Role of the medial prefrontal cortex in the extinction of conditioned appetitive behaviour in rats

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Abstract

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The infralimbic medial prefrontal cortex (IL-PFC) has been posited as a common node in distinct neural circuits that mediate the extinction of appetitive and aversive conditioning. However, appetitive extinction is typically assessed using instrumental conditioning procedures, whereas the extinction of aversive conditioning is studied using Pavlovian fear-conditioning. The role of the IL-PFC in the extinction of appetitive conditioning acquired through Pavlovian learning remains largely unexplored. The present studies utilized animal models of Pavlovian- and instrumental-conditioning with sucrose to study the involvement of the IL-PFC in appetitive extinction. Based on fear-extinction we predicted that inactivating the IL-PFC before extinction would have minimal effect on within-session extinction, but would impair the storage of extinction memory. Control studies were conducted in the prelimbic prefrontal cortex (PL-PFC), which is not involved in extinction. PL-PFC inactivation did not affect the acquisition or recall of extinction memory. Counter to our predictions, inactivating the IL-PFC facilitated the extinction of conditioned Pavlovian- and instrumental sucrose-seeking, with no effect on extinction recall tested 24 hr later. In separate studies, inactivating the IL-PFC during a Pavlovian conditioning session in which cue presentations were paired with sucrose did not affect cue-elicited behaviour, but increased responding during inter-trial intervals. The same manipulation performed during instrumental conditioning did not impact lever pressing for sucrose. These findings contradict a growing body of literature suggesting that the IL-PFC is important for the acquisition and consolidation of extinction memory in appetitive conditioning tasks.

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General Introduction

Pavlovian conditioning is a fundamental form of learning that is important for adaptive behaviour. The ability to predict, acquire, terminate, or avoid salient environmental events are all examples of flexibility in behaviour that helps animals to survive. Equally adaptive is the capacity to withhold behaviour when an expected outcome no longer occurs. This process is mediated by extinction learning, a form of inhibitory learning that manifests as a gradual reduction in conditioned behaviour in the absence of an anticipated outcome. In humans, failing to inhibit responses to environmental stimuli that no longer predict an outcome is a characteristic observed in drug addicts (Garavan & Hester, 2007; Kiefer & Dinter, 2013) and individuals suffering from anxiety disorders (Andero & Ressler, 2012; Bouton, Mineka, & Barlow, 2001; Rothbaum & Davis, 2003). Such behaviour is maladaptive, and may be attributed to malfunctioning neural circuits that mediate Pavlovian learning. Thus, it is of value to investigate the neural mechanisms involved in extinction learning, as it may lead to the development of more efficient therapeutic treatments of addiction and anxiety disorders.

The neural mechanisms that mediate extinction learning have been studied extensively using animal models (for review see Quirk & Mueller, 2008). Studies investigating the extinction of Pavlovian conditioned responses typically involve two phases; a conditioning phase and an extinction phase. During conditioning, training consists of repeatedly pairing a conditioned stimulus (CS) with an unconditioned stimulus (US). After several CS-US pairings, animals learn the predictive properties of the CS. This learning is expressed behaviourally in animals when the CS elicits a conditioned response (CR). Extinction is then conducted by repeatedly presenting the CS in the absence of the US, which results in a gradual reduction in conditioned responding. Observing how quickly conditioned responses diminish across CS trials during extinction

provides a measure of extinction performance. Additionally, extinction recall can be assessed by observing the level of conditioned responding recovered during a subsequent extinction session, as well as by examining how quickly recovered conditioned responses diminish across CS trials. Studies investigating the neural mechanisms that regulate the acquisition of extinction manipulate neuronal activity in the brain region of interest immediately before the first extinction session. By comparison, studies investigating the neural substrates involved in the memory of extinction manipulate neuronal activity at several time-points immediately, or shortly after the first extinction session, and assess extinction performance during a recall test session which is typically conducted either 1 hr or 24 hrs after the initial extinction session.

Instrumental conditioning is the process by which an organism learns to perform an action to obtain a reinforcer. For example, pressing a lever to obtain a drug-reinforcer is a form of instrumental conditioning that is typically used in appetitive conditioning. Akin to Pavlovian conditioned behaviour, instrumental conditioned behaviour can be extinguished by no longer delivering the reinforcer, causing a reduction in instrumental responses. However, most of the work on extinction has utilized models of Pavlovian conditioning. Thus, numerous hypotheses have been proposed to describe the behavioural and neurobiological mechanisms that underlie extinction within the context of Pavlovian learning. For example, it has been proposed that conditioned responding decreases during extinction because the CS-US association formed during Pavlovian conditioning is forgotten, unlearned, or erased (Rescorla & Wagner, 1972). However, there is evidence against these hypotheses. For instance, extinguished conditioned responding re-emerges following an extended period of rest (Corty & Coon, 1995; Hammersley, 1992; Tobeña et al., 1993). This phenomenon is known as spontaneous recovery, and is one of several pieces of evidence suggesting that the CS-US memory that was formed during Pavlovian

conditioning is not erased, unlearned, or forgotten during extinction. Instead, it has been proposed that decreased conditioned responding during extinction is the result of a new association (CS-no US) that is formed when a CS is repeatedly presented in the absence of the US (Bouton, 2004). Thus, animals learn to inhibit their behaviour in response to the CS. This hypothesis suggests that specific neural substrates required for learning the new CS-no US association are recruited during extinction and are responsible for inhibiting conditioned behaviour.

Much of what is currently known about the neural mechanisms involved in extinction originates from fear conditioning studies, in which organisms learn to predict aversive stimuli via Pavlovian conditioning. Such studies have suggested that the medial prefrontal cortex (mPFC) is a key component of the neural circuitry that mediates extinction (Morgan, Romanski, & Ledoux, 1993; Milad & Quirk, 2002; Thompson et al., 2010; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011). In addition, subdivisions of the mPFC, including the infralimbic PFC (IL-PFC) and prelimbic PFC (PL-PFC) have been shown to play differential roles in behaviour. Whereas the IL-PFC mediates the acquisition and recall of extinction, the PL-PFC has been shown to promote conditioned responding (Laurent & Westbrook, 2009; Milad, Vidal-Gonzalez, & Quirk, 2004; Sierra-Mercado et al., 2011; Thompson et al., 2010; Vidal-Gonzalez, Vidal-Gonzalez, Rauch, & Quirk, 2006).

Converging evidence in fear conditioning studies supports the idea that the IL-PFC promotes extinction by inhibiting conditioned fear behaviour (Laurent & Westbrook, 2009; Milad et al., 2004; Sierra-Mercado et al., 2011; Thompson et al., 2010; Vidal Gonzalez et al., 2006). Similarly, the IL-PFC has been shown to inhibit conditioned appetitive behaviour (Ovari & Leri, 2008; Lalumiere, Smith, & Kalivas, 2012; Peters, Lalumiere, & Kalivas, 2008). Indeed,

it has recently been proposed that the IL-PFC mediates the extinction of behaviour acquired through either aversive or appetitive conditioning (Peters, Kalivas, & Quirk, 2009). However, conditioned fear behaviour is typically studied using Pavlovian learning models, whereas conditioned appetitive behaviour predominantly employs instrumental procedures. Thus, the role of the IL-PFC in mediating the extinction of conditioned Pavlovian appetitive behaviour has not yet been examined. The experiments presented in this thesis investigated the neural mechanism involved in extinction of conditioned Pavlovian and instrumental appetitive behaviour. More specifically, the role of the IL-PFC and PL-PFC in extinction of a conditioned sucrose-seeking response in rats was examined. We predicted that IL-PFC, but not PL-PFC inactivation would delay the acquisition and recall of extinction.

Behavioural evidence that extinction results in new learning

It has previously been suggested that extinction is a process that involves the erasure, unlearning, or forgetting of a previously formed memory (Rescorla & Wagner, 1972). However, several behavioural phenomena suggest that extinction involves new learning. These phenomena include spontaneous recovery, renewal, and reinstatement.

Spontaneous recovery involves a reappearance of a previously extinguished response, and is induced by passage of time (Corty & Coon, 1995; Hammersley, 1992; Tobeña et al., 1993). For example, the behaviour of pressing a lever to obtain a reward can be extinguished by omitting reward delivery. Following a period of rest in which the animal does not have access to the lever, a recovery in lever-pressing occurs when the lever is presented again. Thus, the sudden reappearance of the conditioned response indicates that extinction does not eliminate what was learned during training.

Renewal is defined by a recovery in conditioned responding when an organism is removed from the extinction context. For example, if Pavlovian training is conducted in one context and extinction is conducted in another, subsequent re-exposure to the Pavlovian training context results in the reappearance of the extinguished conditioned response. Interestingly, exposure to a novel context following training and extinction in different contexts also results in renewal (Bouton, Todd, Vurbic, & Winterbauer, 2011; Neumann & Kitlertsirivatana, 2010). This context-induced increase in conditioned responding demonstrates that the original CS-US association is not erased, unlearned, or forgotten during extinction, and that animals learn to associate the environment in which extinction occurs with the absence of the US. Renewal studies in particular suggest that extinction results in the formation of a new CS-no US association, and if extinction is conducted in a context that differs from the training context, then that association becomes linked to the environment in which extinction occurred (Bouton, 2000, 2002, 2004; Chaudhri, Sahuque, & Janak, 2008).

The reinstatement phenomenon also provides evidence that extinction does not eliminate conditioned behaviour. Following extinction, conditioned responding can be reactivated by presenting the organism with different stimuli, including exposure to the US, a US-predictive cue, or a stressor (Kalivas, Peters, & Knackstedt, 2006; Shalev, Grimm, & Shaham, 2002; Sinha, Fuse, Aubin, & O'Malley, 2000; Stewart, 2000). For example, an extinguished drug-seeking response can be restored by presenting the animal with an auditory cue that was previously paired with drug delivery.

The examples described above indicate that extinction does not 'erase' or result in the 'unlearning' of the original learning acquired during conditioning. The prevailing interpretation of these behavioural findings is that repeated presentation of the CS alone during extinction leads

to the acquisition of a new inhibitory CS-no US association, causing a gradual reduction in CS-elicited responses. Thus, extinction is said to involve 'new learning' through which organisms come to inhibit behaviour in response to a CS. A similar explanation is used to describe extinction of instrumental responses, such that during extinction the organism learns to inhibit behaviour that no longer results in the expected outcome. Important extensions of the hypothesis that extinction results in new learning are that (a) there must be underlying neural machinery that supports this new learning, and that (b) this machinery might be similar to that which is utilized to form the original associations during conditioning. Considerable effort has gone into identifying the mechanisms that are important for extinction learning and memory using Pavlovian fear-conditioning studies, and more recently, appetitive conditioning studies. Research in both these domains has converged on the medial prefrontal cortex as being an important region for the acquisition and storage of extinction memory.

Involvement of the IL-PFC in the extinction of conditioned fear behaviour

A number of studies have demonstrated that the infralimbic medial prefrontal cortex (IL-PFC) is important for inhibiting conditioned fear behaviour during the acquisition of extinction (Quirk, Russo, Barron, & Lebron, 2000; Sierra-Mercado et al., 2011). For example, Sierra-Mercado et al. (2011) found that pharmacological inactivation of the IL-PFC delays fear extinction in rats. To assess the role of the IL-PFC in extinction, rats were initially trained to associate an auditory tone (CS) with a foot-shock (US) by repeatedly presenting tone-shock pairings. Prior to the first extinction session in which the tone was repeatedly presented in the absence of a foot-shock, animals were either infused with a Gamma-aminobutyric acid (GABA) agonist (muscimol) or saline in the IL-PFC. Inactivating the IL-PFC via muscimol infusions caused persistent freezing to CS presentations during extinction, resulting in significantly slower

extinction compared to controls. Consistent with these findings, activation of IL-PFC neurons via GABAa antagonist infusions (Thompson et al., 2010) or electrical stimulation (Milad & Quirk, 2002; Milad et al., 2004; Vidal-Gonzalez et al., 2006) has been shown to inhibit conditioned fear responses during the acquisition of extinction. Together, these findings suggest that neuronal activity in the IL-PFC is essential for inhibiting conditioned fear behaviour during this acquisition of extinction learning.

