Psychopharmacology (2023) 240:575–594
https://doi.org/10.1007/s00213-022-06287-2

ORIGINAL INVESTIGATION

Prelimbic and infralimbic medial prefrontal cortex neuron activity signals cocaine seeking variables across multiple timescales

David E. Moorman[1] · **Gary Aston-Jones**[2]

Received: 20 July 2022 / Accepted: 23 November 2022 / Published online: 5 December 2022

Abstract

Rationale and Objectives The prefrontal cortex is critical for execution and inhibition of reward seeking. Neural manipulation of rodent medial prefrontal cortex (mPFC) subregions differentially impacts execution and inhibition of cocaine seeking. Dorsal, or prelimbic (PL), and ventral, or infralimbic (IL) mPFC are implicated in cocaine seeking or extinction of cocaine seeking, respectively. This differentiation is not seen across all studies, indicating that further research is needed to understand specific mPFC contributions to drug seeking.

Methods We recorded neuronal activity in mPFC subregions during cocaine self-administration, extinction, and cue- and cocaine-induced reinstatement of cocaine seeking.

Results Both PL and IL neurons were phasically responsive around lever presses during cocaine self-administration, and activity in both areas was reduced during extinction. During both cue- and, to a greater extent, cocaine-induced reinstatement, PL neurons exhibited significantly elevated responses, in line with previous studies demonstrating a role for the region in relapse. The enhanced PL signaling in cocaine-induced reinstatement was driven by strong excitation and inhibition in different groups of neurons. Both of these response types were stronger in PL vs. IL neurons. Finally, we observed tonic changes in activity in all tasks phases, reflecting both session-long contextual modulation as well as minute-to-minute activity changes that were highly correlated with brain cocaine levels and motivation associated with cocaine seeking.

Conclusions Although some differences were observed between PL and IL neuron activity across sessions, we found no evidence of a go/stop dichotomy in PL/IL function. Instead, our results demonstrate temporally heterogeneous prefrontal signaling during cocaine seeking and extinction in both PL and IL, revealing novel and complex functions for both regions during these behaviors. This combination of findings argues that mPFC neurons, in both PL and IL, provide multifaceted contributions to the regulation of drug seeking and addiction.

Keywords Psychostimulant · Extinction · Reinstatement · Relapse · Dependence · Drug abuse · Ensemble · Learning

Introduction

The prefrontal cortex plays a complex role in regulating executive function (Balleine and O'Doherty 2010; Dalley et al. 2004; Euston et al. 2012; Miller and Cohen 2001). In the rodent, different frontal cortical subregions have been associated with different aspects of motivated behavior (Howland et al. 2022; Kaminska et al. 2021). In particular, the dorsal medial prefrontal cortex (mPFC), or prelimbic cortex (PL), is hypothesized to regulate active behavioral processes, whereas the ventral mPFC, or infralimbic cortex (IL), is proposed to mediate inhibitory behavioral control (Gourley and Taylor 2016; Peters et al. 2009). This has been shown most conclusively in the study of fear conditioning and extinction. PL neurons fire during expression

This article belongs to a Special Issue on Conditioned Determinants of Reward Seeking

✉ David E. Moorman
moorman@umass.edu

[1] Department of Psychological and Brain Sciences & Neuroscience and Behavior Graduate Program, University of Massachusetts Amherst, Amherst, MA 01003, USA

[2] Brain Health Institute, Rutgers University and Rutgers Biomedical and Health Sciences, Piscataway, NJ 08854, USA

of conditioned fear responses, and activation or inhibition of PL drives or blocks fear-related freezing, respectively. Conversely, IL neurons fire for extinguished cues that once predicted footshock, most notably when animals no longer freeze to the cues, and activation or inhibition of IL facilitates or inhibits extinction of fear conditioning, respectively (Giustino and Maren 2015; Maren and Quirk 2004; Morgan and LeDoux 1995; Rozeske and Herry 2018).

A PL/IL "go/stop" dichotomy has also been described for appetitively directed behaviors (Gourley and Taylor 2016; Mendoza et al. 2015; Peters et al. 2009). Inactivation of dorsal mPFC (including PL) during a discriminative stimulus (DS)-driven operant reward seeking task reduced cue-driven responses, whereas inactivation of ventral mPFC (IL) during performance of a DS-task increased responses generally, including those triggered by the non-rewarded stimulus (Ghazizadeh et al. 2012; Ishikawa et al. 2008); and increased spontaneous recovery and reinstatement in an appetitive Pavlovian task (Rhodes and Killcross 2007, 2004). D-cycloserine administration directly into IL enhanced extinction of sucrose seeking (Peters and De Vries 2013). In a discriminative-stimulus-driven reward seeking task, PL neurons were more excited, and IL neurons were more inhibited during sucrose seeking (Moorman and Aston-Jones 2015). Optogenetic manipulation of the projection from IL to the nucleus accumbens (NAc) shell suppressed expression of appetitive Pavlovian conditioning (Villaruel et al. 2018, 2022). Thus, across Pavlovian and instrumental tasks, there appear to be differences in PL and IL influences on appetitive behavior.

Other models for contrasting PL and IL contributions to behavior have been characterized, particularly in the context of appetitive motivated behaviors (Green and Bouton 2021; Nett and LaLumiere 2021). For example, inhibition or inactivation of neural activity in PL and IL appears to disrupt either goal-directed or habitual behavior, respectively (Coutureau and Killcross 2003; Killcross and Coutureau 2003; Smith et al. 2012), and neural activity recorded from these brain regions indicate selectively stronger responses in each context (Smith and Graybiel 2013). Other lines of research have emphasized differential roles in minimally vs. extensively trained behaviors (Green and Bouton 2021; Shipman et al. 2018). These different framings of divergent PL and IL function differ somewhat from the go/stop model, but emphasize categorically different contributions of these brain areas, in line with that observed in fear conditioning and reward seeking.

The differences in PL and IL function has been well-characterized in the context of drug seeking, particularly cocaine. Prelimbic projections to the nucleus accumbens (NAc), particularly to the core, play an important role in modulating drug seeking (Kalivas and Volkow 2011; Peters et al. 2009; Stefanik et al. 2013). The contributions of this projection have been frequently described in the context of reinstatement or relapse (Hearing et al. 2008; James et al. 2018; McFarland et al. 2004, 2003; McGlinchey et al. 2016; McLaughlin and See 2003). In contrast, the IL, and in particular its projections to the NAc shell, is important for regulating extinction and inhibition of cocaine seeking (Augur et al. 2016; Marchant et al. 2010; Muller Ewald and LaLumiere 2017; Peters et al. 2009, 2008). These studies are well-aligned with fear conditioning literature and, to some degree, natural reward seeking literature. Thus, a wide range of research supports the hypothesis that PL regulates activation of both reward seeking and fear-related behaviors and that IL inhibits such behaviors (Peters et al. 2009).

Other studies, however, argue that a strict PL/IL dichotomy may have limitations both with respect to natural reward and drug seeking behavior (Caballero et al. 2019; Capuzzo and Floresco 2020; Howland et al. 2022; Moorman and Aston-Jones 2015; Moorman et al. 2015; Riaz et al. 2019). PL inactivation can enhance operant responding for drugs and natural rewards (Caballero et al. 2019; Ishikawa et al. 2008; Jonkman et al. 2009; Willcocks and McNally 2013). Neurons in both PL and IL are modulated during both seeking and extinction of sucrose seeking (Moorman and Aston-Jones 2015). PL and IL neurons also exhibit strong modulation during ethanol seeking (Halladay et al. 2020), though IL neurons were more strongly activated during punishment-associated response withholding. Activation of ensembles in IL is implicated in driving seeking of cocaine, heroin, and methamphetamine (Bossert et al. 2011; Kane et al. 2021; Koya et al. 2009; Madangopal et al. 2021; Peters et al. 2013; Rocha and Kalivas 2010; Warren et al. 2019). In addition, PL appears to be essential for inhibition of cocaine seeking in certain contexts (Chen et al. 2013; Gutman et al. 2017; Martin-Garcia et al. 2014; Mihindou et al. 2013), and IL regulates DS-driven cocaine seeking in some circumstances (Madangopal et al. 2021). Thus, there is reason to question an absolute PL/IL "go/stop" dichotomy, even in the context of cocaine seeking.

Given the complex set of findings related to prefrontal control of cocaine seeking, understanding neural dynamics during cocaine seeking and extinction can add valuable information to our evolving conceptualization of this process. Early studies demonstrated that mPFC neurons are activated during cocaine self-administration, though no association was noted between anatomical localization and response properties or behavior (Chang et al. 1998; 2000; Chang et al. 1997; Sun and Rebec 2006).

More recent studies have focused on mPFC subregion activity during cocaine seeking. Using a discrete trials task, Muller Ewald and colleagues reported increases in LFP and single-unit activity in IL during extinction of cocaine seeking (Muller Ewald et al. 2022), although some activity was observed during self-administration, in line with observations during sucrose seeking and extinction (Moorman and Aston-Jones 2015). Again using a discrete trials behavioral

framework, Cameron and colleagues found greater inhibition than excitation around the time of the lever press in NAc-projecting IL neurons during cocaine self-administration, though some heterogeneity was observed, particularly after 15 days of abstinence (Cameron et al. 2019). Notably, these studies focused on IL, leaving open the question of whether PL and IL exhibit different levels of activity during cocaine self-administration, extinction, and reinstatement.

Only one previous study has directly compared neuronal activity in PL and IL during cocaine seeking and found that both PL and IL neurons responded to cocaine-related cues and lever presses (West et al. 2014). Neural activity incubated, particularly in PL, following 30 days of abstinence, again providing support for some differences in PL and IL function related to cocaine seeking and extinction, but no clearly opposing roles.

To our knowledge, no study has compared PL and IL activity during self-administration, prolonged extinction, and reinstatement in the same animals, and no study has reported prefrontal activity during cue-induced reinstatement of cocaine seeking. Understanding the functions of PL and IL neurons in these contexts is particularly important given the prominence of this behavioral model in addiction research, the relevance of the reinstatement model to the human clinical relapse condition (Bossert et al. 2013), and the fact that the go/stop roles for PL/IL are most frequently observed using this behavioral framework. To address this absence, we recorded the activity of neurons in PL and IL, while rats (i) self-administered IV cocaine, (ii) underwent multiple days of extinction, (iii) underwent cue-induced reinstatement, and (iv) underwent cocaine prime-induced reinstatement. The results of our study support some previous findings, particularly related to the role of PL in reinstatement. However, our data also reveal new aspects of prefrontal coding of cocaine seeking that have not previously been shown and argue against a simple PL/IL dichotomy with respect to cocaine seeking and extinction.

Materials and methods

Subjects

Male Sprague–Dawley rats (approximately 300–400 g upon arrival; Charles River, Wilmington, MA; $n = 12$) were used in these experiments. Rats were single-housed under temperature- and humidity-controlled conditions on a reversed light cycle (6 a.m. off to 6 p.m. on) and allowed free access to commercial chow and tap water. All protocols and procedures followed the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Medical University of South Carolina Institutional Animal Care and Use Committee, where data were collected.

Surgery

Animals were anesthetized with ketamine/xylazine (56.5/8.7 mg/kg) and received meloxicam (1 mg/kg). A catheter was implanted in the jugular vein and exited through a port between the scapulae (Smith et al. 2009). Animals were then placed in a stereotaxic frame and maintained with 1.5–2.5% isoflurane in air delivered through a nose cone. Body temperature was maintained at approximately 37 °C using a thermistor-controlled electric heating pad. All incision points were infiltrated with a long-lasting anesthetic (2% lidocaine). The skull was exposed, and bilateral holes were drilled above the mPFC (~ 2.2–3.2 mm rostral and ~ 0.2–1.0 mm lateral to bregma). Three or four additional holes were drilled, and screws were implanted to secure array implants. Arrays of 16 stainless-steel microwires (50 μm) arranged in a 4×4 pattern (~ 200 μm spacing) were lowered into the medial prefrontal cortex in each hemisphere (two arrays per animal). Wires were cut before surgery such that 8 wires targeted PL (DV: 3.8) and 8 wires targeted IL (DV: 5.2). Craniotomies were filled with gel foam, secured with cyanoacrylate, and arrays were secured to the skull screws using dental cement. Animals were given antibiotic (cefazolin, 0.1 ml of 100 mg/ml IV) and analgesic (meloxicam, 1 mg/kg, SC) and heparin (0.1 ml of 100 U/ml IV) and were allowed to recover at least 1 week following surgery, during which time weight, activity, and other measures of general health were monitored. During recovery and self-administration, the animals received daily infusions of cefazolin and heparin.

