# The prelimbic cortex uses contextual cues to modulate responding towards predictive stimuli during fear renewal

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## A B S T R A C T

Previous research suggests the prelimbic (PL) cortex is involved in expression of conditioned fear (Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009; Corcoran & Quirk, 2007). However, there is a long history of research in the appetitive domain which implicates this region in using higher-order cues to modulate a behavioural response (Birrell & Brown, 2000; Floresco, Block, & Tse, 2008; Marquis, Killcross, & Haddon, 2007; Sharpe & Killcross, 2014). For example, the PL cortex is necessary to allow animals to use contextual cues to disambiguate response conflict in ambiguous circumstances (Marquis et al., 2007). Using an ABA fear renewal procedure, we assessed the role of the PL cortex in using contextual cues to modulate a response towards a conditioned stimulus (CS) in an aversive setting. We found that pre-training lesions of the PL cortex did not impact on the expression or extinction of conditioned fear. Rather, they selectively abolished renewal. Functional inactivation of the PL cortex during extinction did not disrupt the subsequent renewal of conditioned fear or the ability of animals to exhibit fear towards a CS during the extinction session. However, PL inactivation during the renewal test session disrupted the ability of animals to demonstrate a reinstatement of responding in the renewal context. An analysis of orienting responses showed that renewal deficits were accompanied by a lack of change in attentional responding towards the CS. These data suggest the PL cortex uses contextual cues to modulate both a behavioural and an attentional response during aversive procedures. We argue that the role of the PL cortex in the expression of conditioned fear is to use higher-order information to modulate responding towards predictive cues in ambiguous circumstance.

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## 1. Introduction

The dominant theory in the fear conditioning literature purports that the rodent PL region of the medial prefrontal cortex is involved in the expression of conditioned fear. One of the first studies to suggest this was conducted by Corcoran and Quirk (2007) who found that inactivation of the PL cortex disrupted the expression of conditioned fear to a discrete stimulus and a context. Subsequently, Burgos-Robles, Vidal-Gonzalez, and Quirk, (2009) recorded PL neurons during presentation of a conditioned stimulus (CS) and found sustained responses in these neurons were correlated with freezing. This was in contrast to initial bursts of amygdala activity not capable of supporting a lengthy behavioural response, leading the authors to argue that the PL cortex potentiates initial amygdala activity to promote a sustained freezing response. Since then, other authors have suggested that the PL cortex also receives projections from other structures, such as the amygdala and hippocampus, to promote the expression of conditioned fear as dictated by those structures (Sotres-Bayon, Sierra-Mercado, Pardilla-Delgado, & Quirk, 2012; Zelikowsky et al., 2013).

A view of the PL cortex in the expression of conditioned responding as dictated by other structures is at odds with the current view of PL functioning in the appetitive domain. Within this domain there is a long history of research which suggests that the PL cortex is involved in using higher-order information, such as contextual cues, goal value, or the associative history of a stimulus to modulate responding ( Balleine and Dickinson, 1998; Birrell & Brown, 2000; Chudasama & Muir, 2001; Floresco, Block, & Tse, 2008; Granon et al., 2000; Marquis, Killcross, & Haddon, 2007; Sharpe & Killcross, 2014). In line with this, manipulation of activity in the PL cortex disrupts the ability of animals to respond in a goal-directed manner, use contextual cues to disambiguate response conflict, and change the degree of attention directed towards a stimulus on the basis of how well it predicts an outcome (Balleine and Dickinson, 1998; Marquis et al., 2007; Sharpe & Killcross, 2014). This has led to a theory of functioning of the PL

cortex that proposes that this region is necessary to use complex information garnered from the environment to modulate the ability of a stimulus to elicit a particular response (Haddon and Killcross, 2006; Marquis et al., 2007).