The IL-PFC has also been implicated in the consolidation of extinction memory, and several studies have found a relationship between IL-PFC function and the recall of extinction. For example, lesions of the IL-PFC impair extinction recall (Lebron, Milad, & Quirk, 2004; Quirk et al., 2000). Similarly, pharmacological inactivation of the IL-PFC during the acquisition of extinction results in elevated conditioned freezing responses during an extinction session on the following day (Laurent & Westbrook, 2009; Sierra-Mercado et al., 2011), suggesting that the IL-PFC is essential in the formation of the extinction memory, and that extinction recall is impaired in the absence of the IL-PFC. In contrast, electrical stimulation of the IL-PFC reduces conditioned freezing responses during tests for extinction recall, which suggests that activity in the IL-PFC mediates memory consolidation, and that increasing neuronal activity in this brain region can strengthen the extinction memory (Milad & Quirk, 2002; Milad et al., 2004). Further, antagonists of N-methyl-D-aspartate (NMDA) receptors and protein synthesis infused in the IL-PFC impair extinction recall (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007; Santini, Ge, Ren, Pena, & Quirk, 2004; Sotres-Bayon, Diaz-Mataix, Bush, & Ledoux, 2009). Given that glutamate binding to NMDA receptors leads to protein synthesis and the formation of long-term memories (Kandel, 2001), these results provide further support for the hypothesis that extinction results in the formation of new memories, and that these processes likely occur in the IL-PFC.

The PL-PFC has been shown to be important for promoting, rather than inhibiting, conditioned fear behaviour. Whereas pharmacological inactivation of the IL-PFC causes persistent conditioned fear behaviour during the acquisition of extinction, inactivation of the PL-PFC before an extinction session attenuates conditioned freezing responses (Sierra-Mercado et al., 2011). Moreover, electrical stimulation of PL-PFC neurons during extinction causes sustained freezing responses, resulting in extinction impairment (Vidal-Gonzalez et al., 2006). Consistent with this finding, neuronal activity in the PL-PFC after extinction training has been shown to be significantly correlated with poor extinction performance in rats (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009). Unlike the IL-PFC, inactivation of the PL-PFC during extinction has no effect on extinction recall (Laurent & Westbrook, 2009; Sierra-Mercado et al., 2011), suggesting a lack of involvement in extinction memory (but see Vidal-Gonzalez et al., 2006).

Involvement of the IL-PFC in the extinction of conditioned appetitive behaviour

As in fear conditioning, the IL-PFC has been shown to be important for inhibiting conditioned appetitive behaviour under extinction conditions. For example, lesions to the IL-PFC enhance spontaneous recovery, renewal, and reinstatement of Pavlovian-conditioned food seeking behaviour in rats (Rhodes & Killcross, 2004; Rhodes & Killcross, 2007). In line with these findings, Marchant, Furlong, and McNally (2010) found that IL-PFC neurons are recruited during extinction, as evidenced by robust c-fos expression in the IL-PFC following extinction of nose-poking behaviour for alcoholic beer-seeking in rats. In addition, studies employing pharmacological manipulation of neuronal activity have demonstrated the involvement of the IL-PFC in the extinction of appetitive instrumental behaviour. Whereas IL-PFC inactivation reinstates the extinguished behaviour of lever pressing for cocaine (Peters et al., 2008),

stimulating IL-PFC neurons suppresses cue-induced reinstatement of cocaine-seeking in rats (Lalumiere et al., 2012).

Consistent with the findings outlined above, molecular studies have also implicated the IL-PFC in the formation of extinction memory. Based on evidence that glutamatergic transmission is required for learning and the formation of memories (Miyamoto, 2006; Rao & Finkbeiner, 2007; Robbins & Murphy, 2006), investigators have examined the effects of enhanced glutamate transmission on extinction learning. Several studies have found an enhancement in the extinction of appetitive behaviour following pharmacological potentiation of glutamate transmission (Botreau, Paolone, & Stewart, 2006; Cleva, Hicks, Gass, Wischerath, & Plasters, 2011; Gass & Olive, 2009; Lalumiere et al., 2010; Nic Dhonnchadha et al., 2010). Moreover, infusing the IL-PFC with the NMDA partial agnoist D-cycloserine (DCS) immediately after the first extinction session has been shown to enhance extinction recall of instrumental sucrose-seeking in rats (Peters & De Vries, 2013). Similarly, Lalumiere, Niehoff, and Kalivas (2010) demonstrated that post-session infusions of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor potentiator 4-[2-(phenylsulfonylamino)-ethylthio]-2,6-difluorophenoxyacetamide (PEPA) over 5 extinction sessions caused a decrease in active lever responding for cocaine during the last 2 days of extinction training, suggesting that potentiating glutamatergic transmission in the IL-PFC enhances extinction recall. Overall, these findings indicate that the IL-PFC is a key brain region responsible for the consolidation of extinction memory and are consequently in agreement with fear conditioning studies.

Specific aims of the present research

Converging evidence from fear and appetitive conditioning studies support the hypothesis that the IL-PFC has an important role in response inhibition, which is essential for the extinction of conditioned behaviour. It should be noted that while fear extinction has typically been examined using Pavlovian fear conditioning, appetitive extinction has largely been studied using instrumental conditioning procedures. Thus, less is known about the neural mechanisms that mediate extinction of Pavlovian conditioned reward-seeking behaviour. In addition, the underlying neural mechanisms that mediate the extinction of appetitive behaviour have mostly been investigated using drug, but not natural reinforcers.

The experiments in this thesis investigated the role of the IL-PFC in the extinction of Pavlovian and instrumental conditioned sucrose-seeking behaviour in rats. Given the putative role of the PL-PFC in promoting conditioned behaviour, the role of the PL-PFC on extinction was also examined. Rats were given several sessions of Pavlovian conditioning in which a white noise (CS) was repeatedly paired with the delivery of sucrose (US). Based on the procedures of Sierra-Mercado et al. (2011), the role of the IL-PFC and PL-PFC in extinction learning was assessed by pharmacologically inactivating these brain regions before the first extinction session. On the following day, rats received a subsequent session of extinction to assess the effect of IL-PFC inactivation on extinction memory .We hypothesized that inactivating the IL-PFC, but not the PL-PFC, would have an impact on Pavlovian conditioned sucrose-seeking. Given that IL-PFC inactivation has been shown to impair the acquisition of extinction (Sierra-Mercado et al., 2011), we predicted that reversible inactivation of the IL-PFC would cause a similar impairment in extinction learning in the present study. Moreover, based on the observation that activity in the IL-PFC is essential for the consolidation of the extinction memory (Lalumiere et al., 2010), we

expected an impairment in extinction recall in rats that had received M/B in the IL-PFC on the previous day.

Upon completion of the Pavlovian study described above, animals were given several instrumental training sessions in which active lever presses delivered sucrose, followed by IL-PFC or PL-PFC inactivation prior to an initial extinction session. The purpose of this experiment was to examine the role of these brain areas on the extinction of behaviour acquired through instrumental conditioning. Based on the finding that IL-PFC inactivation promotes instrumental cocaine-seeking (Peters et al., 2008), we predicted that inactivation of the IL-PFC would result in persistent sucrose-seeking, causing an impairment in the acquisition of extinction. Moreover, extinction memory was tested on the following day by administering a further infusion-free extinction session. We hypothesized that the IL-PFC would play a significant role in the consolidation of the extinction memory, and predicted that inactivating IL-PFC on the previous day would increase instrumental responding for sucrose during extinction recall.

Finally, we evaluated the effect of IL-PFC inactivation on the ability to make port-entries by inactivating the IL-PFC prior to a Pavlovian conditioning session in which CS trials were paired with sucrose delivery. Inactivation of the IL-PFC was also conducted prior to a sucrose self-administration session, in which active lever pressing initiated the delivery of sucrose. We predicted that there would be no effect of IL-PFC inactivation in well-trained animals, as motor impairments are typically not observed in the absence of the IL-PFC (McLaughlin & See, 2003; Fuchs et al., 2005).

Method

Subjects

Male Long-Evans rats (Charles River, QC, Canada; N=42; 220-240g on arrival) were single-housed in plastic shoebox cages (44.5 cm x 25.8 cm x 21.7 cm) containing beta chip bedding. They were kept in a temperature-controlled room (21° C) on a 12 hr light/dark cycle with lights on at 7:00 AM. All behavioural procedures were conducted during the light phase. Rats were handled daily and given a minimum of 7 days to acclimate to the animal colony before surgery. They had unrestricted access to standard rat chow (Ralston Purina, Canada) and water throughout the experiments, except as outlined below. All procedures were approved by the Animal Research Ethics Committee at Concordia University, and are in accordance with recommendations by the Canadian Council on Animal Care.

Apparatus

Equipment used for behavioural testing was obtained from Med Associates Inc. (St Albans, VT, USA). Behavioural testing was conducted in operant conditioning chambers (ENV-009A; 32.8 cm x 32.8 cm x 32.8 cm) housed within custom-made, ventilated, sound-attenuating melamine boxes (53.6 cm x 68.2 cm x 62.8 cm). Each chamber had a clear Plexiglas front door, back-wall and ceiling, and side-walls made of stainless steel panels. Floors were comprised of stainless steel bars that extended from front to rear. A waste pan lined with absorbent paper was located beneath the floor. To prevent rats from manipulating the paper during behavioural sessions, a stainless steel floor insert comprised of a 0.5-inch grids was placed over the bar floor. A dual cup liquid receptacle (ENV-200R3AM; 5.3 cm x 3.4 cm x 5.3 cm) was located 2 cm above the floor in the center of the right wall. One cup in the receptacle was connected via polyethylene tubing (Tygon; Fisher Scientific, #141691A) to a 20 ml syringe mounted in a pump

(PHM-100, 3.33 RPM) located outside the sound-attenuating box. Entries into the receptacle were measured via infrared detectors (ENV-254-CB) located across the entrance. A retractable lever (ENV-112BM) was positioned on each side of the receptacle. The center of the left wall contained a white house-light (75W, 100 mA, ENV-215M) located 30 cm above the bar floor. A white noise generator (ENV-225SM) and clicker stimulus (ENV-135M) were located to the left and right of the house-light, respectively. The white noise generator was calibrated to produce a noise that was 6-8 decibels above background noise (75-78 dB). Stimulus presentations, pump activation, and extension of levers were controlled by a PC computer using Med PC IV software. Port entries and lever presses were counted and registered by the same computer using the same program.

Drugs

Pharmacological inactivation was conducted using the gamma-aminobutyric acid (GABA) agonists muscimol (Sigma-Aldrich; M1523) and baclofen (Sigma-Aldrich, B5399). A solution (M/B) was prepared by dissolving 5 mg of muscimol and 93.65 mg of baclofen in 438 ml of sterile 0.9% saline (0.03 nmol muscimol; 0.3 nmol baclofen). These agonists have been shown to inhibit neural firing without affecting fibers of passage (Martin & Ghez, 1999; van Duuren et al., 2007) and the doses used are behaviourally effective in studies on extinction (Peters et al., 2008). Sucrose (Anachemia Canada Inc., #87688-380) was dissolved in tap water to obtain a final concentration of 10% (w/v).

Surgery

Surgery was conducted 1-2 weeks after arrival on rats weighing 320-415 g. Animals were anaesthetized with isoflurane and implanted bilaterally with stainless steel, double-barrelled (1.2 mm apart) guide cannulae (26 gauge, Plastics One, Roanoke, VA; C235G) targeting the IL-PFC

(AP = +2.7, ML = ±0.6, DV = -3.1) or PL-PFC (AP = +2.7, ML = ±0.6, DV = -1.6) using standard stereotaxic procedures. Cannulae were occluded using 33 gauge obturators and secured to the skull using dental acrylic and four metal screws. After surgery, 2 ml of 0.9% saline and an analgesic (Anafen; 0.1 ml/kg) were administered by subcutaneous injection. Powdered rat chow mixed with sugar and tap water was provided post-surgery to promote feeding. Rats received 14 days to recover from surgery before training.