Behavioral training and task

Following recovery, rats were trained to self-administer IV cocaine using a fixed ratio (FR1) paradigm in sound-attenuated operant chambers (Med-Associates, St. Albans, VT). Stimulus presentations, cocaine infusions, and data recording were controlled via MED-PC IV software (Med-Associates, St. Albans, Vermont). During 2-h daily sessions, presses on an active lever resulted in a cocaine infusion (fixed ratio – 1; 0.2 mg in 50 µL via motorized pump) paired with tone and light cues (78 dB, 2900 Hz; white stimulus light above the active lever), followed by a 20-s timeout. Presses on an inactive lever had no consequence.

Rats were given at least 10 self-administration sessions in which they earned at least 10 infusions per session. Rats then received two "extinction/self-administration" sessions, during which the first hour was an extinction session (lever presses had no consequences) followed by cocaine self-administration during the second hour. These sessions were separated by at least 1 day with a 2-h cocaine self-administration session and were followed by at least 1 day of cocaine self-administration. Due to issues with analysis,

data from these sessions are not presented in the current report, but are noted here as part of the behavioral regimen. Rats were then given daily extinction sessions, during which lever presses had no consequence (no drug or cues). Before reinstatement testing, rats were required to meet an extinction criterion of < 25 active lever presses for 2 consecutive days. Rats underwent at least 7 extinction sessions before the first reinstatement session. Rats performed 1–2 cue-induced reinstatement and 1–2 cocaine prime-induced reinstatement sessions. For cue-induced reinstatement, active lever presses resulted in presentation of tone and light cues in the same manner as during self-administration, but no drug was delivered. For cocaine-induced reinstatement, the following design was used to control for injection effects. Rats were first injected with 0.9% saline (1 ml/kg IP). Two minutes later, levers were extended, and rats were allowed to press for 30 min at which point levers were withdrawn. Rats were then injected with a priming dose of cocaine (10 mg/kg, 1 ml/kg IP). Two minutes later, levers were extended, and rats were allowed to press for 90 min. Rats almost never pressed the active lever after saline injections (mean active lever presses = 4.3 + / − 1.2), so neural analysis focused on behavior after the cocaine priming dose, during which we observed significant elevations in lever pressing (Fig. 1,

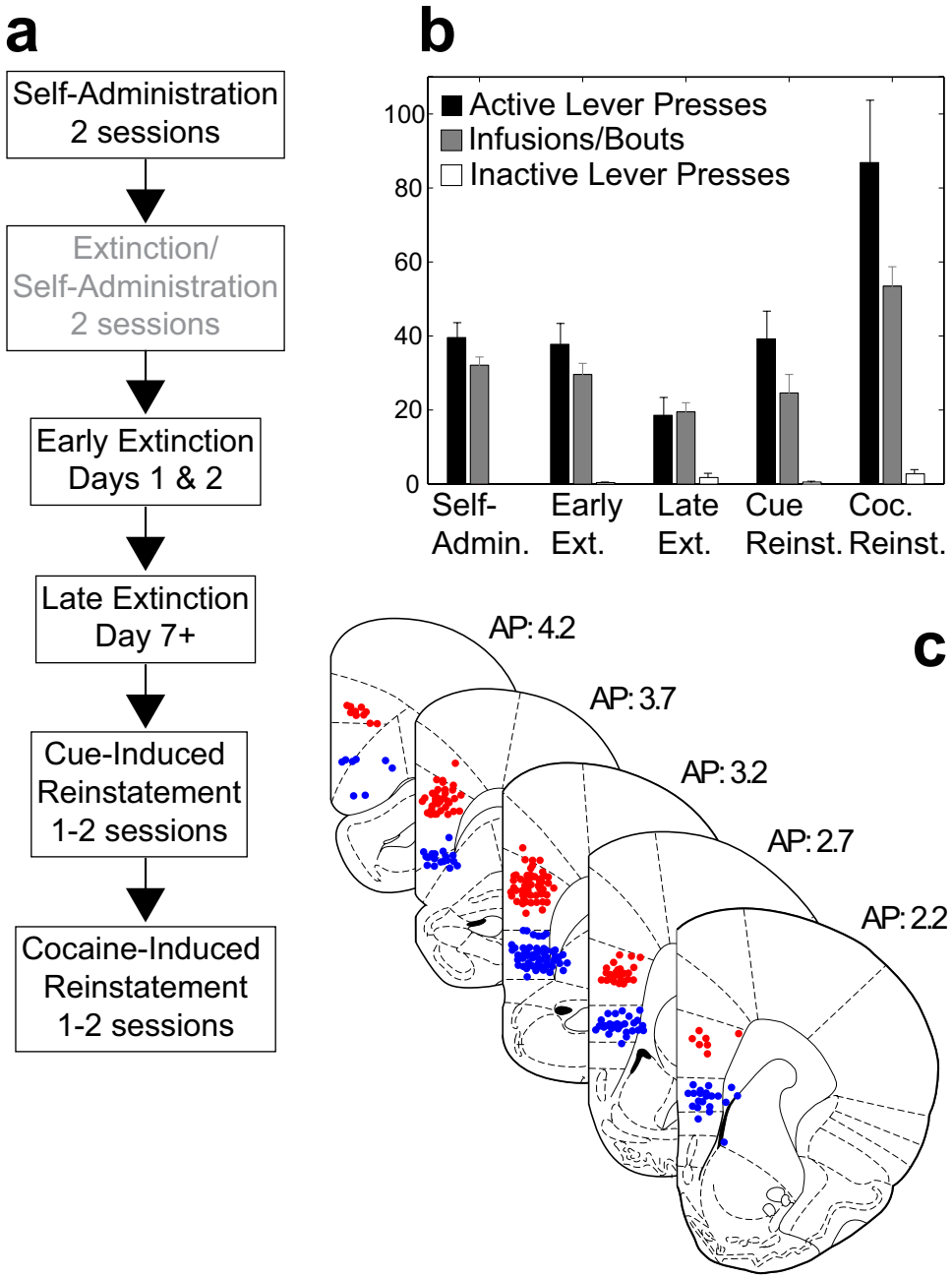

Fig. 1 **a** Timeline of recording sessions producing the data used in the current report. Recordings were obtained during self-administration, early and late extinction, and cue- and cocaine-induced reinstatement sessions. An intervening session consisting of a single day of extinction and self-administration was conducted between the self-administration, and extinction session is shown for completeness, but data are not included here due to analysis issues. **b** Behavior in each stage of the study. Animals reliably pressed the active lever and received infusions during self-administration. Pressing decreased during extinction training, and animals reliably reinstated in both cue- and prime-induced reinstatement sessions. Inactive levers were rarely pressed. Numbers of presses in cocaine-induced reinstatement are from 90-min session post-cocaine injection (see Methods). **c** Recording sites were plotted based on histological processing of tissue following electrolytic lesions generated by recording wires. Recording sites reliably formed two clusters located in dorsal and ventral mPFC (primarily PL and IL), respectively. Four rats had a small number (1–4) of wire placements slightly posterior to those shown here (~ AP 2.0). Red, PL recording sites. Blue, IL recording sites

Results). For all sessions (self-administration, extinction, reinstatement), rats were placed in the chambers 5 min before session onset and remained in the chambers 15 min after session offset to provide baseline measurements for unit activity.

Electrophysiological recording

Neurons were recorded multiple times per rat during performance of all stages of the task. As frequently as possible, we performed 2 days of recording per session, one for each implanted array. Results here focus on mPFC activity in late self-administration (> 10-day post-acquisition), early (first 2 days) and late (> 7 days extinction, within 1 – 2 days prior to reinstatement) extinction, and cue-induced and cocaine-induced reinstatement. Not all rats completed the complete suite of behaviors. We recorded neural activity from 12 rats during cocaine self-administration, 12 during early extinction, 10 during late extinction, 10 during cue-induced reinstatement, and 8 during cocaine-primed reinstatement.

During recording sessions, arrays were connected to a headstage (20 × gain, Plexon, Dallas, TX). Signals were passed through a cable (Omnetics Connector Corporation, Minneapolis, MN) to an electrical commutator (Keyo Electric Co., Ltd., Wenzhou, Zhejiang, CN) to allow free movement. Commutator output was delivered to a Plexon recording system (MAP/16, Plexon), where signals were amplified (50 ×), filtered (100 Hz–8 kHz), and sampled at 40 kHz. Action potentials were recorded using RASPUTIN software (Plexon) where gain and thresholds were set to isolate single neuron activity. Recorded spikes were further sorted offline using Offline Sorter (Plexon) using a combination of template-matching and principal components analyses. Well-isolated single units that fired consistently throughout the recording session were included for analysis. Timestamps for behavioral events were sent from the Med-Associates behavioral control system to the Plexon recording system for use in aligning neural activity to behavior. All experimental sessions were recorded onto digital video (Cineplex, Plexon) for additional behavioral analyses as necessary.

Histology

Following experimental testing and recording, rats were anesthetized with 1.5–2.5% isoflurane, and constant current (25 μA) was delivered to each recording wire for 15 s to produce lesions and iron deposits to mark the tips of recording electrodes. Rats recovered from anesthesia and were sacrificed and perfused 1 day later to allow development of lesion-induced gliosis. Animals were perfused with ~ 50 ml of 0.9% NaCl solution followed by 400–500 ml of 4% paraformaldehyde followed by 50–100 ml of a 5% potassium ferricyanide/5% HCl solution to stain iron deposits with the Prussian blue method. Brains were post-fixed overnight in 4% paraformaldehyde and transferred to a 20% solution of sucrose/0.1% sodium azide in phosphate buffer at 4 °C for at least 3 days. Coronal, 40-μm-thick sections of brains were cut on a cryostat. Prefrontal sections were transferred to slides, counterstained with neutral red (Fisher, Fairlawn, NJ), dehydrated with graded alcohol solutions, cleared with xylene, and coverslipped with Permount (Fisher). Prussian blue-stained iron deposits were used to confirm accurate electrode placements (Fig. 1c).

Data analysis

Timestamps of sorted spikes and behavioral events were imported into Matlab (MathWorks, Natick, MA), where custom analyses were used to assess the relationship of neuronal activity to behavior. All well-isolated neurons were included in analyses, although subsets were selected for additional analysis based on response criteria (e.g., significant increase or decrease of firing rate above or below baseline in relation to task events). Three main measures of neuron activity were analyzed. First, we measured phasic responses related to lever pressing. Neuronal activity aligned on pressing was grouped in 100-ms bins, and spike density functions (SDFs) were generated by Gaussian smoothing of the resulting event-related histogram. For population measures, neuronal activity was z-score normalized against a 200-ms baseline epoch 8-s preceding lever presses for comparisons across neurons by subtracting mean and dividing by standard deviation of baseline. Neurons were characterized as significantly modulated based on paired Wilcoxon signed rank tests comparing (non-normalized) firing rate in a – 10 to – 8-s prepress baseline epoch to 2-s epochs either immediately pre- (– 2 to 0 s) or post-press (0 to 2 s) across trials. Comparisons of press-related activity (e.g., between PL and IL) were made using normalized data during an epoch + / – 1 s peripress. We also analyzed neural responses to the first press in a bout, which consisted of any press not preceded by another press within 5 s (to avoid counting multiple presses in a single burst of pressing), particularly during extinction and reinstatement, when lever presses were less regular than during self-administration. Second, we measured slow changes in activity (30-s bins) associated with either calculated brain cocaine levels during self-administration or general motivation levels. Neural activity during cocaine self-administration was also compared to brain cocaine levels using the equation from (Bentzley et al. 2013; Pan et al. 1991; Peoples and Cavanaugh 2003; Zimmer et al. 2011): $c = \frac{dk}{v(\alpha-\beta)}\left(e^{-\beta t} - e^{-\alpha t}\right)$ where c is the brain cocaine concentration, d is the dose (in mg/kg), k is the rate constant for transfer from blood to brain (0.233), v is the volume of the brain (0.15 L/kg), α is the constant related to flow of cocaine

between blood and brain (0.642), and β is a constant that is related to the elimination of cocaine (0.097). We also examined the relationship of activity to motivation, measured as the summed number of active and inactive lever presses in 30-s bins throughout the session. Binned presses were Gaussian smoothed and correlated with 30-bin spike density functions used for comparisons above. Third, we investigated "tonic" activity during the sessions using 30-s bins to detect significant changes in activity that started at the onset of the session and persisted throughout (Fabbricatore et al. 2009; Guillem et al. 2010; Kravitz et al. 2006; Kravitz and Peoples 2008; Peoples et al. 1999, 1998; Root et al. 2012).