For example, Marquis et al. (2007) demonstrated that the PL cortex is necessary for animals to use contextual cues to disambiguate conflict between two competing responses in a rodent version of the Stroop task. In this task, rats are trained on two bi-conditional discriminations in two distinct contexts. In the 'auditory' context, one auditory cue predicts that a left lever press will result in reinforcement, while presentation of a different auditory stimulus dictates that a right lever press will be reinforced. Similarly, in the 'visual' context, two visual stimuli dictate that either a left or right lever press will be reinforced. During an extinction test session in either context, rats are presented with novel audio–visual compounds. These compounds can be either congruent or incongruent with the contingencies presented during training. In the congruent case, both elements of the audio–visual compound predicted the same lever press would be reinforced during training. In contrast, incongruent compounds comprised two elements which dictated alternate lever-press responses during training. Under these circumstances rats are capable of using the present contextual cues to disambiguate the response conflict evoked by presentation of the incongruent compound and perform the lever press associated with the stimulus trained in the test context (e.g. when given an incongruent audio–visual compound in the auditory context, rats will perform the response associated with the auditory stimulus during training and disregard the visual stimulus). However, when the PL cortex is inactivated prior to the test session, rats are not able to use the contextual cues to disambiguate the response conflict and perform the lever press associated with the cue trained in the test context. These data demonstrate that the PL cortex is necessary to use task-setting contextual cues to disambiguate response conflict, interpreted as an ability to allow present contextual cues to influence the ability of a stimulus to elicit a response (Haddon and Killcross, 2006; Marquis et al., 2007).

Given the PL cortex is theorized to use higher-order cues to modulate responding in appetitively motivated procedures, it begs the question of whether the PL cortex may also serve a similar role in the aversive domain. There have been a few studies that have suggested this may be the case. For example, Orsini, Kim, Knapska, and Maren (2011) found that disconnection of the hippocampus and the PL cortex disrupted the ability of animals to exhibit renewal of conditioned fear following extinction. Orsini et al. (2011) paired a CS with shock in one context (context A), extinguished the CS in an alternative context (context B), and tested animals for levels of fear expressed towards the CS in another familiar context (context C; i.e. an ABC renewal paradigm). Under normal circumstances, rats will again express fear when they are placed in context C. This effect has been argued to be due to the context dependence of extinction. That is, when the CS is presented in context B in the absence of shock, rats attribute the lack of shock to the contextual cues present in extinction. Consequently, it is argued that animals form a modulatory association whereby the extinction context exerts an inhibitory influence over conditioned responding (Bouton, 1993). Thus, when they are placed in context C they again express fear as the contextual cues observed during extinction are no longer present and the inhibitory association is not in effect (Bouton, 1993). Prior to the extinction test session, Orsini et al. (2011) gave rats unilateral lesions (contralateral or ipsilateral) of the hippocampus and PL cortex. Rats with contralateral lesions, where the hippocampus and PL cortex are functionally disconnected, failed to exhibit the renewal of fear when the context was different from that experienced in extinction. This suggests that these animals were not capable of using the contextual cues to exhibit a renewed fear response to the extinguished conditioned stimulus. These data have recently been extended by Kim, Kim, Kim, and Choi (2013) who have also demonstrated that specific lesions of the PL cortex prior to a renewal test session also disrupts the subsequent expression of renewal. These data are consistent with a role for the PL cortex in allowing contextual cues to modulate responding in an aversive setting, as is the case in appetitive procedures.

Though these studies have suggested that the PL cortex may be involved in allowing contextual cues to modulate responding, given these manipulations involved disconnection or specific lesions prior to the renewal test session it is not possible to distinguish between a role for the PL cortex in using contexts to modulate responding from a role for this region fear expression. That is, given an absence of fear renewal necessarily entails a reduction of fear responding, findings that disruption of signaling in the PL cortex produces an absence of renewal may also be interpreted as a role for promoting a fear response, as is often argued to be the case (Zelikowsky et al., 2013). Using an ABA renewal procedure, we attempted to elucidate the role of the PL cortex in using contextual cues to modulate fear responding. In the first experiment, we examined the impact of pre-training lesions of the PL cortex on the ability of animals to exhibit the renewal of conditioned fear. If the PL cortex is necessary for animals to express fear towards a CS, we would expect that these animals would exhibit lower levels of conditioned fear during extinction. However, if the PL cortex is involved in using higher-order information to modulate responding, we would expect that these animals initially exhibit fear towards the CS but fail to renew levels of fear following context change. Additionally, we used functional inactivation of the PL cortex during either extinction or renewal of conditioned fear in order to investigate the temporal dynamics of the involvement of the PL cortex in fear renewal. We hypothesised that the PL cortex would not be involved in the expression of fear per se but, rather, the ability to use contextual cues to modulate responding following extinction.