Intracranial microinfusions

Microinfusions were conducted in the room where the operant conditioning chambers were located. Solutions were infused through a double-barrelled (1.2 mm apart) 33 gauge injector (Plastics One, Roanoke, VA; C235I), which was connected to 2 Hamilton syringes (10 µl; Fisher Scientific, 1701 RNR- #14-815-279) via PE-50 tubing (VWR International Co.). Syringes were placed in a microinfusion pump (Harvard Apparatus, PHD 2000) that infused at a rate of 0.3 µl/min, for a total volume of 0.3 µl. Following the 1 min infusion, the injector was kept inside the cannula for 2 min to optimize diffusion. Throughout the 3 min microinfusion procedure, rats were gently restrained to prevent them from detaching the injector. Behavioural testing commenced 5-20 min after the microinjection.

Sucrose consumption in the home cage

Fourteen days after surgery, a bottle containing 10% sucrose was placed on the home cage. Rat weights and sucrose consumption were recorded every 24 hr. After 48 hr the bottle was removed.

Experiment 1a. Effect of IL-PFC and PL-PFC inactivation on the extinction of appetitive Pavlovian conditioning

This study tested the hypothesis that the IL-PFC and PL-PFC have distinct roles in the extinction of appetitive Pavlovian conditioning.

Twenty-four hours after sucrose exposure in the home cage, rats were handled in the behaviour testing room for 30 min in order to habituate them to the testing environment. On the following day, Pavlovian conditioning sessions commenced. Rats received 8 daily 30 min Pavlovian conditioning sessions (consecutive days; 8:00 AM – 1:00 PM). Each session consisted of 14 trials in which a 15 sec white-noise CS was paired with the delivery of 0.3 ml of sucrose into the fluid port for oral consumption. Sucrose delivery began 6 sec after CS onset and co-terminated with the CS. CS trials were controlled by a variable-time 120 sec schedule. The house-lights were turned on manually at the start of the session and turned off automatically at the end of the session. Immediately before Pavlovian training session 4, rats received a sham microinfusion using the procedure described above, except with an injector that was cut so as not to protrude beyond the cannula tip, and without any fluid in the lines. In addition, a saline microinfusion was conducted before training session 6. These infusions were performed to habituate the rats to the microinfusion procedure.

The acquisition of extinction was examined 24 hr after the last Pavlovian training session. Rats received a bilateral microinfusion (0.3 µl/hemisphere) of either saline or M/B into the IL-PFC (Saline, n=7; M/B, n=7) or PL-PFC (Saline, n=7; M/B, n=7) using a between-subjects design. At 20 min after the infusion they were placed into the operant conditioning chambers for a 30 min session that was identical to a Pavlovian conditioning session, except that the pumps were turned off and did not contain sucrose syringes. Extinction memory was tested across 4

subsequent daily extinction sessions conducted in the absence of intracranial injections.

Experiment 1b. Effect of IL-PFC and PL-PFC inactivation on the extinction of appetitive instrumental conditioning

This study tested the hypothesis that the IL-PFC, but not PL-PFC, is important for the extinction of appetitive instrumental behaviour. Upon completion of the study above, rats from Experiment 1 underwent the procedures outlined below.

Rats were water deprived for 24 hr and then placed in operant conditioning chambers for a 12 hr lever-press training session. Session onset was indicated by illumination of the house-light and extension of the left lever into the chamber. Each lever press resulted in a 0.1 ml delivery of 10% sucrose into the fluid port on a fixed-ratio 1 (FR1) schedule. After 200 sucrose deliveries the lever was retracted and the house-light turned off to indicate the end of the session. Rats were returned to their home-cages where unrestricted access to water was restored.

Sucrose self-administration training began 24 hr after the lever-press training session. In each daily 45 min session (consecutive days; 8:00 AM – 1:00 PM) responding on the left (active) lever resulted in the delivery of 0.1 ml of 10% sucrose into the fluid port on an FR1 schedule. Responding on the right (inactive) lever was recorded but had no programmed consequence. Because of scheduling constraints, rats with cannulae targeting the IL-PFC received 5 self-administration training sessions, whereas rats with PL-PFC cannulae placements received 6 training sessions. A saline sham microinfusion was administered before training session 4.

The acquisition of extinction was examined 24 hr after the last sucrose self-administration session in a session that was identical to a self-administration session, except that the pumps were turned off and did not contain sucrose syringes. At 20 min before test, rats received a bilateral microinfusion (0.3 µl/hemisphere) of either saline or M/B into the IL-PFC

(Saline, n=7; M/B, n=7) or PL-PFC (Saline, n=6; M/B, n=7) using a between-subjects design. In order to control for order effects, rats from each group from experiment 1a were equally distributed into the saline and M/B treatment conditions for this extinction test. Extinction memory was tested across 4 subsequent daily extinction sessions conducted in the absence of intracranial injections.

Experiment 2. Effect of IL-PFC inactivation on appetitive Pavlovian and instrumental conditioning

A separate group of animals were utilized to investigate the impact IL-PFC inactivation on appetitive conditioning in both Pavlovian and instrumental procedures.

Rats received 8 Pavlovian training sessions as in experiment 1, wherein CS trials were paired with 10% sucrose. A saline sham microinfusion was conducted prior to training day 7. Before Pavlovian training session 9, rats received rats received a bilateral microinfusion (0.3 µl/hemisphere) of either saline (n=7) or M/B (n=7) into the IL-PFC using a between subject design.

After the test described above, rats underwent lever-press training and sucrose self-administration sessions as described in experiment 2. A sham microinfusion with a cut injector occurred before session 4 and a saline microinfusion before session 7. The effect of IL-PFC inactivation on sucrose self-administration was tested on session 9, in which rats received a pre-session bilateral microinjection of saline (n=7) or M/B (n=7) into the IL-PFC using a between subject design.

Histological verification of cannulae placements

Following experiments 2 and 3, rats were anesthetized with isoflurane and decapitated. Brains were removed and immersed in formalin for 24 hr, followed by 25% sucrose for 7 days,

and then sectioned on a cryostat (60 microns, coronal). Sections were collected onto glass slides and stained with cresyl violet. Placement of cannulae and verification of injector tips was examined using light microscopy for rats utilized in experiment 1 (Fig 1d & 2d) and experiment 2 (Fig 6). Subjects were excluded if 1 or both injector tips were located outside the boundaries of the IL-PFC or PL-PFC, as delineated in the Paxinos and Watson (1997) rat brain atlas. Based on this criterion, seven rats with guide cannulae targeting the IL-PFC and 2 rats with PL-PFC cannulae were excluded from experiment 1 as injectors were located outside the IL-PFC or PL-PFC, respectively.

Statistical analysis

During Pavlovian conditioning entries into the fluid receptacle (referred to as port-entries) during each 15 sec CS trial (CS responses) as well as during a 15 sec interval immediately before each CS (preCS) were recorded. CS responses were normalized to account for differences in baseline responding by subtracting preCS responses from responses during the corresponding CS. Port-entries made when the CS was not presented (non-CS responses) were also calculated. Pavlovian training sessions and extinction recall sessions were analyzed separately using ANOVA with *Session* (Pavlovian training sessions 1-8; Recall sessions 1-4) as a within-subject variable and *Group* (saline; M/B) as a between-subject variable. Independent samples *t*-tests were used to analyze group differences in port-entry responses during the extinction test. Repeated measures ANOVA was used to analyze port-entry responses per CS trial and latency to respond to each CS at test with *Trial* (trials 1-14) as the within-subject variable and *Group* (saline; M/B) as the between subject variable.

During instrumental conditioning responses on the left (active) and right (inactive) levers, and port-entries made into the fluid port were recorded. Sucrose self-administration sessions and

recall sessions were analyzed independently using ANOVA with *Session* (Self-administration sessions 4-5; Recall sessions 1-4) and *Lever* (active, inactive) as within-subjects variables and *Group* (saline, M/B) as a between-subjects variable. The extinction test session was analyzed using ANOVA with *Lever* (active, inactive) as the within-subjects variable, and *Group* (saline, M/B) as the between-subjects variable. To further characterize within-session extinction of instrumental responding, the number of active lever presses made during 1-min time bins across the test session were analysed using ANOVA with *Time* (Min 1-45) as a within-subjects variable, and *Group* (saline, M/B) as a between-subjects variable. In Experiment 1, one rat was excluded following instrumental training as it did not learn to self-administer sucrose.

Violations of homogeneity of variance were determined by Mauchly's test of sphericity and were corrected using the Greenhouse-Geisser correction. Analyses were conducted using SPSS software (version 20). The alpha level was set to $\alpha = 0.05$ for all statistical analyses.

Results

Experiment 1a. Effect of IL-PFC and PL-PFC inactivation on the extinction of appetitive Pavlovian conditioning

IL-PFC inactivation

Rats learned the association between the CS and sucrose, as shown by an increase in normalized conditioned port-entries elicited by the CS across session during Pavlovian conditioning [Fig 1a; Session, $F(7,84) = 26.245$, $p = 0.000$]. Both groups acquired Pavlovian learning at a similar rate [Group, $F(1,12) = 0.925$, $p = 0.854$; Group x Session, $F(7,84) = 2.210$, $p = 0.083$]. Compared to saline, inactivating the IL-PFC significantly reduced CS responses during the first extinction session in which the CS was presented without sucrose [Fig 1a; $t(12) = 2.867$,

$p = 0.014$]. This effect was specific to CS responses as port-entries made during time intervals that were not signalled by the CS were not affected by IL-PFC inactivation (Appendix A). There was no impact of prior IL-PFC inactivation on the subsequent recall of extinction memory. Responding to the CS decreased across 4 extinction recall sessions [Session, $F(3,36) = 41.931$, $p = 0.000$], with no main effects or interactions with group [Group, $F(1,12) = 0.126$, $p = 0.729$; Group x Session, $F(3,36) = 0.733$, $p = 0.539$].

In order to examine the impact of IL-PFC inactivation on within-session extinction acquisition as well as the within-session expression of extinction memory during recall, a detailed analysis of extinction sessions 1 (acquisition) and 2 (recall) was conducted. On the first day of extinction (Fig 1b, left panel), the number of port-entries made during each CS trial decreased as a function of trial [Trial, $F(13,156) = 16.541$, $p = 0.000$]. Compared to saline, IL-PFC inactivation reduced the number of CS responses overall [Group, $F(1,12) = 8.218$, $p = 0.014$]. Interestingly, ANOVA revealed a significant Group x Trial interaction [Group x Trial, $F(13,156) = 2.098$, $p = 0.017$] suggesting that inactivating the IL-PFC caused a more rapid extinction of CS responses across CS trials. Follow-up t-tests conducted to investigate this interaction found that there was no difference in the number of port-entries made during the first 2 CS trials in saline or M/B infused rats [Trial 1, $t(12) = 1.106$, $p = 0.290$; Trial 2 $t(12) = 0.825$, $p = 0.425$]. However, the number of responses elicited by CS trials 3 and 4 was significantly reduced following IL-PFC inactivation compared to saline [Trial 3, $t(12) = 3.482$, $p = 0.005$; Trial 4 $t(12) = 3.695$, $p = 0.003$]. During extinction recall (Fig 1b, right panel) port-entries during each CS presentation decreased as a function of trial [Trial, $F(13,156) = 14.290$, $p = 0.000$] in rats from both groups [Group x Trial, $F(12,156) = 0.426$, $p = 0.959$; Group x Trial, $F(12,156) = 0.426$, $p = 0.959$].

An examination of latency to respond to each CS trial during the first extinction session (Fig 1c, left panel) found that rats were slower to make a port-entry after CS onset following IL-PFC inactivation, compared to saline [Group, $F(1,12) = 15.840$, $p = 0.002$]. However, latency increased as a function of trial [Trial, $F(13,156) = 10.777$, $p = 0.000$] in parallel in rats from both groups [Group x Trial, $F(13,156) = 0.861$, $p = 0.595$].

Given that the impact of IL-PFC inactivation on the number of port-entries per CS trial was most pronounced across the first 4 trials of extinction, we conducted t-tests to examine differences in latency to respond to each of the first 4 CS trials as a function of group. Latency to respond was similar across both groups during the first 3 CS trials (all p's > 0.05), however, M/B-infused animals were significantly slower to respond to the 4^{th} CS trial ($p = 0.001$).

During recall (Fig 1c, right panel), latency to respond was similar in both groups [Group, $F(1,12) = 0.008$, $p = 0.932$; Group x Trial, $F(13,156) = 1.162$, $p = 0.313$], with an overall increase in latency across trials [Trial, $F(13,156) = 7.960$, $p = 0.000$].