Additional parametric or nonparametric analyses, as appropriate, were applied to firing rate data as described in "Results" section. Main effects were calculated using ANOVAs, Kruskal–Wallis, and Friedman tests based on whether the data were parametric and whether data were paired. Within-neuron comparisons were made using paired *t*-tests/Wilcoxon signed rank tests when trials were equivalent. Analyses of numbers of significant neurons were made using Fisher's exact test. Non-equivalent or cross-neuron analyses were performed using unpaired *t*-tests or Mann–Whitney *U* tests.

Results

Behavior

Figure 1A shows the time course of behavioral tests used in this study (self-administration, extinction, cue-induced reinstatement, cocaine-induced reinstatement). Figure 1b shows the mean number of active and inactive lever presses at each stage and numbers of infusions received (cocaine + cues in self-administration, cues only in cue-induced reinstatement). During extinction and cocaine prime-induced reinstatement, animals would frequently press in bouts, as opposed to regulated pressing observed during maintenance of self-administration. We considered the leading press of each bout to represent initial seeking of an infusion. We measured such 'infusion seeking' as any press not preceded by another press within 5 s (to avoid counting multiple presses in a single burst of pressing). Animals consistently pressed the active lever, earning cocaine infusions during self-administration, and rarely pressed the inactive lever. Animals exhibited cocaine loading, i.e., high-frequency pressing during the first ~ 1–5 min, followed by stable maintenance pressing. During extinction, active lever presses declined, reaching a nadir during late extinction. Finally, during both cue-induced reinstatement and cocaine-prime-induced reinstatement, animals reliably pressed the active lever at a level approximately equal to (cue-reinstatement) or almost double (cocaine prime-reinstatement) that seen during self-administration. There was a significant effect of session on active lever presses ($x2(4,82) = 19.65$, $p < 0.001$; Kruskal–Wallis), infusions/bouts ($x2(4,82) = 27.52$, $p < 0.001$), and inactive lever presses ($x2(4,82) = 15.60$, $p < 0.005$). Significant changes in inactive presses were largely driven by cocaine prime-induced lever activity during reinstatement testing (Fig. 1b).

Overview of neural activity

Recording sites were localized to PL or IL based on post-recording lesions (Fig. 1c). Neurons in both areas were active during all phases of the study. In the following sections, we describe analyses of neural activity during each phase: self-administration (Figs. 2, 3, and 4), early and late extinction (Fig. 5), and cue- and cocaine prime-induced reinstatement (Fig. 6). Details related to lever press-associated phasic responses are provided in Tables 1 (PL) and 2 (IL). Figure 7 summarizes the findings across sessions for comparison.

PL and IL neural activity during cocaine self-administration

During self-administration, a significant proportion of both PL and IL neurons exhibited changes in firing rate, consistent with previous studies (West et al. 2014). Due to large, variable fluctuations in activity during cocaine loading in the first 5 min, we focused on analysis of press-related activity during the maintenance phase of cocaine self-administration, > 5 min after session onset (Root et al. 2012). Activity of single neurons in PL and IL during cocaine self-administration is shown in Fig. 2. Among 259 PL and 307 IL neurons analyzed from recordings during self-administration, 42.6% and 36.9%, respectively, were phasically modulated in relation to the cocaine lever press (Fig. 3a and b). There were no significant differences in either the proportion of significantly modulated neurons ($p > 0.05$, Fisher's exact test) or in the strength of signaling in PL vs. IL neurons during cocaine self-administration (all neurons: $Z = 1.21$, $p > 0.05$; significantly modulated neurons: $Z = 1.77$, $p > 0.05$, Mann–Whitney *U*). Importantly, neuronal signaling was heterogeneous in both PL and IL, with neurons in both regions exhibiting phasic excitation and inhibition preceding, following, or surrounding the lever press (Fig. 2; Tables 1 and 2). The absolute value of press-related activity, which measures overall signaling strength irrespective of direction, was not significantly different between PL and IL (Fig. 3a; all neurons: $Z = 1.48$, $p > 0.05$; significantly modulated neurons: $Z = 0.24$, $p > 0.05$). The proportions of neurons in PL and IL that were excited vs. inhibited were very similar (Fig. 3b; Tables 1 and 2). Thus, both PL and IL exhibited significant

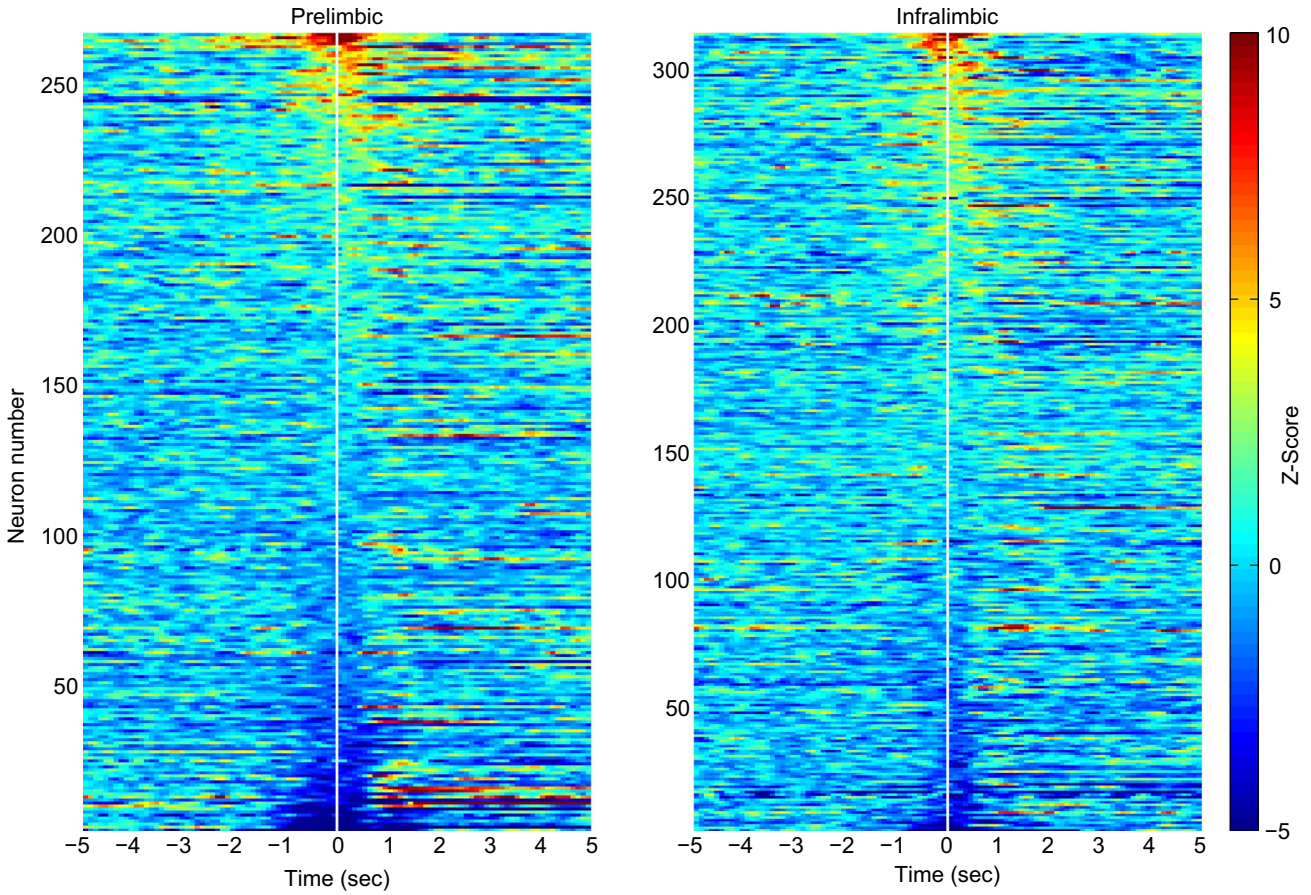

Fig. 2 Lever press-aligned activity in all recorded PL ($n=259$) and IL ($n=307$) neurons during cocaine self-administration. Neurons are sorted along the *y*-axis based on mean activity at the time of the lever press. Both PL and IL neurons exhibited prominent and significant lever press-related excitation and inhibition. Temporal profiles of responses were somewhat heterogeneous. Although significant modulation occurred leading up to and following the lever press, many neurons exhibited only pre- or only post-press excitation or inhibition, and many neurons exhibited delayed responses that correspond to the onset of cue-presentation or cocaine delivery. Thus PL and IL neurons both exhibit robust, but highly diverse response properties during cocaine self-administration. See Tables 1 and 2 for quantification of this heterogeneity

seeking-related changes in activity during cocaine self-administration, and there were no significant differences between the two regions.

In addition to activity changes time locked to the lever press, we also observed a relationship between calculated brain cocaine levels and both PL and IL activity over the course of the session (see Methods). Activity and drug levels were binned in 30-s intervals and focused on the maintenance epoch. Out of 266 PL neurons analyzed, 72 (27.1%) and 90 (33.8%) exhibited positive and negative significant correlations with calculated brain cocaine levels, respectively. Among 313 IL neurons analyzed, 80 (25.6%) and 121 (38.7%) were positively and negatively correlated with brain cocaine levels, respectively. Figure 3c shows two examples of neurons exhibiting a strong correlation between neural activity and cocaine levels. Session recordings for a single IL (top) and PL neuron (bottom) are shown on the left, and 30-s data points used to calculate correlations for each of these two neurons are shown on the right. Figure 3d, left, shows the overall distribution of positive and negative drug vs. firing rate correlation coefficients across neurons. Despite a hint that slightly more IL neurons were inversely correlated with calculated cocaine levels, the differences between proportions across PL and IL were not significant ($p>0.05$, Fisher's exact test).

We further examined the relationship between neural activity and lever pressing binned in 30-s intervals for two reasons—first to determine if neural activity was more closely related to seeking behavior as compared to drug levels and second to compare activity to later sessions in which lever pressing was present, but no drug was delivered. During self-administration sessions,we found an overall greater positive