## 2. Materials and methods

### 2.1. Subjects

All animals were experimentally naïve male Long–Evans rats (Monash animal services, Australia), weighing between 280 and 360 g. Animals were housed 8 rats per cage (26 cm × 59 cm × 37 cm), in a temperature- and humidity-controlled environment (22 °C) operating on a 12 h light/dark cycle (lights on at 7:00 a.m.). All behavioural and surgical procedures took place during the light cycle. All rats were handled by the experimenter for 3 days prior to surgery.

All animal procedures, both experimental and routine care, were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-123, revised 1996) and were approved by the University of New South Wales Animals Care and Ethics Committee (ACE: 10/100A).

### 2.2. Surgery

Surgery was conducted under complete anesthesia which was induced by inhalation of isoflurane in oxygen carrier (5% induction; 1–2% maintenance). Following the onset of anesthesia, rats were placed in a stereotaxic frame (World Precision Instruments, FL). An incision was made into the scalp, and the skin was retracted to expose the skull. For each rat, the incisor bar was adjusted such that bregma and lambda were level. Small holes above the intended lesion site were made with a high-speed dental drill, and the dura mater was severed to reveal the cortical parenchyma.

*Pre-training lesions*: Excitotoxic lesions were induced through the injection of the neurotoxic drug *N*-methyl-D-aspartic acid (NMDA; Sigma–Aldrich, Australia). Rats received bilateral injections of 0.35 μL of 0.067 M NMDA using a 5-μL syringe (co-ordinates relative to bregma; anteroposterior, +3.0; mediolateral, ±0.7; dorsoventral, −3.8; Hamilton syringes, NV). NMDA was infused at a rate of 0.1 μL per minute, 1 min after lowering the needle and an additional 4 min of diffusion time was given prior to elevating the needle. Rats receiving sham surgery underwent an identical procedure without injection of NMDA.

*Cannulae implanation and drug infusion*: Bilateral stainless steel guide cannulae (26 gauge; Plastics One, VA) were lowered 0.5 mm dorsal to the infusion site (co-ordinates relative to bregma; anteroposterior, +3.0; mediolateral, ±0/7; dorsoventral, −3.3). Cannulae were held in place by dental cement and anchored to the skull with four fixing screws located on different bone plates. Removable dummy cannulae were inserted into the guide cannulae to prevent the cannulae from blocking. Dummy cannulae and dust caps were removed prior to infusions. Muscimol (5-amino-methyl-3-hydroxyisoxazole; Sigma–Aldrich, Australia) was dissolved in nonpyrogenic saline (0.9% w/v) to obtain a final concentration of 0.5 μg/μl and was infused bilaterally into the PL cortex by inserting a 33 gauge internal cannula into the guide cannula. The internal cannula was connected to a 25 μl glass syringe (Hamilton syringes, NV) attached to an infusion pump (World Precision Instruments, FL) and projected an additional 0.5 mm from the tip of the guide cannula. A total volume of 0.5 μl was infused bilaterally at a rate of 0.5 μl/min. This amount was in line with those used in appetitive experiments demonstrating effects selective to the PL cortex (Marquis et al., 2007). The internal cannula remained in place for an additional 60 s following infusions, allowing the bolus to be absorbed. The infusion cannulae were then removed and the dummy cannulae and dust caps replaced. The infusions occurred 10 min before the onset of the test sessions.