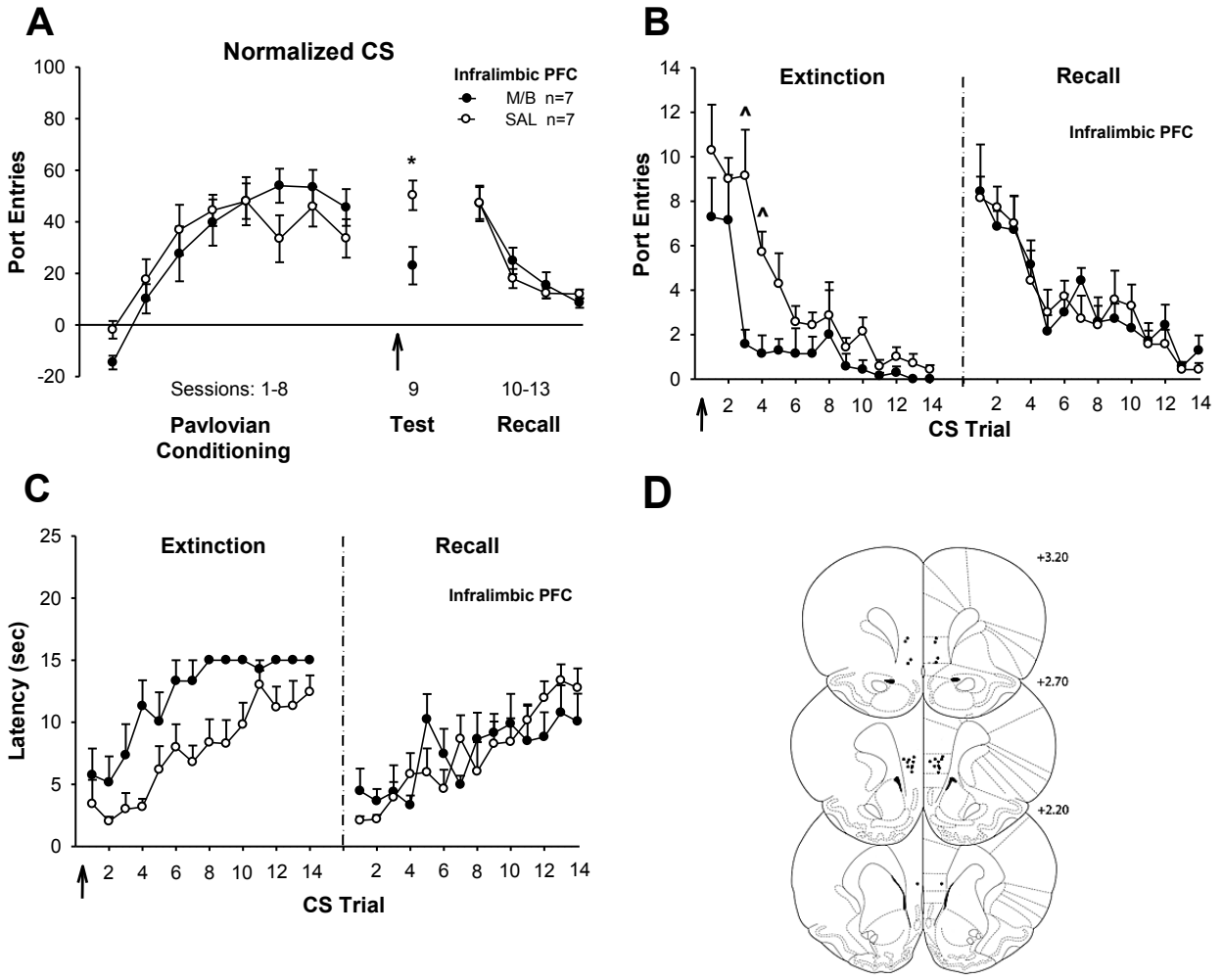

Figure 1. Inactivating the IL-PFC before an extinction session in which a Pavlovian, sucrose-predictive CS was presented without sucrose facilitated the within-session acquisition of extinction, but had no effect on extinction recall. Rats received Pavlovian conditioning sessions, followed by a single extinction session that was preceded by an intracranial infusion into the IL-PFC, and 4 subsequent sessions conducted to assess the recall of extinction memory. In Figures A, B, and C, filled circles represent rats that received M/B before extinction session 1 (n=7) and open circles represent rats that received saline (n=7). **A** Mean (±SEM) normalized CS responses (CS – PreCS) during Pavlovian conditioning, a single extinction test, and 4 sessions to assess the recall of extinction. **B** Mean (±SEM) port-entries made during each CS trial across the extinction test and the first recall session. **C** Mean (±SEM) latency to first port-entry response after the onset of each CS trial during the extinction test and recall session 1. **D** Placement of injector tips within the IL-PFC. Distance from bregma is indicated to the right of each coronal section. Symbols indicate statistical significance from independent samples t-test comparisons: ^$P < 0.01$, *$P < 0.05$ significant difference between saline and M/B. Arrows indicate infusions of saline or M/B in the IL-PFC in this, and subsequent figures.

PL-PFC inactivation

Responses to the CS increased across conditioning sessions in rats with guide cannulae targeting the PL-PFC [Fig 2a; Session, $F(7,84) = 30.124$, $p = 0.000$], with no main effects or interactions involving group [Group, $F(1,12) = 0.182$, $p = 0.677$; Group x Session, $F(7,84) = 0.703$, $p = 0.669$]. Unlike the IL-PFC, inactivating the PL-PFC had no impact on CS responding compared to saline at test. [Fig. 3a; $t(12) = 1.624$, $p = 0.130$]. Likewise, PL-PFC inactivation had no impact on responses made outside CS presentations (Appendix A). Furthermore, CS responses decreased comparably across the 4 extinction recall sessions [Fig 3a; Session, $F(3,36) = 42.569$, $p = 0.000$] in rats from both groups [Group, $F(1,12) = 0.909$, $p = 0.359$; Group x Session, $F(3,36) = 0.374$, $p = 0.640$].

That PL-PFC inactivation had no impact on the extinction of CS responding was verified by a within-session analysis on day 1 of extinction (Fig 2b, left panel), which revealed an overall decrease in port-entry responses as a function of CS trial [Trial, $F(13,156) = 6.793$, $p = 0.000$] in rats from both groups [Group x Trial, $F(13,156) = 0.979$, $p = 0.475$; Group, $F(1,12) = 2.572$, $p = 0.135$]. Likewise, during extinction recall (Fig 2b, right panel), port-entries decreased as a function of trial [Fig 2b; Trial, $F(13,156) = 10.587$, $p = 0.000$], and there were no main effects or interactions involving group [Group, $F(1,12) = 0.431$, $p = 0.524$; Group x Trial, $F(13,156) = 0.686$, $p = 0.775$].

Latency to make a port-entry response increased across trial during the first extinction session [Fig 2c, left panel; Trial, $F(13,156) = 5.921$, $p = 0.000$], and PL-PFC inactivation had no impact on this measure [Group, $F(1,12) = 1.314$, $p = 0.274$; Group x Trial, $F(13,156) = 1.250$, $p = 0.249$]. Similarly, during extinction recall, latency to make a port-entry increased as a function of trial [Fig 2c right panel; Trial, $F(13,156) = 5.539$, $p = 0.000$], with no main effects or

interactions with group [Group, $F(1,12) = 0.963$, $p = 0.346$; Group x Trial, $F(13,156) = 0.545$, $p = 0.893$.

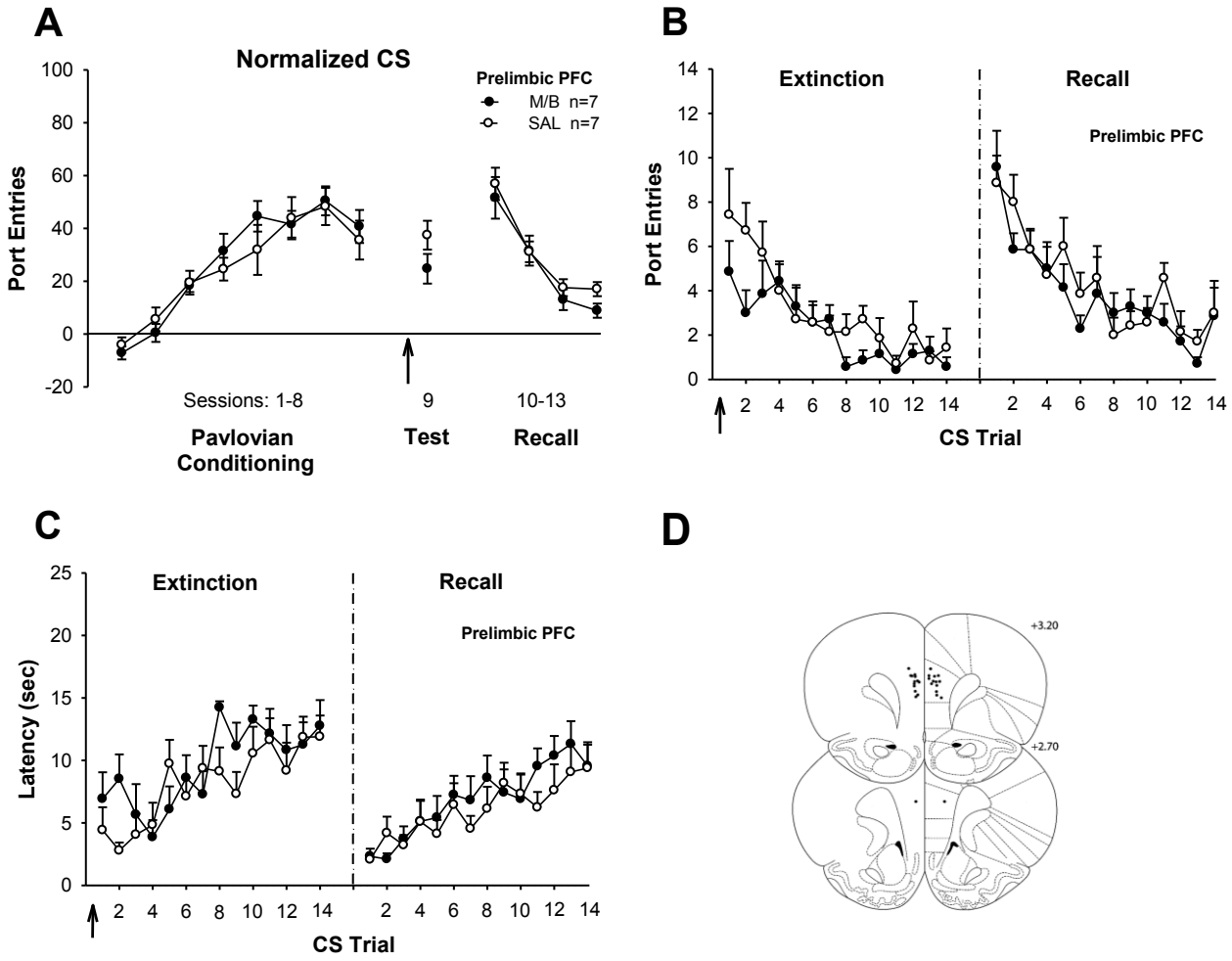

Figure 2. Inactivating the PL-PFC before an extinction session in which a Pavlovian, sucrose-predictive CS was presented without sucrose had no effect on the acquisition or recall of extinction. Rats received Pavlovian conditioning sessions, followed by a single extinction session that was preceded by an intracranial infusion into the PL-PFC, and 4 subsequent sessions conducted to assess the recall of extinction memory. In Figures A, B, and C, filled circles represent rats that received M/B before extinction session 1 (n=7) and open circles represent rats that received saline (n=7). **A** Mean (±SEM) normalized CS responses (CS – PreCS) during Pavlovian conditioning, a single extinction test, and 4 sessions to assess the recall of extinction. **B** Mean (±SEM) port-entries made during each CS trial across the extinction test and the first recall session. **C** Mean (±SEM) latency to first port-entry response after the onset of each CS trial across the extinction test and recall session 1. **D** Placement of injector tips within the PL-PFC. Distance from bregma is indicated to the right of each coronal section.