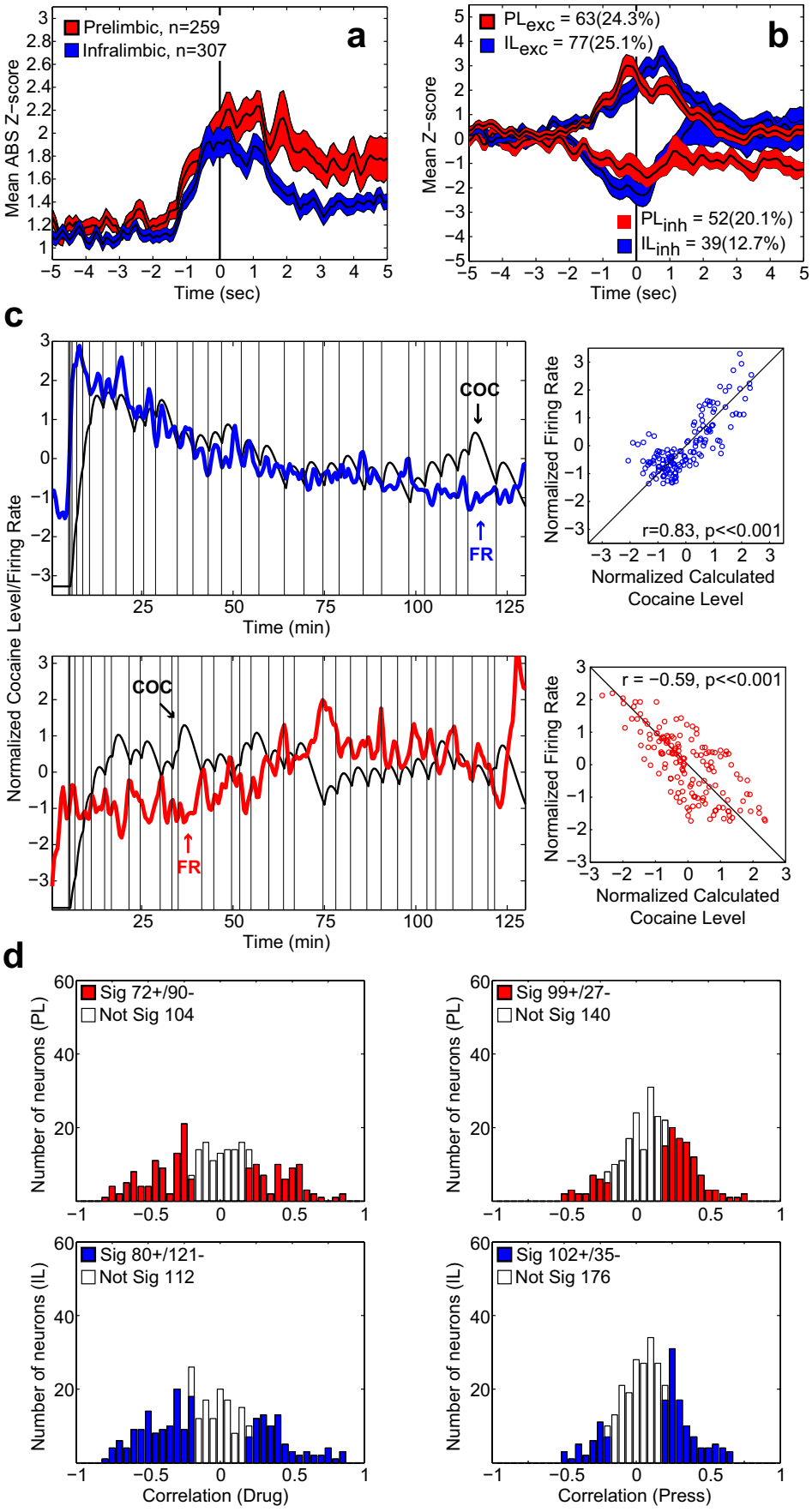

Fig. 3 Activity of PL and IL neurons during cocaine self-administration sessions. **a** Activity of all recorded PL and IL neurons aligned on cocaine-reinforced lever press. Activity is *Z*-score normalized and absolute value transformed to assess the magnitude of signaling across the heterogeneous responses seen in PL and IL (see Methods and Fig. 2). Shaded regions represent standard error across neurons. Both PL and IL exhibited responses of similar magnitude related to the lever press. b *Z*-scores (not absolute value transformed) of neurons exhibiting significant activation around the time of the lever press. More neurons were significantly excited than inhibited, particularly in IL, but both types of signaling were prominently observed. **c** (Top) session-long activity of a single IL neuron (thick blue line) in 30-s bins across the whole self-administration session. Vertical lines represent times of lever presses. Both neural activity and calculated cocaine levels were normalized to themselves to facilitate comparison. Two details are notable in the activity of this neuron. First, the neuron exhibits strong session-related activation that decreases over the course of the self-administration session. Second, this neuron exhibits a highly significantly positive correlation with calculated brain cocaine levels. This is reflected in the overlap between the two curves in (**c** top left) and in the scatter plot in (**c** top right) in which mean values of normalized activity and cocaine levels are plotted against one another in 30-s bins. **c** (Bottom) session-long activity of a single PL neuron exhibiting a highly significant inverse correlation between firing rate and calculated brain cocaine levels. Both PL and IL neurons exhibited both positive and negative correlations with calculated brain cocaine levels. Other examples of session-related excitation and inhibition in PL and IL are shown in Fig. 4. **d** Histograms of correlation coefficients for neurons recorded from PL (top) and IL (bottom) with respect to calculated cocaine levels (left) and counts of active lever presses in 30-s bins (middle). Shaded bars represent statistically significant ($p < 0.05$) positive or negative correlation

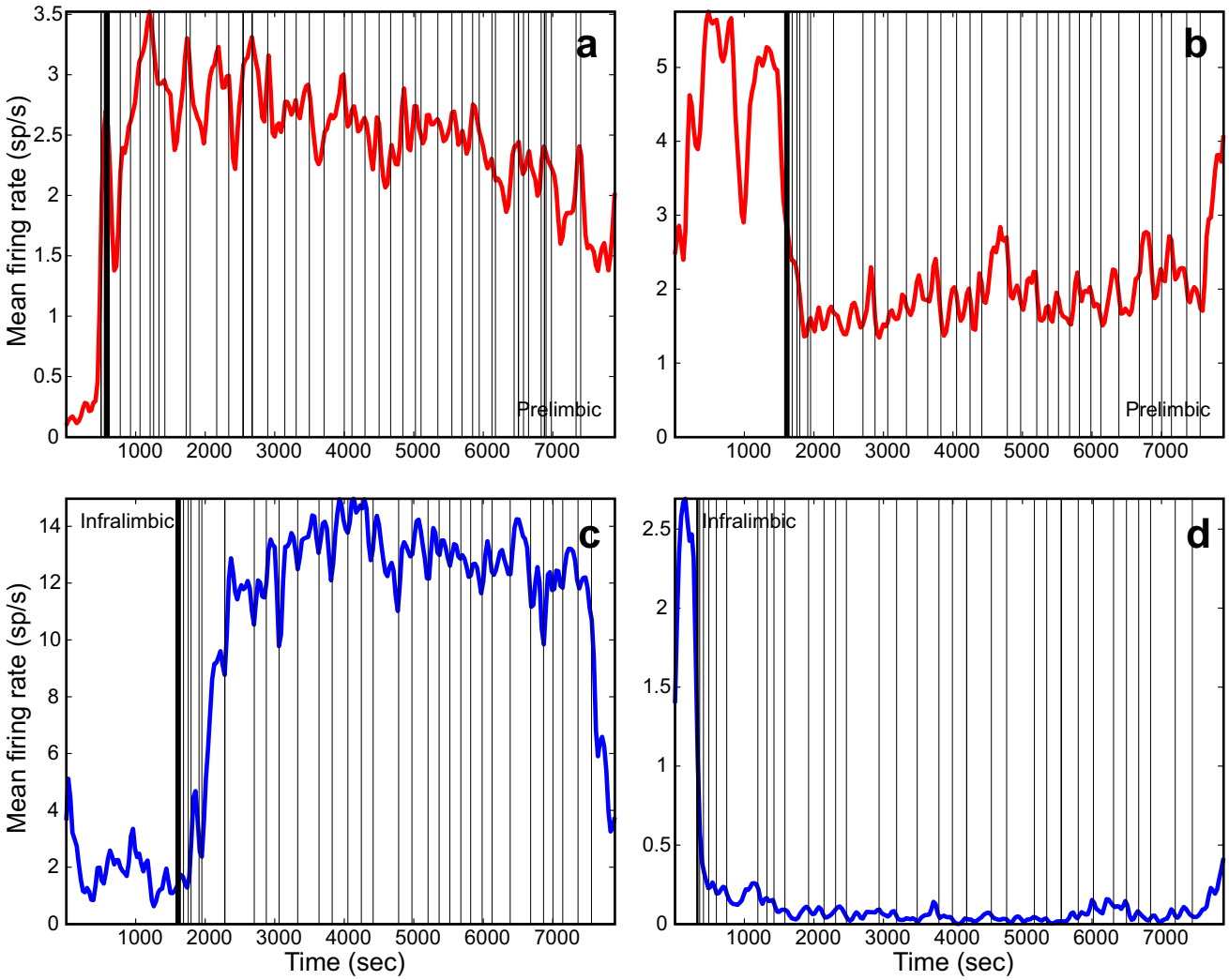

Fig. 4 Examples of four neurons (2 PL, top and 2 IL, bottom) that exhibit robust session-long changes in tonic firing rate during cocaine self-administration. Thick colored lines represent Gaussian-smoothed spike density functions generated from average firing rate in 30-s bins. Vertical lines show times of lever presses. Of note, both PL and IL neurons exhibited instances of strong session-long tonic excitation (left) or inhibition (right). See Fig. 7 for proportions of neurons in each region exhibiting significant modulation in each direction. As in example neurons shown in Fig. 3, press- or cocaine-related fluctuations in activity can also be observed in some cases, indicating that information is multiplexed across multiple timescales at the level of single neurons

than negative relationship for both PL and IL between neural activity and presses in 30-s bins over the course of the session (Fig. 3d (right); PL: 99 (37.2%) significantly positive, 27 (10.2%) significantly negative; IL: 102 (32.6%) significantly positive, 35 (11.2%) significantly negative).

Based on previous findings of session-long tonic changes in activity during cocaine self-administration in NAc, ventral pallidum (VP), and orbitofrontal cortex (OFC) (Fabbricatore et al. 2009; Guillem et al. 2010; Kravitz et al. 2006; Kravitz and Peoples 2008; Peoples et al. 1999, 1998; Root et al. 2012), we investigated whether PL and IL exhibited tonic changes in activity across the session. We compared 5 min of activity during the baseline (in 30-s bins) to 5 min of activity 50- and 100-min post-session-start. To be considered tonically modulated, neurons had to demonstrate significant changes from baseline in both session epochs in the same direction (i.e., both significantly inhibited and excited relative to baseline). We found that 30.5% of 266 PL neurons (11.7% significantly excited and 18.8% significantly inhibited) and 28.4% of 313 IL (11.5% significantly excited and 16.9% significantly inhibited) neurons were tonically modulated over the course of the self-administration session. Examples of tonic excitation and inhibition in both PL and IL neurons are shown in Fig. 4.

PL and IL neural activity during extinction

We next measured PL and IL activity during early and late extinction. Early extinction was the first 2 days of extinction

