic strength in the vmPFC (31). Additionally, lesions of the vmPFC enhance spontaneous recovery, reinstatement, and contextual renewal of Pavlovian food seeking (32,33). Together, these studies indicate that following extinction learning, the vmPFC exerts behavioral inhibition over responding to reward-related cues. Note that this does not exclude the coexistence of vmPFC ensembles that promote conditioned responses before extinction learning (12,26). To our knowledge, the monosynaptic innervation of dmPFC PV-INs by vmPFC projections has not been previously reported, but independent studies have shown that vmPFC-to-dmPFC projections and PV-INs mediate extinction of conditioned fear (16,22) and extinction of natural reward seeking (21). In line with our observations, extinction of cue-evoked food seeking drives recruitment of GABAergic interneuron activity in the dmPFC (34). Furthermore, excitatory projections in the opposite direction, from dmPFC to vmPFC, also mediate extinction of conditioned fear (14). Together with our findings, this indicates that a reciprocal intrinsic mPFC circuit serves to provide important adaptive control over conditioned behavior, in particular when an originally learned association (context → reinforcer) does not match with the conditions during re-exposure to the same context and an alternative association (e.g., context → no reinforcer) is learned.

To conclude, we discovered that monosynaptic interaction between the vmPFC and dmPFC mediates extinction of cocaine reward memory through activation of dmPFC PV-INs. This sheds new light on the architecture of the neuronal circuit that enables adaptive responding to cocaine-contextual cues and may provide a new therapeutic target for strengthening of behavioral inhibition on context-evoked retrieval of cocaine memories.

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