Experiment 1b. Effect of IL-PFC and PL-PFC inactivation on extinction of appetitive instrumental conditioning

IL-PFC inactivation

Rats learned to discriminate between the active and inactive levers during instrumental conditioning sessions (Fig 3a). ANOVA was conducted on active and inactive lever responding across the last 2 sessions of self-administration to verify that responding was stable immediately before the extinction test. Responding was higher on the active than the inactive lever [Lever, $F(1,12) = 134.491$, $p = 0.000$] in rats from both group [Lever x Group, $F(1,12) = 1.271$, $p = 0.282$]. There was no main effect of session [Session, $F(1,12) = 1.786$, $p = 0.206$] or group [$F(1,12) = 1.143$, $p = 0.306$], and no statistically significant interactions [Session x Group, $F(1,12) = 0.001$, $p = 0.971$; Session x Lever, $F(1,12) = 2.481$, $p = 0.141$; Session x Group x Lever, $F(1,12) = 0.002$, $p = 0.964$].

Compared to saline, inactivating the IL-PFC did not affect lever responses on session 1 of extinction where active lever responding no longer resulted in sucrose (Fig 3a). Rats responded more on the active than the inactive lever [Lever, $F(1,12) = 60.343$, $p = 0.000$] and there was no effect of IL-PFC inactivation on responding on either lever [Group, $F(1,12) = 0.330$, $p = 0.330$; Lever x Group, $F(1,12) = 0.334$, $p = 0.574$].

Collapsed across 4 recall sessions (Fig 3a) active lever pressing was higher than inactive lever responding [Lever, $F(1,12) = 24.406$, $p = 0.000$]. However, across session there was a decrease in lever pressing [Session, $F(3,36) = 8.829$, $p = 0.000$] in both groups [Group, $F(1,12) = 1.323$, $p = 0.272$; Session x Group, $F(3,36) = 1.197$, $p = 0.325$]. There was an across-session reduction in active lever pressing, but not inactive lever pressing [Session x Lever, $F(3,36) = 4.796$, $p = 0.035$] in rats from both groups [Session x Lever x Group, $F(3,36) = 2.650$, $p = 0.063$]

Active lever responses were averaged into 1-min time-bins across extinction sessions 1 and 2 in order to investigate the effect of IL-PFC inactivation on the acquisition and recall of extinction, respectively. For clarity, only responding across the first 5 min of each session is depicted in Figure 3b (see Appendix B for entire session). Active lever responses decreased across the first 5 min of extinction session 1 [Fig 3b, left panel; Time, $F(4,48) = 3.956$, $p = 0.007$]. There was no overall difference in the number of active lever presses as a function of group [Group, $F(1,12) = 1.675$, $p = 0.220$]. However, responding diminished at a different rate between the groups [Time x Group, $F(4,48) = 6.229$, $p = 0.000$]. Follow-up t-tests for independent samples revealed that at minute 1 of the test session, M/B-infused rats responded more on the active lever compared to saline-infused rats [$t(12) = -2.239$, $p = 0.045$]. At minute 2, active lever pressing was similar in both groups [$t(12) = -0.186$, $p = 0.856$]. However, M/B-infused rats responded significantly less on the active lever compared with control animals at minute 3 [$t(12) = 2.628$, $p = 0.022$] and minute 4 [$t(12) = 3.328$, $p = 0.006$].

During extinction recall (Fig 3b, right panel) there was a near-significant decline in active lever responses across the first 5 min [Fig 3b; Time, $F(4,48) = 3.413$, $p = 0.066$]. However, unlike extinction session 1, there was no difference between the groups in active lever responses as a function of time [Group, $F(1,12) = 0.015$, $p = 0.905$; Time x Group, $F(4,48) = 0.894$, $p = 0.475$].

PL-PFC inactivation

Rats learned to discriminate between the active and inactive levers during instrumental conditioning sessions (Fig 3c). Overall, rats pressed more on the active than the inactive lever [Lever, $F(1,11) = 101.909$, $p = 0.000$].The number of lever presses did not change across the last

2 sessions of instrumental training [Fig 3c; Session, $F(1,11) = 0.003$, $p = 0.954$] with no interactions as a function of group [Group, $F(1,11) = 0.016$, $p = 0.903$; Session x Group, $F(1,11) = 2.697$, $p = 0.129$; Session x Lever, $F(1,11) = 0.040$, $p = 0.845$; Session x Group x Lever, $F(1,11) = 3.050$, $p = 0.109$].

Compared to saline, PL-PFC inactivation had no impact on lever pressing during extinction (Fig 3c). Animals continued to discriminate between the active and inactive lever [Lever, $F(1,11) = 29.869$, $p = 0.000$], but there were no main effects or interactions with group [Group, $F(1,11) = 0.416$, $p = 0.532$; Group x Lever, $F(1,11) = 0.861$, $p = 0.373$].

Across 4 sessions of extinction recall, more responses were made on the active lever than the inactive lever [Lever, $F(3,33) = 15.415$, $p = 0.000$]. However, active lever responses decreased across session [Lever x Session, $F(3,33) = 8.804$, $p = 0.003$], in rats from both groups [Session x Group, $F(3,33) = 0.375$, $p = 0.652$; Session x Lever x Group, $F(3,33) = 0.559$, $p = 0.646$]. There was no impact of prior treatment on overall responding [Group, $F(1,11) = 0.024$, $p = 0.880$].

During the first 5 min of extinction session 1 (Fig 3d, left panel) active lever responses decreased as a function of time [Fig 3d; Time, $F(4,44) = 3.205$, $p = 0.022$]. PL-PFC inactivation had no impact on this measure [Group, $F(1,11) = 0.063$, $p = 0.806$; Time x Group, $F(4,44) = 0.714$, $p = 0.587$]. Likewise, active lever responses diminished during the first 5 min of extinction recall [Fig 3d, right panel; Time, $F(4,44) = 11.373$, $p = 0.000$]. Prior PL-PFC inactivation had no effect on active lever pressing during the first 5 min of recall [Group, $F(1,11) = 0.117$, $p = 0.739$], but there was a significant group x time interaction [Group x Time, $F(4,44) = 4.207$, $p = 0.006$]. Follow-up t-tests for independent samples revealed no group differences in active lever pressing at any time bin during the first 5 min of extinction (all p's > 0.05).

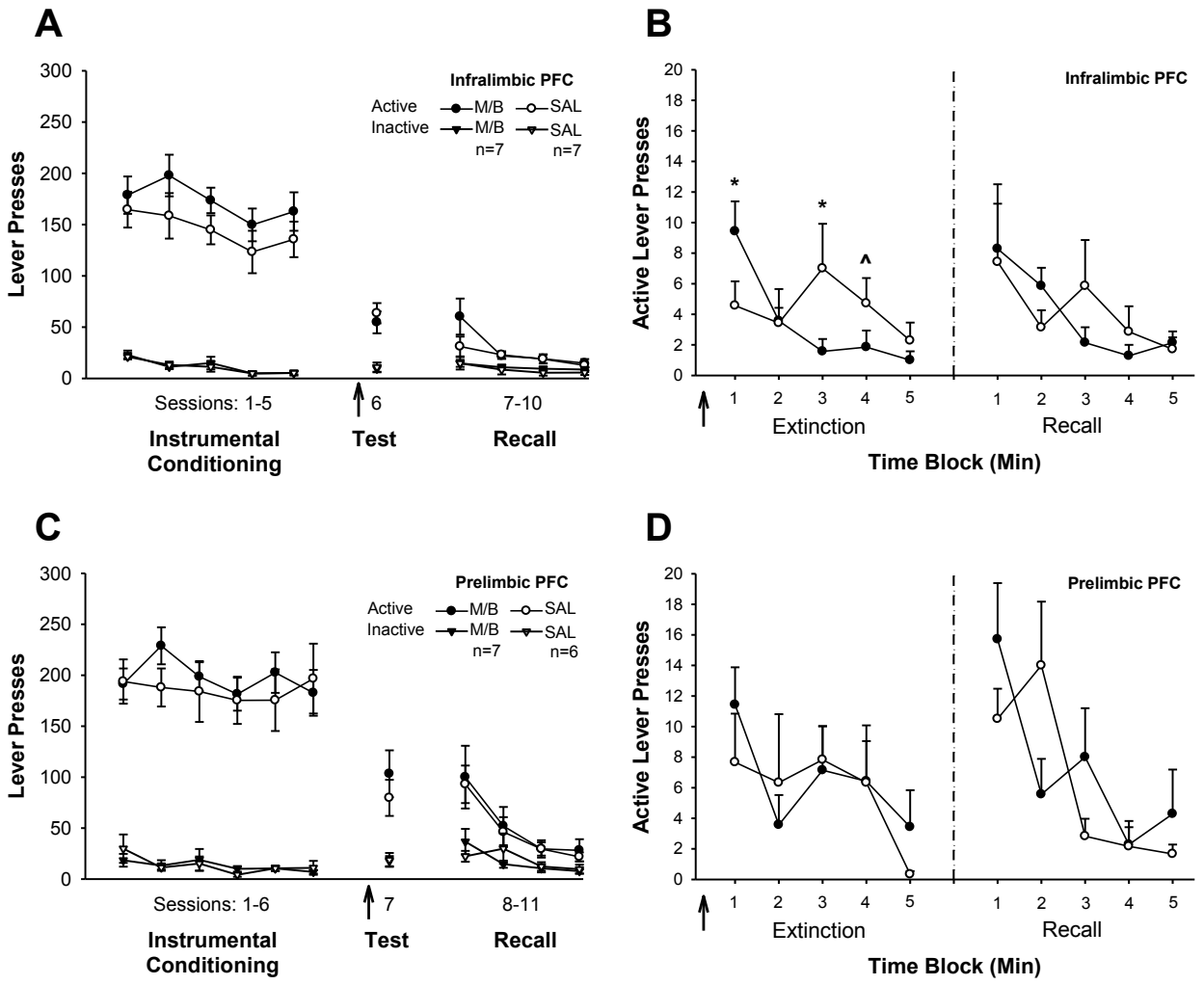

Figure 3. Inactivating the IL-PFC, but not the PL-PFC, caused a more rapid decline in active lever responses during the first 5 min of the extinction test session, compared to saline. Rats received instrumental conditioning sessions, followed by a single extinction session that was preceded by an intracranial infusion into the IL-PFC, and 4 subsequent sessions conducted to assess the recall of extinction memory. Filled symbols represent rats that received M/B before extinction session 1 (n=7), and open symbols represent rats that received saline (n=6). Circles represent active lever presses and triangles represent presses on the inactive lever. **A** Mean (±SEM) active and inactive lever presses made by rats with guide cannulae targeting the IL-PFC across all experimental phases. **B** Mean (+SEM) active lever presses made by rats with IL-PFC infusions during the first 5 min of the extinction test and the first recall session. **C** Mean (±SEM) active and inactive lever presses made by rats with guide cannulae targeting the PL-PFC across all experimental phases. **D** Mean (+SEM) active lever presses made by rats with PL-PFC infusions across the first 5 min of the extinction test and the first recall session. Symbols indicate statistical significance from independent samples t-test comparisons: ^$P < 0.001$, *$P < 0.05$ significant difference between saline and M/B.

Experiment 2. Effect of IL-PFC inactivation on appetitive Pavlovian and instrumental conditioning

Across training, normalized CS responses increased [Fig 4a; Session, $F(7,84) = 16.071$, $p = 0.000$] in rats from both groups [Group, $F(1,12) = 0.004$, $p = 0.951$; Session x Group, $F(7,84) = 0.239$, $p = 0.883$]. Inactivating the IL-PFC reduced normalized CS responding (Fig 4a), compared to saline [$t(12) = 2.933$, $p = 0.013$]. However, additional analyses (Fig 4b) revealed that this effect was attributable to IL-PFC inactivation producing a significant increase in preCS responding [$t(12) = -3.101$, $p = 0.009$], with no change in CS responding [$t(12) = 1.356$, $p = 0.200$]. Thus, IL-PFC inactivation promoted port-entry responses during time periods that were not explicitly signalled by the CS. This effect was also evident in an analysis of the number of responses made during non-CS intervals (Fig 4c). There was no change in non-CS responses across Pavlovian conditioning as a function of session [Session, $F(7,84) = 1.431$, $p = 0.204$] or group [Group, $F(1,12) = 0.093$, $p = 0.765$; Group x Session, $F(7,84) = 0.714$, $p = 0.660$]. However, at test IL-PFC inactivation significantly increased non-CS responses, compared to saline [$t(12) = -5.704$, $p = 0.000$].