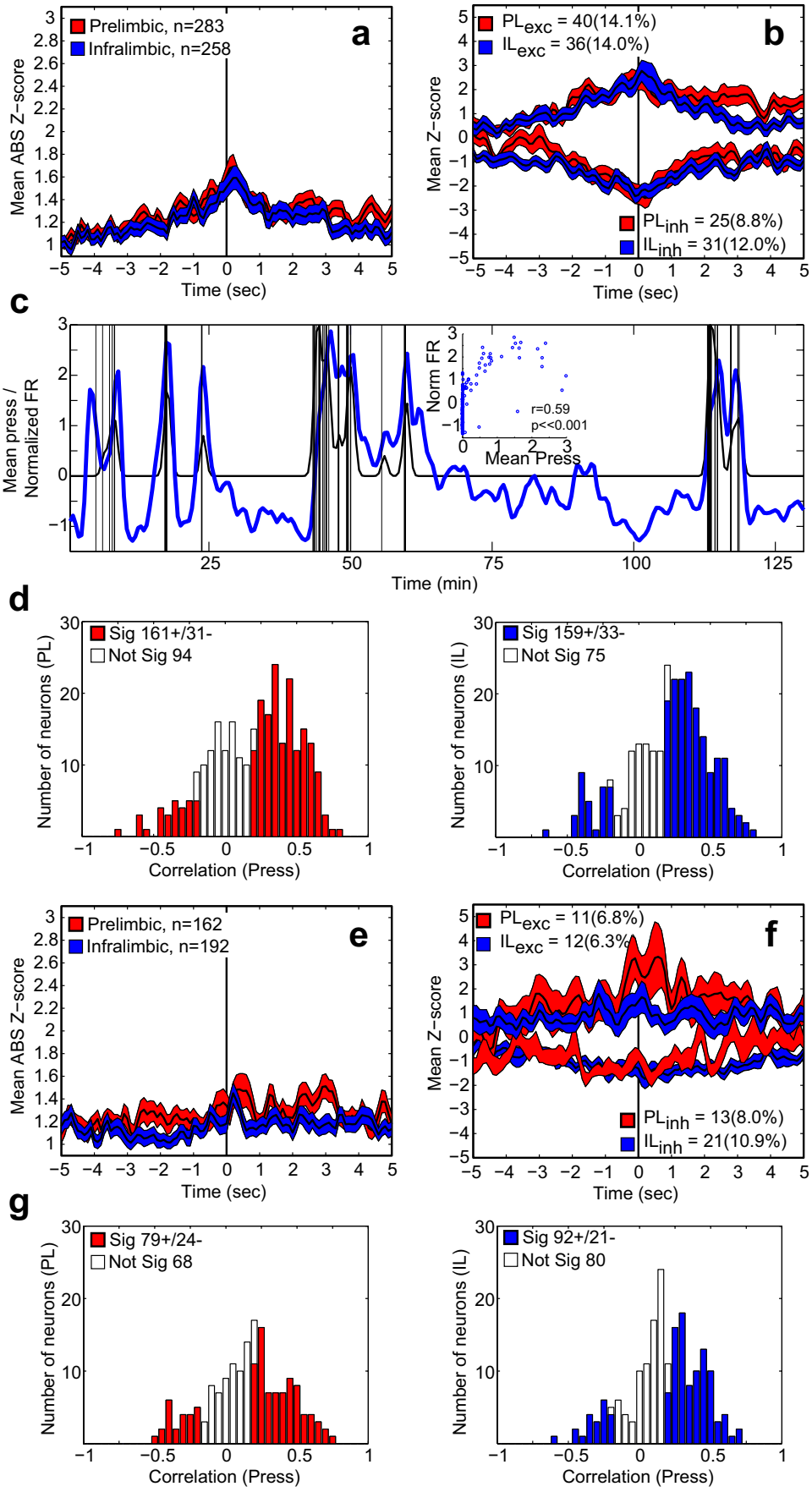

Fig. 5 Activity of PL and IL neurons during early (**a**–**d**) and late (**e**–**g**) extinction, aligned on the lever press (**a**, **b**, **e**, **f**) or correlated with counts of lever presses (**c**, **d**, **g**). Both the absolute value magnitude of signaling (**a**, **e**) and the magnitude of excitation/inhibition in significantly modulated neurons (**b**, **f**) were strongly reduced compared to that observed during self-administration (Fig. 3) or reinstatement (Fig. 6). The decreased proportions of significantly modulated neurons are shown in (**b**, **f**) and in Fig. 7. Interestingly, neurons continued to exhibit tonic (~30 s) fluctuations that were positively correlated with lever pressing (**c**, **d**, and **g**). An example of a single IL neuron with activity strongly positively correlated with lever pressing is shown in **c**. Vertical lines represent times of lever presses. Neural activity was normalized to itself to facilitate comparison. Although the intensity of activity phasically related to lever presses was diminished in extinction, tonic activity tracked bouts of initiation of responding on a slower timeframe

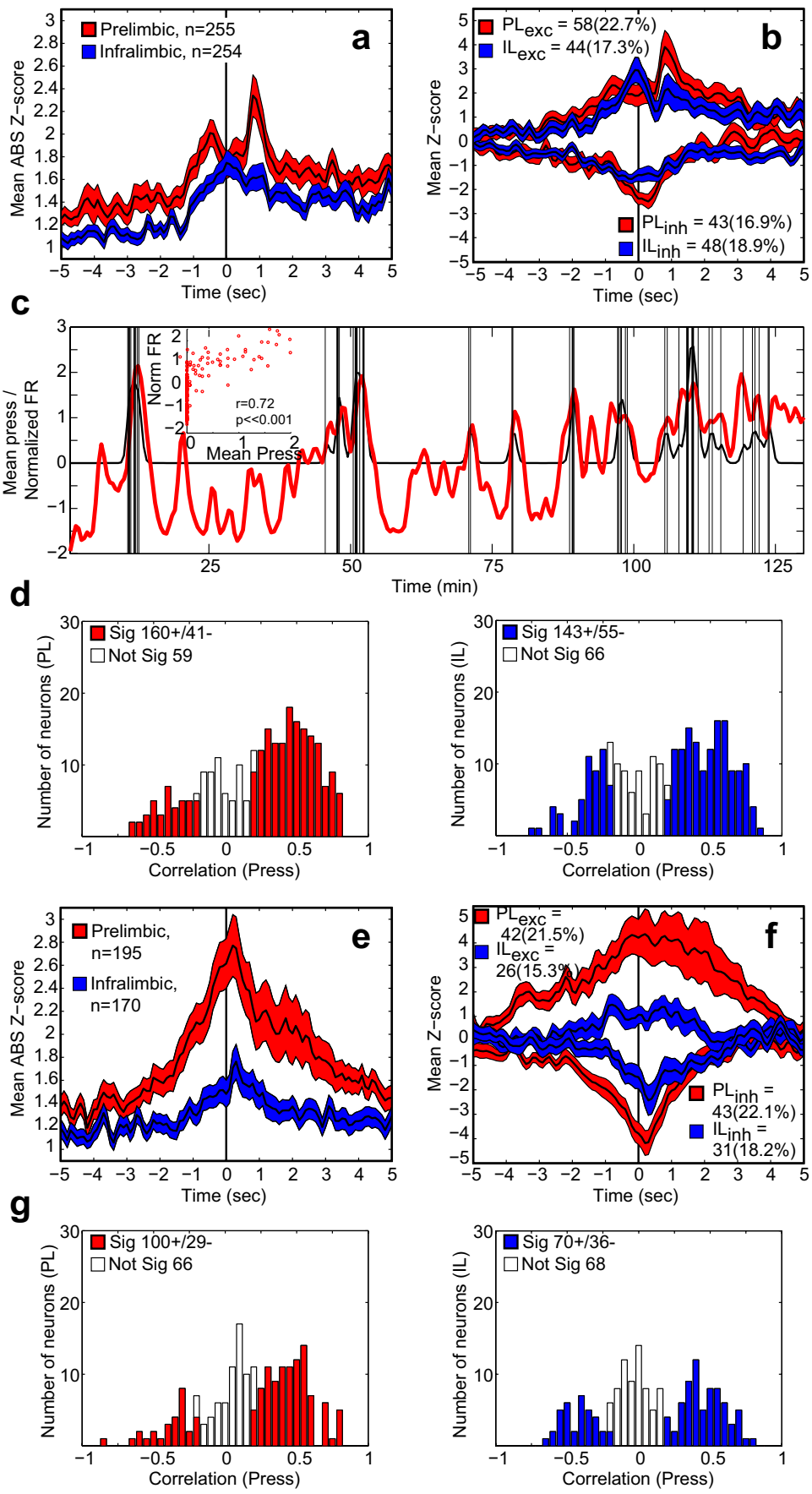

Fig. 6 Activity of PL and IL neurons during cue-induced (**a**–**d**) and cocaine prime-induced (**e**–**g**) reinstatement, aligned on lever press (**a**, **b**, **e**, **f**) or correlated with counts of lever presses (**c**, **d**, **g**). Absolute value signaling is shown in **a** and **e**, and mean *Z*-scores for significantly modulated neurons are shown in **b** and **f**. Activity in both PL and IL was enhanced in cue-induced reinstatement to approximately the same level as seen during cocaine self-administration. During cocaine prime-induced reinstatement, PL excitation and inhibition were both dramatically enhanced, whereas IL activity was not. As in self-administration and extinction, tonic activity was strongly correlated, largely positively, with lever pressing (**c**, **d**, **g**). An example of a single PL neuron with activity strongly positively correlated with lever pressing during cued reinstatement behavior is shown in **c**. Vertical lines represent times of lever presses. Neural activity was normalized to itself to facilitate comparison

Table 1 Phasic responses of prelimbic neurons separated into classes based on epoch (pre- vs. post-lever press) and direction of response (excitation (↑), inhibition (↓), not significant (-))

Self-Admin		Significant: 110/259 (42.6%)					
Pre	Post	Pre	Post	Pre	Post	Pre	Post
↑	↑	↑	↓	↑	-	-	↑
16 (6.2%)		2 (0.8%)		14 (5.4%)		28 (10.8%)	
↓	↓	↓	↑	↓	-	-	↓
15 (5.8%)		3 (1.2%)		14 (5.4%)		18 (7.0%)	
Early Ext		Significant: 65/283 (23.0%)					
↑	↑	↑	↓	↑	-	-	↑
15 (5.3%)		0 (0%)		12 (4.2%)		13 (4.6%)	
↓	↓	↓	↑	↓	-	-	↓
7 (2.5%)		0 (0%)		9 (3.2%)		9 (3.2%)	
Late Ext		Significant: 24/162 (14.9%)					
↑	↑	↑	↓	↑	-	-	↑
7 (4.3%)		0 (0%)		2 (1.2%)		2 (1.2%)	
↓	↓	↓	↑	↓	-	-	↓
2 (1.2%)		0 (0%)		6 (3.7%)		5 (3.1%)	
Cue Reinst		Significant: 98/255 (38.5%)					
↑	↑	↑	↓	↑	-	-	↑
17 (6.7%)		2 (0.8%)		14 (5.5%)		24 (9.4%)	
↓	↓	↓	↑	↓	-	-	↓
15 (5.9%)		1 (0.4%)		12 (4.7%)		13 (5.1%)	
Coc Reinstat		Significant: 82/195 (42.2%)					
↑	↑	↑	↓	↑	-	-	↑
14 (7.2%)		2 (1.0%)		15 (7.7%)		10 (5.1%)	
↓	↓	↓	↑	↓	-	-	↓
11 (5.6%)		1 (0.5%)		16 (8.2%)		13 (6.7%)	

For each stage of the study (self-administration, early and late extinction, and cue-induced and cocaine-induced reinstatement), neurons are separated into 8 categories based on whether or not they were significantly excited or inhibited in the 2-s epoch pre and/or post lever press

(one per recording array) and late extinction was the last 1–3 days of extinction preceding reinstatement (after at least 7 days of extinction). Lever press-related activity was diminished in both PL and IL during early extinction (Fig. 5a, b; PL: 23.0%, IL: 26.0% significantly modulated) and late extinction (Fig. 5e, f; PL: 14.8%, IL: 17.2% significantly modulated). During extinction, when cues/infusions were not delivered and timeouts were not enforced, animals would frequently press in bouts. When considering the leading press of these bouts as a measure of "infusion seeking" during extinction (see Methods), we found even fewer neurons modulated in both early (PL: 13.7%, IL: 18.8% significantly modulated) and late (PL: 14.8%, IL: 8.9%) extinction. We found no significant differences in either frequency or strength of press-related activity between PL and IL cells, whether considering all neurons, significantly modulated neurons, or absolute value of signaling (all $z < 1.8$, all $p > 0.05$).

The overall strength of press-related activity was also decreased in both PL and IL during extinction relative to self-administration. Using a rank-ordered two-factor ANOVA, we found a main effect of session (self-administration, early extinction, late extinction: $F(2,1455) = 3.85$; $p < 0.05$) but no effect of PL vs. IL ($F(1,1455) = 0.08$; $p > 0.05$) and no interaction ($F(2,1455) = 1.62$; $p > 0.05$). The absolute value of press-related activity across all neurons was substantially smaller during both early and late extinction compared to self-administration (Fig. 5a and e; $F(2,1455) = 7.22$, $p < 0.001$; rank-ordered two-factor ANOVA), and again, there were no effects of PL vs. IL ($F(1,1455) = 1.66$, $p > 0.05$) or interaction ($F(2,1455) = 0.07$, $p > 0.05$). The magnitude of press-related activity was not significantly different in neurons significantly modulated during extinction as compared to neurons that were significantly modulated during self-administration (PL or IL, excited or inhibited, all $p > 0.05$; rank-ordered two-factor ANOVA), even though the number of significantly modulated press-related neurons was decreased during extinction, demonstrating that diminished signaling during extinction was primarily due to decreased numbers of significant responses.

To determine whether neural activity during extinction was associated with overall motivation or seeking behavior, we correlated smoothed neural activity in 30-s bins with

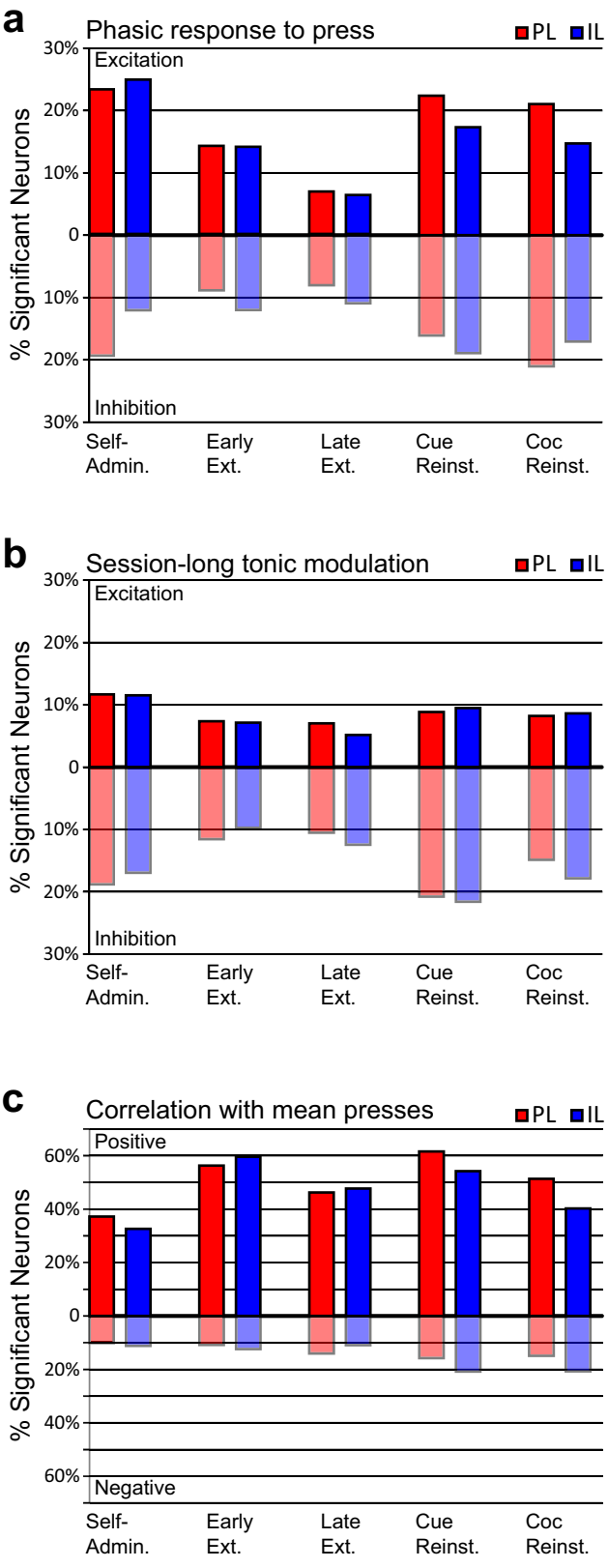

Fig. 7 Summary of proportions of neurons modulated across timeframes in PL and IL during self-administration, early and late extinction, and cue- and cocaine prime-induced reinstatement (see Methods for analysis epochs). **a** Proportions of neurons significantly excited (positive bars) and inhibited (negative bars) in each area at each stage. Of note, many neurons in both PL and IL exhibit activity significantly related to the lever press during self-administration, and this declines (in an equivalent fashion) over the course of extinction. In both cue- and prime-induced reinstatement, both PL and IL neurons are significantly responsive although, as shown in Fig. 6, the magnitude of response is significantly greater in PL vs. IL during cocaine-primed and, to a lesser degree, during cue-primed reinstatement. **b** Proportions of neurons significantly excited (positive bars) or inhibited (negative bars) across the entire session relative to pre-session baseline. Note the large proportion of neurons with significant session-long inhibition during cocaine-seeking epochs (self-administration and reinstatement sessions), and the overall diminution of modulation during extinction. **c** Proportions of neurons with tonic activity that was significantly positively (positive bars) or negatively (negative bars) correlated with patterns lever press at each stage of recording. Note that during extinction and reinstatement cocaine was not delivered (except for a priming injection in cocaine-induced reinstatement). Thus, significantly correlated activity in these stages likely reflects motivation- or response execution-related mPFC activity

smoothed press counts in 30-s bins in both early and late extinction. Consistent with the proposal that both PL and IL neuronal activity reflects motivational components of drug seeking even in the absence of drug, we found that large proportions of neurons in both PL and IL exhibited activity that was primarily positively correlated with pressing (Fig. 5c, d, and g). An example of a single IL neuron whose activity was highly correlated with mean lever presses is shown in Fig. 5c. Interestingly, correlation between activity and pressing persisted into late extinction (Fig. 5g) despite low numbers of presses overall (Fig. 1). These results indicate that, on moderate timescales (30 s), both PL and IL activity are associated with drug seeking behavior, even in extinction sessions.

PL and IL neural activity during reinstatement

We next measured mPFC activity during cue- and cocaine prime-induced reinstatement. All reinstatement sessions were separated by extinction sessions, and reinstatement sessions produced elevations in active lever pressing relative to extinction (Fig. 1). During both cue- and cocaine-induced reinstatement, the number of neurons significantly modulated at the time of lever press increased relative to activity during extinction (Fig. 6b, Tables 1 and 2). During cue-induced reinstatement, a similar proportion of PL and IL neurons responded around the time of lever press, and there were no significant differences in the strength of signaling between the two regions (all neurons, significantly modulated neurons, and absolute value of signaling: all $p > 0.05$).

During cocaine prime-induced reinstatement, the numbers of significantly modulated neurons in both PL and IL

Table 2 Phasic responses of infralimbic neurons separated the using the same categories as Table 1

Self-Admin		Significant: 113/307 (36.9%)					
Pre	Post	Pre	Post	Pre	Post	Pre	Post
↑	↑	↑	↓	↑	-	-	↑
13 (4.2%)		2 (0.7%)		29 (9.5%)		32 (10.4%)	
↓	↓	↓	↑	↓	-	-	↓
6 (2.0%)		1 (0.3%)		13 (4.2%)		17 (5.5%)	
Early Ext		Significant: 67/258 (26.0%)					
↑	↑	↑	↓	↑	-	-	↑
8 (3.1%)		0 (0.0%)		14 (5.4%)		14 (5.4%)	
↓	↓	↓	↑	↓	-	-	↓
11 (4.3%)		0 (0.0%)		12 (4.7%)		8 (3.1%)	
Late Ext		Significant: 33/192 (17.2%)					
↑	↑	↑	↓	↑	-	-	↑
0 (0.0%)		0 (0.0%)		5 (2.6%)		7 (3.7%)	
↓	↓	↓	↑	↓	-	-	↓
5 (2.6%)		0 (0.0%)		6 (3.1%)		10 (5.2%)	
Cue Reinst		Significant: 92/254 (36.3%)					
↑	↑	↑	↓	↑	-	-	↑
6 (2.4%)		0 (0.0%)		16 (6.3%)		22 (8.7%)	
↓	↓	↓	↑	↓	-	-	↓
16 (6.3%)		0 (0.0%)		17 (6.7%)		15 (5.9%)	
Coc Reinst		Significant: 54/170 (31.9%)					
↑	↑	↑	↓	↑	-	-	↑
2 (1.2%)		2 (1.2%)		12 (7.1%)		9 (5.3%)	
↓	↓	↓	↑	↓	-	-	↓
6 (3.5%)		1 (0.6%)		5 (2.9%)		17 (10.0%)	

were elevated compared to extinction, but a greater number of significantly modulated neurons were observed in PL than IL (Fig. 6f, Tables 1 and 2). The overall strength of signaling (measured as absolute value) was also significantly greater in PL than in IL during primed reinstatement (Fig. 6e; $z = 347$, $p < 0.001$). Interestingly, this difference was driven by neurons both phasically excited and inhibited at the time of lever press (Fig. 6f). When analyzing neurons that were significantly excited or inhibited around the press during primed reinstatement, we found that the magnitude of excitation of PL neurons was greater than that of IL neurons ($z = 3.22$, $p < 0.001$). There also was greater inhibition in PL neurons than in IL neurons, although this was not statistically significant ($z = 1.92$. $p = 0.055$). Thus, during cocaine prime-induced reinstatement, PL signaling was stronger than IL, and the strength of this signaling was driven by both excitatory and inhibitory responses around the time of lever press.

We characterized the relationship between slow changes (30 s) in activity and motivation based on binned lever presses in both types of reinstatement. An example of a single PL neuron whose activity was highly correlated with mean lever presses during cue-induced reinstatement is shown in Fig. 6c. Activity of neurons in both PL and IL was strongly correlated with pressing during both cue- and cocaine-induced reinstatement (Fig. 6d and g). As during self-administration and extinction, these correlations in activity were primarily positive, further supporting the direct relationship between neural activity and drug seeking independent of pharmacological effects of cocaine.

Comparison of significant effects across sessions

Figure 7 summarizes the percentages of PL and IL neurons exhibiting significant changes in phasic lever responses (Fig. 7a), significant session-long changes in activity (Fig. 7b), and significant correlations with mean pressing rates across the session (Fig. 7c). In addition to summarizing the findings of Figs. 3, 4, 5, and 6, inspection of these data shows a few additional points of interest. First, Fig. 7a makes clear that the proportion of significantly press-modulated (excited and inhibited) neurons in both PL and IL decreases over the course of extinction and rebounds during reinstatement. Interestingly, the proportion of neurons modulated is similar during cue- and cocaine prime-induced reinstatement even though the magnitude of responses is greater during prime reinstatement (compare Fig. 6a and b to Fig. 6e and f). Similar changes between extinction and self-administration/

reinstatement are seen in proportions of neurons exhibiting session-long modulation (Fig. 7b). Intriguingly, a slightly different pattern is seen when assessing the correlation of activity with mean pressing (Fig. 7c). In this case, the predominant pattern across neurons, positive correlation with pressing behavior, is amplified during extinction and reinstatement, potentially due to the decrease in pressing regularity in extinction/reinstatement vs. self-administration. Further interpretations are considered below.

Discussion

The prefrontal cortex has been repeatedly implicated regulation of cocaine seeking, but specific functions of mPFC subregions have been hard to isolate. We recorded neuronal activity in PL and IL, while rats self-administered cocaine, during extinction of cocaine seeking, and finally during cue- and cocaine-primed reinstatement conditions. Neural activity in both areas was strongly associated with cocaine seeking. PL and IL neuronal activity was significantly modulated during cocaine self-administration, activity in both areas was equivalently decreased during extinction, and activity was strongly enhanced during reinstatement, particularly in PL during cocaine prime-induced reinstatement. Although our results support previous findings for different roles of PL and IL in drug seeking (Gourley and Taylor 2016; Kalivas 2009; Kalivas et al. 2005; Peters et al. 2009), our findings also indicate that generalized hypotheses of PL/IL dichotomies in execution vs. inhibition of behavior do not reflect the full extent of the complex relationships between mPFC neurons and behavior (Caballero et al. 2019; Moorman and Aston-Jones 2015; Moorman et al. 2015; Riaz et al. 2019). Furthermore, we demonstrated two new signaling mechanisms by which PL and IL neurons encode information during self-administration, extinction, and reinstatement. First, we showed correlations between neural activity and calculated brain cocaine levels (during self-administration) or bouts of lever pressing, presumed to measure overall levels of motivation (during extinction and reinstatement). Second, we showed that mPFC activity shows tonic, session-related changes in activity that start at the onset of the session and persist until the end. Neural activity on these timescales has been reported in NAc, VP, and OFC (Fabbricatore et al. 2009; Guillem et al. 2010; Kravitz et al. 2006; Kravitz and Peoples 2008; Peoples et al. 1999, 1998; Root et al. 2012), but to our knowledge, never in mPFC. Our results indicate that mPFC neurons multiplex information related to lever presses, drug levels, motivation, and the overall task context—all within a single behavioral session, in line with the role of the mPFC as a highly integrative structure.

PL and IL neurons are significantly modulated during cocaine self-administration

We found that both PL and IL exhibit significant changes in activity during cocaine seeking. We found no significant differences between activity in PL and IL during cocaine self-administration. This observation was true for lever press-related activity, session-long tonic activity, and activity aligned to calculated cocaine levels. Neural responses were heterogeneous, exhibiting both excitation and inhibition at all timescales measured (Fig. 7, Tables 1 and 2). More neurons in both PL and IL were excited than inhibited during cocaine self-administration around the time of the lever press, though this excitation bias was more substantial for IL than PL neurons. These results support previous findings that PL is important during drug seeking behaviors, but they demonstrate that modulation of IL activity is prevalent during drug seeking as well. This finding is well-aligned with previous recording studies showing activation of both PL and IL neurons during cocaine self-administration (Muller Ewald et al. 2022; West et al. 2014), and does not support the perspective that PL neurons are selectively involved in execution of behaviors (e.g., cocaine seeking), or that IL neurons are selectively involved in inhibition of behaviors (e.g., extinction of cocaine seeking) (Moorman et al. 2015). That both PL and IL neurons exhibited heterogeneous response properties indicates that there is not a single functional role for either PL or IL and argues that networks of neurons within each region play different roles with respect to drug seeking (Warren et al. 2019).