An analysis of the number of port-entries made during each CS trial (Fig 4d) at test revealed no impact of IL-PFC inactivation on port-entries per CS trial across the session [Trial, $F(13, 156) = 1.540$, $p = 0.109$; Group, $F(1,12) = 1.840$, $p = 0.200$; Group x Trial, $F(13,156) = 0.755$, $p = 0.706$].

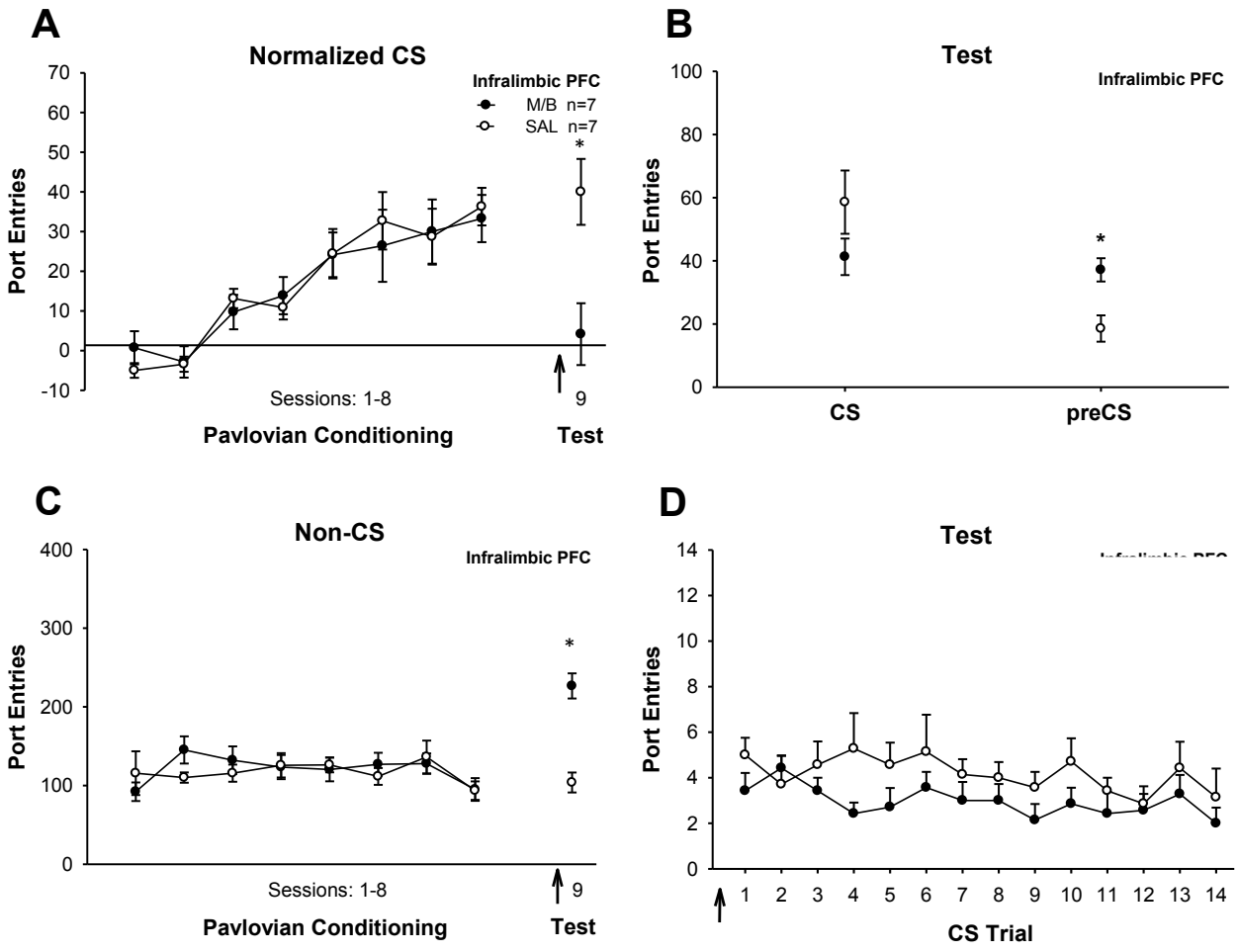

Figure 4. Inactivating the IL-PFC before a Pavlovian conditioning session in which CS trials were paired with sucrose delivery caused an increase in port-entries made during time intervals that were not signalled by the CS (Non-CS), but did not affect CS-elicited port-entries. Rats received Pavlovian conditioning sessions, followed by a subsequent conditioning session that was preceded by an intracranial infusion into the IL-PFC. Filled circles represent rats that received M/B before the test session (n=7) and open circles represent rats that received saline (n=7). **A** Mean (±SEM) normalized CS responses (CS – PreCS) during Pavlovian conditioning and at test. **B** Mean (±SEM) port-entries made during preCS intervals and CS trials at test. **C** Mean **(**±SEM) port-entries made during non-CS intervals across Pavlovian conditioning and at test. **D** Mean (±SEM) port-entries made across each CS trial at test. Symbols indicate statistical significance from independent samples t-test comparisons: *P < 0.001 significant difference between saline and M/B. Arrow indicates infusions of saline or M/B in the IL-PFC.

During instrumental training more responses were made on active than the inactive lever across training [Lever, $F(1,12) = 211.155$, $p = 0.000$]. Lever responding remained stable across self-administration sessions [Session, $F(7,84) = 1.239$, $p = 0.291$; Session x Lever, $F(7,84) = 1.554$, $p = 0.161$] in rats from both groups [Group, $F(1,12) = 0.006$, $p = 0.938$; Session x Group, $F(7,84) = 0.412$, $p = 0.893$; Session x Lever x Group, $F(7,84) = 0.601$, $p = 0.754$].

Inactivating the IL-PFC had no effect on instrumental responding for sucrose (Fig 5a). Rats responded more on the active than the inactive lever [Lever, $F(1,12) = 161.477$, $p = 0.000$], with no impact of IL-PFC inactivation in either measure [Group, $F(1,12) = 0.316$, $p = 0.584$; Group x Lever, $F(1,12) = 0.481$, $p = 0.501$]. A detailed examination of the test session (Figure 5b) indicated that IL-PFC inactivation did not influence within-session responding on either lever [Time, $F(43,516) = 25.108$, $p = 0.000$; Group, $F(1,12) = 0.399$, $p = 0.539$; Group x Time, $F(43, 516) = 0.756$, $p = 0.871$].

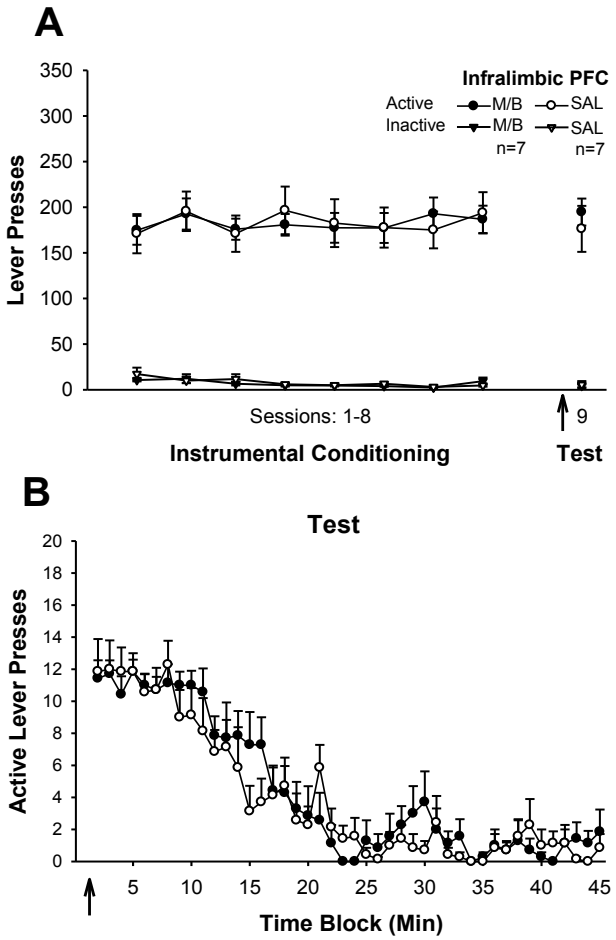

Figure 5. Pre-session inactivation of the IL-PFC had no impact on instrumental responding during a sucrose self-administration session in which pressing on the active lever delivered sucrose. Rats received instrumental conditioning sessions, followed by a subsequent conditioning session that was preceded by an intracranial infusion into the IL-PFC. Filled symbols represent rats that received M/B before extinction session 1 (n=7), and open symbols represent rats that received saline (n=7). Circles represent active lever presses and triangles represent presses on the inactive lever. **A** Mean (±SEM) active and inactive lever presses during instrumental conditioning and at test. **B** Mean (±SEM) active lever responses across 1 min time bins at test. Arrow indicates infusions of saline or M/B in the IL-PFC.

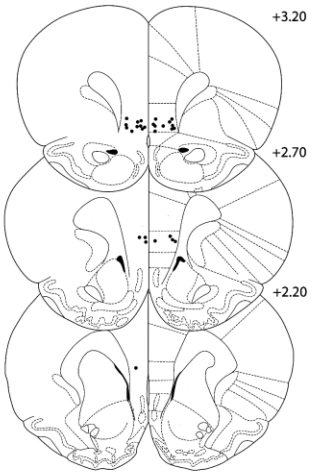

Figure 6. Placement of injector tips within the IL-PFC in experiment 2. Distance from bregma is indicated to the right of each coronal section.

Discussion

The present experiments examined the role of the medial prefrontal cortex in the extinction of Pavlovian and instrumental conditioned sucrose-seeking behaviour. Following Pavlovian or instrumental conditioning, bilateral, pharmacological inactivation of the IL-PFC or PL-PFC was conducted prior to extinction in order to assess the involvement of these brain regions in extinction learning in rats. Inactivation of the IL-PFC caused a more rapid decline in cue-driven sucrose-seeking behaviour, compared to saline. In contrast, PL-PFC inactivation had no effect on conditioned responding during extinction, and inactivation of either brain region had no impact on the recall of extinction when tested on the following day. When examining the role of the IL-PFC in the extinction of instrumental sucrose-seeking, we found that IL-PFC inactivation caused a brief increase in active lever pressing at the beginning of the extinction session, followed by a rapid decline in active lever responding. Unlike the IL-PFC, inactivating the PL-PFC had no effect on active lever pressing during extinction. When assessing extinction memory 24 hrs later, neither IL-PFC nor PL-PFC inactivation affected extinction memory. In a separate experiment, compared to saline, IL-PFC inactivation had no impact on port-entries elicited by a CS that was paired with sucrose delivery. However, the same inactivation procedure significantly increased the number of port-entries made during time intervals that were not signalled by the CS. Inactivating the IL-PFC did not affect active lever pressing during sucrose self-administration. Thus, IL-PFC inactivation appears to reduce conditioned Pavlovian and instrumental sucrose-seeking during the acquisition of extinction, and this effect is not attributed to a motor deficit. Instead, we propose that inactivating the IL-PFC enhances the detection of change in contingencies, thereby facilitating extinction.

Pavlovian & instrumental conditioning

During Pavlovian conditioning, rats learned to associate the CS with sucrose delivery, as evidenced by a progressive increase in port-entry responses elicited by the CS across Pavlovian training sessions. During instrumental training, animals pressed more on the active than the inactive lever, which demonstrates that animals learned the discrimination task. Across training sessions, there were no group differences in Pavlovian or instrumental conditioned responding as a function of the treatment administered during future extinction tests.