In addition to phasic responses associated with lever pressing, we also observed two slower forms of firing rate changes. First, both PL and IL neurons exhibited strong session-related changes in activity that started immediately at the onset of the session and persisted throughout the duration of self-administration. Second, neurons also exhibited changes in activity that were highly correlated with fluctuations in brain cocaine levels during self-administration and with bouts of active lever presses during self-administration, extinction, and reinstatement. Importantly, there were very few neurons demonstrating correlated activity with inactive lever pressing behavior, arguing against a nonspecific motoric component of this signaling. These results are strikingly similar to findings from previous studies demonstrating slow modulation of neural activity in related brain areas such as NAc, VP, and OFC during cocaine seeking (Fabbricatore et al. 2009; Guillem et al. 2010; Peoples and Cavanaugh 2003; Peoples et al. 1999, 1998; Root et al. 2012), although this is the first time that these dynamics have been characterized in the mPFC. The potential role of tonic inhibition/excitation during task is unclear but intriguing, particularly given the observed bias towards tonic inhibition (Fig. 7b). It is clearly not exclusively driven by cocaine since the

relative proportions are similar in extinction and reinstatement (though reduced in extinction overall). Cocaine history, as experienced by the rats here, could result in mPFC hypofunction (Chen et al. 2013). However, tonic inhibition has been seen in OFC and NAc during sucrose self-administration sessions (Kravitz et al. 2006; Kravitz and Peoples 2008), indicating that multiple factors, motivational, cognitive, pharmacological, or otherwise, are driving this tonic change in firing.

Neuronal activity, in both PL and IL, that was inversely correlated with calculated cocaine levels during self-administration may be related to a direct inhibitory influence of cocaine on mPFC neurons (Qiao et al. 1990) and/or complex excitatory and inhibitory effects of dopamine itself on mPFC neurons (Seamans and Yang 2004). Inverse correlations may also represent indirect modulation resulting from drug acquisition, subjective "high," or satiety (Risinger et al. 2005). The relationship between activity positively correlated with response execution and negatively (and in some cases positively) correlated with drug levels may reflect an inherent heterogeneity in prefrontal neural networks whereby some neurons participate in representation of drug reward, whereas others are more directly involved in driving drug seeking behavior. The interaction among these networks of neurons is an important issue for future study. Importantly, correlation with pressing persisted and was even increased during extinction and reinstatement vs. during self-administration (Fig. 7c), indicating that a major feature of this temporal signaling framework is associated with motivation or action, even in the absence of primary drug reinforcement.

Together, these results indicate that interrelated networks may encode drug seeking information and behaviors across multiple timeframes. Given the complexity of mPFC representations, neural activity at different timescales allows networks of mPFC neurons to multiplex information related to specific behaviors (e.g., phasic activity), drug levels or motivation (cocaine-level and press-correlated activity), and context (session-long activity). Future research will be critical in understanding how different networks are delineated and in understanding which ones are disrupted in cocaine addiction.

PL and IL neuronal activity diminished across extinction sessions

During extinction, we observed an overall diminution of task-related activity in both PL and IL neurons that became more prevalent over days of extinction learning. Phasic excitation and inhibition as well as tonic excitation and inhibition were decreased in both PL and IL during extinction. This was true when activity was measured either as a proportion of neurons exhibiting significant responses or as the magnitudes of responses. The finding that neither PL nor IL neurons selectively represented extinction of cocaine seeking, either early or late in extinction learning, was surprising. Based on the role of IL in extinction of cocaine seeking and conditioning (Muller Ewald and LaLumiere 2017; Peters et al. 2008) and in extinction of fear conditioning (Milad and Quirk 2002), we expected to see enhanced IL activity during extinction. It is possible that the small population of neurons we found to be responsive during extinction sessions was responsible for extinction learning and suppression of behavior. Another possibility is that different tasks engage mPFC neurons differently, even when the overall phenomenon (e.g., extinction learning) is the same. However, recent studies demonstrated that PL may participate in inhibiting cocaine seeking (Chen et al. 2013; Martin-Garcia et al. 2014; Mihindou et al. 2013), and IL may help drive seeking of cocaine and other drugs of abuse (Bossert et al. 2011; Koya et al. 2009; Peters et al. 2013; Rocha and Kalivas 2010); these results support our findings for a complex relationship between PL/IL and drug self-administration and extinction.

Importantly, two previous studies measured PL and IL neuronal activity during extinction. West and colleagues measured activity in both areas during lever pressing under extinction and found press-related excitation and inhibition in both areas, with a slight bias towards a higher proportion of responsive neurons in IL (West et al. 2014). Of note, however, is that neural activity was not recorded after multiple extinction sessions, so these results do not speak to the impact of extended extinction learning on mPFC activity. Muller Ewald and colleagues recorded from IL during extinction sessions in a trial-based task in which animals could press or withhold pressing a cocaine-associated lever on each trial (Muller Ewald et al. 2022). These authors found reported changes in IL neuronal activity and theta local field potentials during extinction, although PL neural activity was not recorded. Intriguingly, activity was strong on trials where rats withheld pressing a lever during extinction, confirming a similar observation seen in both PL and IL neurons during withholding of lever presses in extinction of sucrose seeking (Moorman and Aston-Jones 2015). The authors also reported changes in activity for non-lever related events, in line with the tonic changes in activity reported here.

In our study, neurons continued to exhibit activity correlated with bouts of active lever pressing during extinction or reinstatement despite the absence of cocaine delivery. These results indicate that, at slower timescales (e.g., ~ 30 s), activity in both PL and IL may reflect behavioral functions such as goal-directed response execution or motivation, independent of drug availability. Interestingly, whereas correlations between activity and calculated drug levels were frequently negative during self-administration, correlations with pressing were typically positive. These results indicate that neurons whose activity is positively correlated with lever

pressing may be related to cocaine seeking behaviors such as the initiation of a seeking response, even after prolonged extinction. An alternate explanation is that activity in these neurons signals expectation of cocaine reward, even in the absence of cocaine.

PL and, to a lesser extent, IL activity was significantly elevated during reinstatement, particularly during cocaine-primed reinstatement

During reinstatement, mPFC neural signaling increased to or above levels observed during cocaine self-administration. The strongest reinstatement-related enhancement was primarily observed in PL rather than IL neurons, particularly during cocaine prime-induced reinstatement. This indicates that important differences exist between PL and IL, particularly during reinstatement or relapse conditions. These results support previously observed roles for PL neurons in reinstatement of cocaine seeking (James et al. 2018; McFarland et al. 2004, 2003; McGlinchey et al. 2016; McLaughlin and See 2003; Stefanik et al. 2013). Our observation of increased activity during reinstatement, particularly in PL neurons, is in line with the finding of West and colleagues who reported increased PL (and not IL) activity during cocaine seeking after 30 days of abstinence (West et al. 2014), as well as with that of Muller Ewald and colleagues who reported limited IL activity during cocaine-primed reinstatement (Muller Ewald et al. 2022). An important novel aspect our results can be seen in Figs. 6 and 7. Although the overall proportion of neurons responding during cocaine-primed reinstatement is similar to that seen during self-administration and cue-induced reinstatement (Fig. 7a), the magnitude of responding is significantly greater (Fig. 6e and f). This suggests that, rather than recruiting additional neurons to produce a population effect to drive reinstatement, PL neuron activity is augmented. Exactly what this enhanced PL activation corresponds to (e.g., craving, increased behavioral activation, etc.) remains to be determined and would benefit from direct comparisons of the same neuron in different conditions. To our knowledge, a comparison of PL and IL neuronal activity, specifically during reinstatement of cocaine seeking, has not been previously reported, thus extending findings reported in previous studies.

Although a sizeable proportion of PL neurons was excited during reinstatement, some PL (and some IL) neurons were significantly inhibited. This prominent inhibition in PL during relapse corresponds with findings that PL activation inhibits, and PL inactivation enhances, cocaine seeking in certain circumstances (Chen et al. 2013; Martin-Garcia et al. 2014; Mihindou et al. 2013). Thus, suppressed activity in PL neurons may correspond to the loss of behavioral inhibitory control associated with relapse seen in these previous papers.

Differences in behavioral context may also account for differences observed across studies. In studies in which behavioral response inhibition is critical to, e.g., avoid an aversive outcome (Chen et al. 2013), PL neurons appear to play a major role. In our previous work, both PL and IL neurons exhibited context-dependent modulation—firing more strongly for sucrose-rewarded lever presses and for withheld presses during extinction (Moorman and Aston-Jones 2015) although, as observed here, there were differences in firing properties across regions in different phases of the task. Additionally, context may be signaled by session-long tonic modulations—for example, indicating whether the rat is either in or out of the behavioral session. Given the role of mPFC in representation and use of contextual information (Euston et al. 2012; Hyman et al. 2012; Thomas et al. 2020), understanding how this cognitive property of mPFC interacts with models of drug seeking will be highly valuable in developing sophisticated models of addiction. In addition, aligning behavioral assays to test drug seeking in different contexts (e.g., reinstatement, abstinence, discrete trials) will be essential in understanding specific neuronal contributions to these behaviors.

Taken together, our findings demonstrate a complex heterogeneity of PL and IL neural activity during cocaine self-administration, extinction, and reinstatement. This heterogeneity is not surprising given the diverse range of connectivity of these cortical regions (Gabbott et al. 2005; Heidbreder and Groenewegen 2003; Sesack et al. 1989), and previous observations that different ensembles in these areas are activated by execution or inhibition of drug seeking (Warren et al. 2019). It is very likely the case that the observed differences in neuronal activity between PL and IL activity, in both our study and in others, are at least a product of different connectivity profiles. In the light of the divergent connectivity of PL and IL, it is almost more striking that we observed so many similar responses between the areas. An important future direction will be to characterize similar vs. different response profiles of specific mPFC ensembles based, for example, on anatomical connectivity.

A major goal of this study was to test for a strictly dichotomous contribution of PL and IL to drug seeking and inhibition of seeking during extinction, something that was not apparent from our results. However, beyond this lack of go/stop dichotomy, we revealed multifaceted signaling patterns of mPFC neurons in which drug level, behavior, and context were integrated in the activity of single neurons in both areas. Drug use and addiction are made up of a complex suite of action, emotion, and cognition, incorporating learning and memory, context, attention, goal-directed and habitual behaviors, and motivation and stress, among many other features. The mPFC has been broadly implicated in all

of these functions, indicating an important but complex contribution of mPFC circuits in the regulation of drug seeking. By increasing the sophistication of our studies via cell-type and circuit-specific analysis across multiple behaviors, the field is well-positioned for major advances in understanding precise roles of mPFC neurons in drug use and dependence.

Funding The study is supported by PHS grants R21-DA032005, P50-DA015369, R01-DA006214, R01-MH092868, P50-AA010761, and UL1-RR029882.

Declarations

Competing interests The authors declare no competing interests.

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. J Neuroscience 36:10174–10180

Balleine BW, O'Doherty JP (2010) Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. Neuropsychopharmacology 35:48–69

Bentzley BS, Fender KM, Aston-Jones G (2013) The behavioral economics of drug self-administration: a review and new analytical approach for within-session procedures. Psychopharmacology 226:113–125

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. Nat Neurosci 14:420–422

Bossert JM, Marchant NJ, Calu DJ, Shaham Y (2013) The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology 229:453–476

Caballero JP, Scarpa GB, Remage-Healey L, Moorman DE (2019) Differential Effects of dorsal and ventral medial prefrontal cortex inactivation during natural reward seeking, extinction, and cue-induced reinstatement. eNeuro 6.