Infralimbic prefrontal cortex

Numerous studies have demonstrated the involvement of the IL-PFC in the suppression of conditioned fear (Laurent & Westbrook, 2009; Milad et al., 2004; Tompson et al., 2010; Vidal Gonzalez et al., 2006) and appetitive behaviour (Ishikawa, Ambroggi, Nicola, & Fields, 2008; Lalumiere et al., 2012; Peters & De Vries, 2013; Rhodes & Killcross, 2004; Rhodes & Killcross, 2007). The inhibitory role of the IL-PFC in conditioned responding is reflected in studies investigating the underlying mechanisms that mediate extinction. While enhancing neuronal activity in the IL-PFC facilitates the acquisition of fear extinction (Thompson et al., 2010; Milad & Quirk, 2002; Milad et al., 2004; Vidal-Gonzalez et al., 2006), inactivation of the IL-PFC impairs conditioned fear extinction (Sierra-Mercado et al., 2011). In addition, pharmacological inactivation of the IL-PFC reinstates extinguished cocaine-seeking behaviour in rats (Peters et al., 2008), indicating that the IL-PFC is important for suppressing conditioned appetitive behaviour during extinction. Together, these findings suggest that the IL-PFC is part of the neural circuitry that mediates the extinction of aversive and appetite behaviour.

It has been proposed that extinction training stimulates the excitatory pathway from IL-

PFC to the nucleus accumbens shell (NaShell) and that this pathway is important for inhibiting conditioned responding for an appetitive cue (Lalumiere et al., 2012; Peters et al., 2009). This hypothesis is based on the observation that activity in the NaShell sends inhibitory signals to the ventral pallidum (VP), which is a brain region important for initiating drug-seeking behaviour (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Kalivas, Churchhill, & Romanides, 1999; McFarland & Kalivas, 2001). Thus, activity in the IL-PFC during extinction indirectly inhibits the VP, thereby causing a reduction in conditioned responding. Alternatively, others have considered the possibility that the IL-PFC may reduce conditioned responding by preventing PL-PFC neurons from firing. This hypothesis is based on recent findings whereby optogenetic stimulation of the IL-PFC inhibited PL-PFC output (Ji & Neugebauer, 2012).

Thus, we predicted that IL-PFC inactivation would delay the acquisition of extinction during an initial session in which the CS was presented in the absence of sucrose. Instead, we found that inactivating the IL-PFC diminished the number of CS responses during the first extinction session. While IL-PFC inactivation had no impact on the number of port-entries made during the first CS trial, there was a rapid decline in the number of port-entry responses made during the next 2 CS trials, compared to saline. Similarly, there was no difference in latency to respond to the first 3 CS trials, however, M/B-infused animals were significantly slower to respond to the 4^{th} CS trial. These results suggest that inactivating the IL-PFC facilitated extinction learning, and contradict fear conditioning findings, in which impaired extinction learning is observed in the absence of the IL-PFC (Sierra-Mercado et al., 2011). Results from the present research are all the more surprising given that activation of the IL-PFC has been shown to suppress conditioned fear responses during the acquisition of extinction (Milad & Quirk, 2002; Milad et al., 2004; Thompson et al., 2010).

Given that the role of the IL-PFC in mediating extinction learning has typically been observed in fear conditioning studies, our results may indicate that the IL-PFC has a different role in appetitive extinction. To our knowledge, our study is the first to have examined the effect of IL-PFC inactivation on extinction learning of appetitive behaviour. Other studies have demonstrated that IL-PFC lesions do not interfere with the acquisition of extinction, but enhance Pavlovian conditioned food-seeking during tests of reinstatement, renewal, and spontaneous recovery (Rhodes & Killcross, 2004; Rhodes & Killcross, 2007). However, it is important to note that in lesion studies Pavlovian conditioning is conducted in the absence of the IL-PFC. Thus, the absence of the IL-PFC during Pavlovian conditioning may have a significant impact on the acquisition and expression of extinction.

Our prediction that IL-PFC inactivation would impair the extinction of instrumental sucrose-seeking was based on the hypothesis that the IL-PFC is a common brain region in mediating aversive and appetitive extinction (Peters et al., 2009). We found that IL-PFC inactivation had no impact on the average number of active lever responses during the acquisition of extinction. Interestingly, an examination of within-session responding revealed that IL-PFC inactivation caused a burst in active lever responses during the first minute of the initial extinction session, followed by a more rapid decline in responding, compared to saline. This finding is consistent with a previous study in which reversible inactivation of the IL-PFC did not affect the average number of cocaine-seeking responses during the acquisition of extinction (Peters et al., 2008). However, the authors of this study did not report within-session behaviour. In designing these experiments, it is important to take into consideration that the extinction of a conditioned response can be observed in the first few minutes of a session, and that averaging responses across a test session could potentially mask an effect on extinction

learning. Thus, the analysis of within-session responding during extinction of appetitive behaviour should be not be overlooked.

That IL-PFC inactivation caused a rapid decline in Pavlovian and instrumental sucrose-seeking during the first extinction suggests that inactivating the IL-PFC may facilitate extinction learning, possibly by enhancing the ability to detect the change in contingencies during extinction. As such, the IL-PFC could potentially be responsible for mediating persistence in responding when an expected outcome fails to occur, as experienced during extinction. This would explain results from previous studies in which IL-PFC inactivation attenuated reinstatement of conditioned heroin (Bossert et al., 2010; Rogers & See, 2008) and methamphetamine seeking in rats (Rocha & Kalivas, 2010). However, others have found that pharmacological inactivation of the IL-PFC reinstates extinguished instrumental cocaine-seeking behaviour (Peters et al., 2008), and increasing IL-PFC function via glutamate transmission enhancement suppresses cue-induced reinstatement of cocaine seeking (Lalumiere et al., 2012). Given the putative role of glutamate transmission in learning and memory consolidation (Miyamoto, 2006; Rao & Finkbeiner, 2007), studies that manipulate glutamate transmission may be more informative than inactivation studies when investigating the underlying neural mechanisms that mediate extinction learning

Although fear conditioning studies have suggested that the IL-PFC is required for the consolidation of the extinction memory (Sierra-Mercado et al., 2011), we found that pre-session inactivation of the IL-PFC does not affect extinction recall. Similarly, IL-PFC inactivation prior to the extinction of cocaine-seeking has been shown to have no impact on the retention of extinction (Peters et al., 2008). However, our findings need to be interpreted with caution given that IL-PFC inactivation was performed before, rather than after extinction. This is important to

highlight as memory consolidation of extinction has been shown to occur within the first few hours after initial extinction learning (Burgos-Robles et al., 2007). Indeed, others have found that post-extinction inhibition of IL-PFC activity impairs extinction recall, and that increasing IL-PFC glutamate transmission within this consolidation window enhanced the retention of extinction learning (Lalumiere et al., 2010).

To investigate whether IL-PFC inactivation impairs the ability to make port-entries, we inactivated the IL-PFC prior to a Pavlovian conditioning session in which CS trials were paired with sucrose delivery. We found that inactivation of the IL-PFC had no impact on CS responding, thereby confirming that animals can execute the Pavlovian task in the absence of the IL-PFC. We also found that inactivating the IL-PFC did not affect active lever pressing during operant responding for sucrose in an instrumental conditioning task. These results extend previous findings that IL-PFC inactivation does not impair locomotor activity (McLaughlin & See, 2003; Fuchs et al., 2005), and confirm that the decrease in conditioned responding following IL-PFC inactivation during extinction was not caused by an inability to respond.

Interestingly, IL-PFC inactivation increased the number of port-entries made outside CS intervals (non-CS). One interpretation of this finding is that inhibiting neuronal activity in the IL-PFC causes an increase in impulsive behaviour in the presence of a reinforcer. This idea is further supported by a number of studies showing increased impulsivity in the absence of the IL-PFC (Chudasama, Passetti, Rhodes, Lopian, Desai, & Robbins, 2003; Murphy, Dalley, & Robbins, 2005; Tsutui-Kimura et al., 2013). These findings suggest that the role of the IL-PFC in conditioned responding may depend on the presence or absence of a reinforcer.

Prelimbic prefrontal cortex

The role of the PL-PFC in promoting conditioned behaviour has been well established in studies of fear conditioning. Whereas stimulation of PL-PFC neurons potentiates conditioned fear behaviour (Vidal-Gonzalez et al., 2006), inactivating these neurons attenuates conditioned freezing responses during extinction (Laurent & Westbrook, 2009; Sierra-Mercado et al., 2011). In addition, PL-PFC neurons are activated during early extinction when conditioned fear behaviour is high, and neuronal activity in the PL-PFC is highly correlated with poor extinction behaviour in rats (Burgos-Robles et al., 2009). However, unlike the IL-PFC, the PL-PFC does not appear to be involved in extinction recall (Laurent & Westbrook, 2009; Sierra-Mercado et al., 2011). Similarly, the PL-PFC has been shown to promote conditioned appetitive behaviour, as indexed by a reduction in cocaine, alcohol, and methamphetamine drug-seeking during tests of reinstatement following PL-PFC inactivation (Capriles Rodaros, Sorge, and Stewart, 2003; Fuchs et al., 2005; McFarland & Kalivas, 2001; McLaughlin & See, 2003; Stefanik et al., 2013; Willcocks & McNally, 2012). There is also evidence showing that in the absence of the PL-PFC, animals require more time to reach stable sucrose and food self-administration (Corbit & Balleine, 2003), which suggests that the PL-PFC is important for initiating reward-seeking behaviour.

Several hypotheses have been proposed to explain how the PL-PFC promotes conditioned appetitive behaviour. For example, the PL-PFC may promote appetitive behaviour via glutamatergic projections to the nucleus accumbens core (Lalumiere & Kalivas, 2008; McFarland et al., 2003), which in turn projects to the pallidum and activates reward-seeking behaviour (Peters et al., 2009). Others have proposed that the PL-PFC can initiate reward-seeking behaviour via its excitatory glutamatergic projections to the basolateral amygdala (BLA;

Brinley-Reed, Mascagni, & McDonald, 1995), which is a brain region that has been implicated in learned stimulus-reward associations (Balleine & Killcross, 2006; Everitt, Cardinal, Parkinson, & Robbins, 2003; See, 2005). Support for this model comes from findings showing that enhanced glutamatergic transmission in the BLA facilitates the extinction of a conditioned preference for a drug-associated context (Schroeder & Packard, 2004), and BLA inactivation attenuates cue-induced reinstatement of a cocaine-seeking response in rats (Grimm & See, 2000).

Based on the findings described above, we predicted that PL-PFC inactivation would decrease conditioned responding during the initial extinction session, without affecting extinction memory when tested on the following day. Contrary to this prediction, PL-PFC inactivation had no effect on Pavlovian or instrumental conditioned responding during extinction. In congruence with the literature, there was no impact of PL-PFC inactivation on extinction recall. As such, our results indicate that the PL-PFC is not involved in the extinction of conditioned appetitive behaviour. Thus, although the PL-PFC is important for the reinstatement of reward-seeking behaviour in extinguished animals (Fuchs et al., 2005; McFarland & Kalivas, 2001; McLaughlin & See, 2003; Willcocks & McNally, 2012), our data suggests that it does not play a key role in extinction acquisition of conditioned appetitive behaviour. Indeed, the idea that the PL-PFC is not involved in the extinction of conditioned reward-seeking is further supported by a study in which PL-PFC lesions did not alter the extinction of conditioned place preference for a cocaine-associated context (Zavala, Weber, Rice, Alleweireldt, & Neisewander, 2003).

Methodological considerations

An important consideration in the interpretation of our data is that subjects utilized in our experiments were Long-Evans rats, whereas most studies that have found the IL-PFC to be

important for suppressing conditioned responding used Sprague-Dawley rats (Vidal-Gonzalez et al., 2006; Milad et al., 2004; Milad & Quirk, 2002; Morgan & Ledoux, 1993; Sierra-Mercado et al., 2011; Thompson et al., 2010; Vidal-Gonzalez et al., 2006). Evidence exists demonstrating different outcomes in extinction behaviour between Long-Evans and Sprague-Dawley rats following IL-PFC lesions. Specifically, it has been found that lesions to the IL-PFC impair extinction memory in Sprague-Dawley rats, but not in the Long-Evans strain (Chang & Maren, 2010). Though strain type may have influenced the effects of IL-PFC inactivation on extinction in our experiments, we consider this possibility to be unlikely since others have failed to find a significant effect on conditioned behaviour during extinction in Sprague-Dawley rats in the absence of the IL-PFC (Barron, & Lebron, 2000; Peters et al., 2008).

Considering that the IL-PFC has typically been shown to inhibit conditioned cocaine-seeking (Lalumiere et al., 2012; Peters et al., 2008) but not heroin (Bossert et al., 2010; Rogers & See, 2008), ecstasy (Ball & Slane, 2012), or methamphetamine (Rocha & Kalivas, 2010) seeking behaviour, a possible explanation for our findings could be attributed to the type of reinforcer used. Indeed, it has been proposed that the function of the IL-PFC in conditioned reward-seeking behaviour may depend on the type of reinforcer that animals are subjected to (Badiani, Belin, Epstein, Calu, & Shaham, 2011). However, cues associated with natural reinforcers recruit the same network of neurons as those recruited by drug-associated cues (Schroeder, Binzak, & Kelley, 2001). In addition, lesions to the IL-PFC potentiate spontaneous recovery, renewal, and reinstatement of Pavlovian conditioned food-seeking behaviour in rats (Rhodes & Killcross, 2004; Rhodes & Killcross, 2007). Thus, these findings are in line with what has been observed in experiments using drugs as reinforcers.

Cannulae targeting the IL-PFC in the present experiments passed through the PL-PFC,

inadvertently destroying a portion of the PL-PFC. This is important to highlight, as the PL-PFC has typically been shown to promote conditioned appetitive behaviour ((Fuchs et al., 2005; McFarland & Kalivas, 2001; McLaughlin & See, 2003; Willcocks & McNally, 2012). Thus, it can be argued that our finding that IL-PFC inactivation diminished conditioned responding during extinction was caused by a partial destruction of PL-PFC neurons. Similarly, the M/B solution infused in the IL-PFC could have made its way into the PL-PFC when removing the injector, thereby inactivating PL-PFC neurons during the extinction test. However, it is unlikely that the IL-PFC effect on extinction was caused by the destruction or inactivation of PL-PFC neurons because infusions of M/B in the PL-PFC had no impact on conditioned responding during extinction.

Future studies

Our finding that IL-PFC inactivation facilitated the acquisition of extinction is surprising given that previous fear conditioning studies have observed an opposite effect, whereby extinction is impaired in the absence of the IL-PFC (Morgan et al., 1993; Sierra-Mercado et al., 2011). In addition, other studies have demonstrated that stimulating IL-PFC neurons inhibits conditioned fear behaviour during extinction (Milad et al., 2004; Vidal Gonzalez et al., 2006), thereby facilitating extinction learning (Thompson et al., 2010). Our data suggests that the role of the IL-PFC in the acquisition of extinction may depend on the type of behaviour being extinguished, notably appetitive versus fear behaviour. To further explore this possibility, it would be of interest to examine the effect of pre-session IL-PFC stimulation on the acquisition of extinction of a conditioned appetitive response. Impairment in extinction learning by IL-PFC stimulation would further support our hypothesis that IL-PFC inactivation enhances the extinction of conditioned appetitive behaviour, and that the IL-PFC promotes persistence in

conditioned responding during extinction. Given the novelty of our results, we have yet to fully understand the neural mechanisms underlying our findings pertaining to the role of the IL-PFC in the extinction of conditioned sucrose-seeking. The IL-PFC is not the only neural substrate involved in extinction. Instead, it is part of a larger circuit involving brain regions such as the BLA, nucleus accumbens, hippocampus, hypothalamus, and ventral pallidum (Gass & Chandler, 2013; Millan, Marchant, & McNally, 2011; Peters et al., 2009; Quirk & Mueller, 2008). Thus, additional studies are required to further understand how these different brain regions interact with each other to form a circuit that mediates the extinction of reward-seeking behaviour.

Conclusions

The present experiments investigated the role of the IL-PFC and PL-PFC in the extinction of Pavlovian and instrumental conditioned sucrose seeking. Contrary to predictions based on fear extinction studies, we found that IL-PFC inactivation facilitated extinction learning**.** These results suggest that the IL-PFC mediates persistence in appetitive conditioned responding during extinction, and more specifically when expectancy is violated. Moreover, our data demonstrated that the PL-PFC does not play a central role in the extinction of conditioned appetitive behaviour, and that neither the IL-PFC nor the PL-PFC appears to be involved in the extinction memory of conditioned sucrose-seeking. When examining the role of the IL-PFC under conditions in which CS presentations delivered sucrose, we found that IL-PFC inactivation caused an increase in impulsivity, which is an effect that has previously been observed in the absence of the IL-PFC under reinforced conditions. These findings suggest that the IL-PFC and PL-PFC have more complex roles, other than being important for inhibiting and promoting conditioned behaviour, respectively. Moreover, our results indicate that the IL-PFC and PL-PFC may have differential functions in the extinction of aversive and appetite behaviour, and

consequently add to the growing literature on neural mechanisms that mediate the extinction of reward-seeking behaviour.

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Appendix A

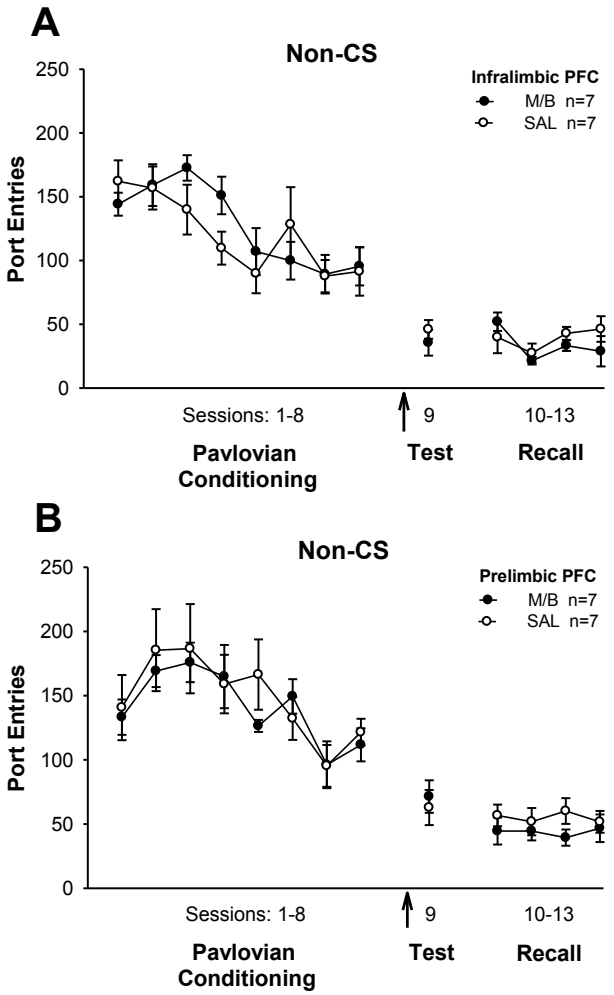

Figure 1. Inactivating the IL-PFC or PL-PFC had no impact on port-entries made during time intervals that were not signaled by the CS (Non-CS) across Pavlovian conditioning, a single extinction session, and 4 subsequent recall sessions. Filled symbols represent rats that received M/B before the extinction test session, and open symbols represent rats that received saline. **A** The average (Mean, ± SEM) number of Non-CS responses decreased across Pavlovian training sessions in rats with cannulae placements in the IL-PFC [Session, $F(7, 84) = 8.717$, $p = 0.000$], and there were no differences as a function of group [Group, $F(1, 12) = 0.212$, $p = 0.654$; Group x Session, $F(7, 84) = 1.402$, $p = 0.248$]. Inactivating the IL-PFC did not affect non-CS responses [$t(12) = 0.833$, $p = 0.421$] during the extinction test. There was no change in non-CS responses across 4 subsequent extinction sessions, [Session, $F(3, 36) = 2.311$, $p = 0.093$], with no main effects or interactions with group [Group, $F(1, 12) = 0.737$, $p = 0.407$; Group x Session, $F(3, 36) = 1.140$, $p = 0.346$]. **B** The average (Mean, ± SEM) number of Non-CS responses decreased across Pavlovian training sessions in rats with cannulae placements in the PL-PFC [Session, $F(7,84) = 4.386$, $p = 0.017$], and there were no differences as a function of group [Group, $F(1,12) = 0.634$, $p = 0.558$; Group x Session, $F(7,84) = 0.369$, $p = 0.732$]. Inactivating the PL-PFC did not affect non-CS responses [$t(12) = -0.460$, $p = 0.653$] during the extinction test. There was no change in non-CS responses across 4 subsequent extinction sessions, [Session, $F(3,36) = 0.044$, $p = 0.988$], with no main effects or interactions with group [Group, $F(1,12) = 1.297$, $p = 0.277$; Group x Session, $F(3,36) = 0.511$, $p = 0.677$].

Appendix B

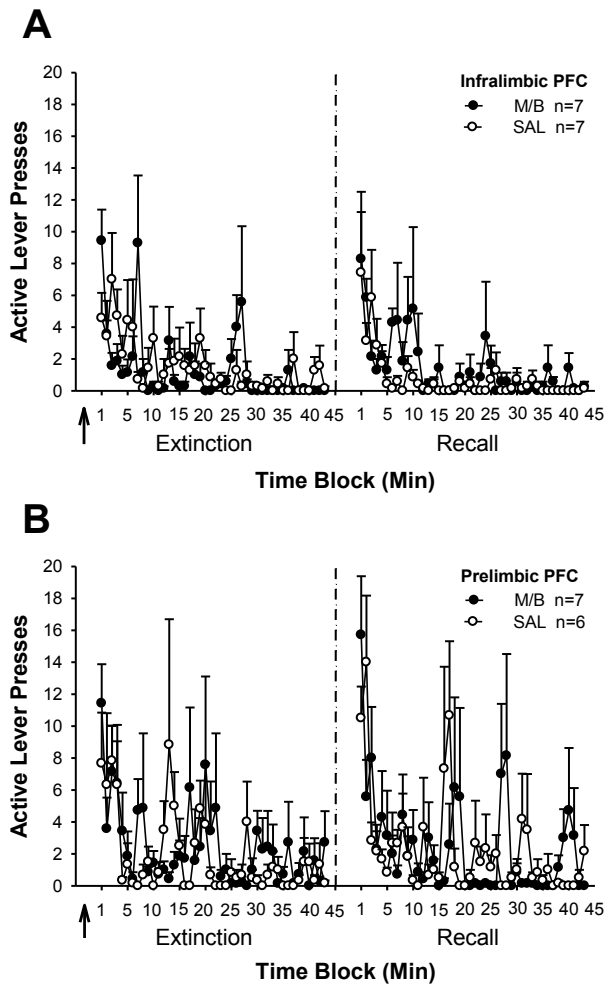

Figure 2. Inactivating the IL-PFC before an extinction session caused a rapid decline in active lever pressing during the extinction test session, but not during recall. Responses on the active lever during extinction diminished as a function of time following infusions in the IL-PFC [Time, F(43, 516) = 3.038, $p = 0.000$] or PL-PFC [Time F(43,473) = 2.100, $p = 0.000$]. There was no main effect of group on active lever presses following infusions in the IL-PFC [Group, F(1, 12) = 2.634, $p = 0.131$] or in the PL-PFC [Group F(1,11) = 0.655, $p = 0.435$]. However, M/B infusions caused a more rapid decrease in active lever pressing in rats with cannulae targeting the IL-PFC [Group x Time, F(43, 516) = 1.674, $p = 0.006$], but not in rats with PL-PFC canulae placements [Time x Group F(43,473) = 0.882, $p = 0.686$]. During recall, active lever pressing diminished as a function of time following infusions in the IL-PFC [Time F(43,516) = 3.243, p = 0.000] or in the PL-PFC [Time F(43,473) = 3.066, $p = 0.000$]. There was no main effect of group on active lever presses following infusions in the IL-PFC [Group F(1,12) = 2.115, p = 0.171] or in the PL-PFC [Group F(1,11) = 0.035, $p = 0.855$]. A significant group x time interaction was found in rats with PL-PFC infusions [Time x Group F(43,473) = 1.505, $p = 0.024$] but not in rats with infusions in the IL-PFC [Time x Group F(43,473) = 0.882, $p = 0.686$]. Mean (± SEM) active lever-pressing following M/B (filled symbols) or saline (open symbols) infusions in the IL-PFC (**A**) or PL-PFC (**B**) across the extinction test and recall session 1.