Cameron CM, Murugan M, Choi JY, Engel EA, Witten IB (2019) Increased cocaine motivation is associated with degraded spatial and temporal representations in IL-NAc neurons. Neuron 103(80–91):e7

Capuzzo G, Floresco SB (2020) Prelimbic and Infralimbic prefrontal regulation of active and inhibitory avoidance and reward-seeking. J Neuroscience 40:4773–4787

Chang JY, Sawyer SF, Paris JM, Kirillov A, Woodward DJ (1997) Single neuronal responses in medial prefrontal cortex during cocaine self-administration in freely moving rats. Synapse 26:22–35

Chang JY, Janak PH, Woodward DJ (1998) Comparison of mesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. J Neuroscience 18:3098–3115

Chang JY, Janak PH, Woodward DJ (2000) Neuronal and behavioral correlations in the medial prefrontal cortex and nucleus accumbens during cocaine self-administration by rats. Neuroscience 99:433–443

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

Coutureau E, Killcross S (2003) Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. Behav Brain Res 146:167–174

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev 28:771–784

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

Fabbricatore AT, Ghitza UE, Prokopenko VF, West MO (2009) Electrophysiological evidence of mediolateral functional dichotomy in the rat accumbens during cocaine self-administration: tonic firing patterns. Eur J Neurosci 30:2387–2400

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145–177

Ghazizadeh A, Ambroggi F, Odean N, Fields HL (2012) Prefrontal cortex mediates extinction of responding by two distinct neural mechanisms in accumbens shell. J Neuroscience 32:726–737

Giustino TF, Maren S (2015) The role of the medial prefrontal cortex in the conditioning and extinction of fear. Front Behav Neurosci 9:298

Gourley SLSL, Taylor JRJR (2016) Going and stopping: dichotomies in behavioral control by the prefrontal cortex. Nat Neurosci 19:656–664

Green JT, Bouton ME (2021) New functions of the rodent prelimbic and infralimbic cortex in instrumental behavior. Neurobiol Learn Mem 185:107533

Guillem K, Kravitz AV, Moorman DE, Peoples LL (2010) Orbitofrontal and insular cortex: neural responses to cocaine-associated cues and cocaine self-administration. Synapse 64:1–13

Gutman AL, Ewald VA, Cosme CV, Worth WR, LaLumiere RT (2017) The infralimbic and prelimbic cortices contribute to the inhibitory control of cocaine-seeking behavior during a discriminative stimulus task in rats. Addict Biol 22:1719–1730

Halladay LR, Kocharian A, Piantadosi PT, Authement ME, Lieberman AG, Spitz NA, Coden K, Glover LR, Costa VD, Alvarez VA, Holmes A (2020) Prefrontal regulation of punished ethanol self-administration. Biol Psychiat 87:967–978

Hearing MC, Miller SW, See RE, McGinty JF (2008) Relapse to cocaine seeking increases activity-regulated gene expression differentially in the prefrontal cortex of abstinent rats. Psychopharmacology 198:77–91

Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27:555–579

Howland JG, Ito R, Lapish CC, Villaruel FR (2022) The rodent medial prefrontal cortex and associated circuits in orchestrating adaptive behavior under variable demands. Neurosci Biobehav Rev 135:104569

Hyman JM, Ma L, Balaguer-Ballester E, Durstewitz D, Seamans JK (2012) Contextual encoding by ensembles of medial prefrontal cortex neurons. Proc Natl Acad Sci U S A 109:5086–5091

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

James MH, McGlinchey EM, Vattikonda A, Mahler SV, Aston-Jones G (2018) Cued reinstatement of cocaine but not sucrose seeking is dependent on dopamine signaling in prelimbic cortex and is associated with recruitment of prelimbic neurons that project to contralateral nucleus accumbens core. Int J Neuropsychopharmacology 21:89–94

Jonkman S, Mar AC, Dickinson A, Robbins TW, Everitt BJ (2009) The rat prelimbic cortex mediates inhibitory response control but

not the consolidation of instrumental learning. Behav Neurosci 123:875–885
Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 10:561–572
Kalivas PW, Volkow ND (2011) New medications for drug addiction hiding in glutamatergic neuroplasticity. Mol Psychiatry 16:974–986
Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45:647–650
Kaminska B, Caballero JP, Moorman DE (2021) Integration of value and action in medial prefrontal neural systems. Int Rev Neurobiol 158:57–82
Kane L, Venniro M, Quintana-Feliciano R, Madangopal R, Rubio FJ, Bossert JM, Caprioli D, Shaham Y, Hope BT, Warren BL (2021) Fos-expressing neuronal ensemble in rat ventromedial prefrontal cortex encodes cocaine seeking but not food seeking in rats. Addict Biol 26:e12943
Killcross S, Coutureau E (2003) Coordination of actions and habits in the medial prefrontal cortex of rats. Cereb Cortex 13:400–408
Koya E, Uejima JL, Wihbey KA, Bossert JM, Hope BT, Shaham Y (2009) Role of ventral medial prefrontal cortex in incubation of cocaine craving. Neuropharmacology 56(Suppl 1):177–185
Kravitz AV, Peoples LL (2008) Background firing rates of orbitofrontal neurons reflect specific characteristics of operant sessions and modulate phasic responses to reward-associated cues and behavior. J Neuroscience 28:1009–1018
Kravitz AV, Moorman DE, Simpson A, Peoples LL (2006) Session-long modulations of accumbal firing during sucrose-reinforced operant behavior. Synapse 60:420–428
Madangopal R, Ramsey LA, Weber SJ, Brenner MB, Lennon VA, Drake OR, Komer LE, Tunstall BJ, Bossert JM, Shaham Y, Hope BT (2021) Inactivation of the infralimbic cortex decreases discriminative stimulus-controlled relapse to cocaine seeking in rats. Neuropsychopharmacology 46:1969–1980
Marchant NJ, Furlong TM, McNally GP (2010) Medial dorsal hypothalamus mediates the inhibition of reward seeking after extinction. J Neuroscience 30:14102–14115
Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. Nat Rev Neurosci 5:844–852
Martin-Garcia E, Courtin J, Renault P, Fiancette JF, Wurtz H, Simonnet A, Levet F, Herry C, Deroche-Gamonet V (2014) Frequency of cocaine self-administration influences drug seeking in the rat: optogenetic evidence for a role of the prelimbic cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.
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. J 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. J 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. J 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. Neurobiol Learn Mem 118:198–208
Mihindou C, Guillem K, Navailles S, Vouillac C, Ahmed SH (2013) Discriminative inhibitory control of cocaine seeking involves the prelimbic prefrontal cortex. Biol Psychiat 73:271–279
Milad MR, Quirk GJ (2002) Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70–74
Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202
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 Res 1628:130–146
Morgan MA, LeDoux JE (1995) Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. Behav Neurosci 109:681–688
Muller Ewald VA, Kim J, Farley SJ, Freeman JH, LaLumiere RT (2022) Theta oscillations in rat infralimbic cortex are associated with the inhibition of cocaine seeking during extinction. Addict Biol 27:e13106
Muller Ewald VA, LaLumiere RT (2017) Neural systems mediating the inhibition of cocaine-seeking behaviors. Pharmacol Biochem Behav.
Nett KE, LaLumiere RT (2021) Infralimbic cortex functioning across motivated behaviors: can the differences be reconciled? Neurosci Biobehav Rev 131:704–721
Pan HT, Menacherry S, Justice JB Jr (1991) Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J Neurochem 56:1299–1306
Peoples LL, Cavanaugh D (2003) Differential changes in signal and background firing of accumbal neurons during cocaine self-administration. J Neurophysiol 90:993–1010
Peoples LL, Uzwiak AJ, Guyette FX, West MO (1998) Tonic inhibition of single nucleus accumbens neurons in the rat: a predominant but not exclusive firing pattern induced by cocaine self-administration sessions. Neuroscience 86:13–22
Peoples LL, Uzwiak AJ, Gee F, West MO (1999) Tonic firing of rat nucleus accumbens neurons: changes during the first 2 weeks of daily cocaine self-administration sessions. Brain Res 822:231–236
Peters J, De Vries TJ (2013) D-cycloserine administered directly to infralimbic medial prefrontal cortex enhances extinction memory in sucrose-seeking animals. Neuroscience 230:24–30
Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neuroscience 28:6046–6053
Peters J, Kalivas PW, Quirk GJ (2009) Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 16:279–288
Peters J, Pattij T, De Vries TJ (2013) Targeting cocaine versus heroin memories: divergent roles within ventromedial prefrontal cortex. Trends Pharmacol Sci 34:689–695
Qiao JT, Dougherty PM, Wiggins RC, Dafny N (1990) Effects of microiontophoretic application of cocaine, alone and with receptor antagonists, upon the neurons of the medial prefrontal cortex, nucleus accumbens and caudate nucleus of rats. Neuropharmacology 29:379–385
Rhodes SE, Killcross S (2004) Lesions of rat infralimbic cortex enhance recovery and reinstatement of an appetitive Pavlovian response. Learn Mem 11:611–616
Rhodes SE, Killcross AS (2007) Lesions of rat infralimbic cortex enhance renewal of extinguished appetitive Pavlovian responding. Eur J Neurosci 25:2498–2503
Riaz S, Puveendrakumaran P, Khan D, Yoon S, Hamel L, Ito R (2019) Prelimbic and infralimbic cortical inactivations attenuate contextually driven discriminative responding for reward. Sci Rep 9:3982

Risinger RC, Salmeron BJ, Ross TJ, Amen SL, Sanfilipo M, Hoffmann RG, Bloom AS, Garavan H, Stein EA (2005) Neural correlates of high and craving during cocaine self-administration using BOLD fMRI. Neuroimage 26:1097–1108

Rocha A, Kalivas PW (2010) Role of the prefrontal cortex and nucleus accumbens in reinstating methamphetamine seeking. Eur J Neurosci 31:903–909

Root DH, Fabbricatore AT, Pawlak AP, Barker DJ, Ma S, West MO (2012) Slow phasic and tonic activity of ventral pallidal neurons during cocaine self-administration. Synapse 66:106–127

Rozeske RR, Herry C (2018) Neuronal coding mechanisms mediating fear behavior. Curr Opin Neurobiol 52:60–64

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 74:1–58

Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213–242

Shipman ML, Trask S, Bouton ME, Green JT (2018) Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. Neurobiol Learn Mem 155:164–172

Smith RJ, See RE, Aston-Jones G (2009) Orexin/hypocretin signaling at the orexin 1 receptor regulates cue-elicited cocaine-seeking. Eur J Neurosci 30:493–503

Smith KS, Virkud A, Deisseroth K, Graybiel AM (2012) Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. Proc Natl Acad Sci USA 109:18932–18937

Smith KS, Graybiel AM (2013) A dual operator view of habitual behavior reflecting cortical and striatal dynamics. Neuron.

Stefanik MT, Moussawi K, Kupchik YM, Smith KC, Miller RL, Huff ML, Deisseroth K, Kalivas PW, LaLumiere RT (2013) Optogenetic inhibition of cocaine seeking in rats. Addict Biol 18:50–53

Sun W, Rebec GV (2006) Repeated cocaine self-administration alters processing of cocaine-related information in rat prefrontal cortex. The Journal of Neuroscience : the Official Journal of the Society for Neuroscience 26:8004–8008

Thomas CMP, Thrailkill EA, Bouton ME, Green JT (2020) Inactivation of the prelimbic cortex attenuates operant responding in both physical and behavioral contexts. Neurobiol Learn Mem 171:107189

Villaruel FR, Lacroix F, Sanio C, Sparks DW, Chapman CA, Chaudhri N (2018) Optogenetic activation of the infralimbic cortex suppresses the return of appetitive Pavlovian-conditioned responding following extinction. Cereb Cortex 28:4210–4221

Villaruel FR, Martins M, Chaudhri N (2022) Corticostriatal suppression of appetitive Pavlovian conditioned responding. J Neuroscience 42:834–849

Warren BL, Kane L, Venniro M, Selvam P, Quintana-Feliciano R, Mendoza MP, Madangopal R, Komer L, Whitaker LR, Rubio FJ, Bossert JM, Caprioli D, Shaham Y, Hope BT (2019) Separate vmPFC ensembles control cocaine self-administration versus extinction in rats. J Neuroscience 39:7394–7407

West EA, Saddoris MP, Kerfoot EC, Carelli RM (2014) Prelimbic and infralimbic cortical regions differentially encode cocaine-associated stimuli and cocaine-seeking before and following abstinence. Eur J Neurosci 39:1891–1902

Willcocks AL, McNally GP (2013) The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. Eur J Neurosci 37:259–268

Zimmer BA, Dobrin CV, Roberts DC (2011) Brain-cocaine concentrations determine the dose self-administered by rats on a novel behaviorally dependent dosing schedule. Neuropsychopharmacology 36:2741–2749