## 2.3. Histology

At the end of all experiments, rats were killed with an overdose of sodium pentobarbitone (Virbac, Sydney, Australia) and decapitated. Brains were removed, immediately placed on a Peltier element of a cryostat (Leica-microsystems, Sydney, Australia), and frozen overnight. Forty microns coronal sections were cut through the region of the PL cortex and mounted onto glass slides. Tissue was stained using 1% cresyl violet nissl stain and cannulae and lesions placement were subsequently assessed by a trained observer. The PL region was defined by the boundaries specified in the atlas of Paxinos and Watson (1998). Lesioned rats with incomplete damage to the PL cortex, or with extensive damage to surrounding areas, were excluded from all analyses. Cannulae placements that were considered outside of the boundaries of the PL cortex were excluded from all analyses.

## 2.4. Behavioural procedures

All rats were given 10 days to recover from surgery, during which they were handled and weighed daily. Prior to all test sessions, rats were taken out of their home cages and transported to the testing laboratory in buckets. Rats remained in the buckets for 10 mins prior to the start of the session. With the exception of the renewal tests, rats received two test sessions every day, one in the morning (AM) and one in the afternoon (PM) separated by at least 3 h.

### 2.4.1. Apparatus

Training and testing took place in 8 operant chambers (30 cm × 24 cm × 22 cm; Med Associates, VT) which were individ-

ually housed in light- and sound-attenuating compartments. Boxes were 30 cm wide × 24 cm deep × 21 cm high and consisted of two aluminium walls and an aluminium ceiling, and two Perspex side walls. The chamber floors constructed of 19 stainless steel rods (3.8 mm in diameter, spaced 1.6 cm apart). These chambers served as context A for the experiment. A second context was constructed by fixing laminated spotted wallpapers to three of the four chamber walls, and placing a thick Perspex flooring over the stainless steel rods. Two panel lights (2 cm in diameter), were located on the right hand wall of the chamber above the magazine. A 3-W house light was located on the upper left hand wall of the chambers and was illuminated for the duration of all experimental sessions. The chambers contained a heavy duty relay that delivered a 5 kHz clicker stimulus. A computer equipped with MED-PC software (Med Associates, VT) controlled the equipment.

During fear conditioning, a 1-s 0.8 mA shock was delivered through the stainless steel rods on the floor of the operant chambers connected to a scrambled shock generator (ENV-412, Med Associates, VT). A camera was mounted on the back wall of each shell containing the operant chamber. The cameras were linked to a remote access DVR video recorder.

### 2.4.2. Context pre-exposure

All animals were pre-exposed to both contexts A and B prior to fear conditioning to enhance the discriminability between the contexts and reduce contextual fear conditioning. Pre-exposure ran across experimental days one and two, with two 30-min sessions each day. The order of pre-exposure to each context was counterbalanced within and between groups. Thus, all rats were exposed to both contexts twice for a duration of 30 min each.

### 2.4.3. Fear conditioning

Animals received two sessions of fear conditioning on experimental day three in context A. Each conditioning session consisted of three 30-s presentations of a clicker stimulus co-terminating with delivery of a 0.8 mA shock. The first shock was delivered an average of 8 min following placement in the chambers, and subsequent pairings were separated by a variable inter-trial interval (ITI) which varied around an 8-min mean. Additionally, rats were left in the chamber for an average of 8 min after the final click-shock pairing, yielding a session of approximately 35 min. Long ITIs were used to reduce competition between the clicker CS and the context, ensuring the amount of conditioned fear attributed to the context was low (Holland, 2000; Mais & Vossen, 1993).

### 2.4.4. Context extinction

Rats received two sessions of extinction in context A on experimental day four to extinguish any residual fear towards the context and ensure delivery of the shock was attributed presentation of the CS and not the context. These sessions were 30 min long. No CS or shock presentations occurred during this session.

### 2.4.5. CS extinction

Rats received two extinction sessions on experimental day five in context B. Extinction sessions comprised five 30-s presentations of the clicker stimulus separated by a variable ITI with a mean of 8 min. Again, rats were left in the chambers for an average of 8 min after the final CS clicker presentation, yielding a session of approximately 50 min. No shock was delivered during extinction sessions.

### 2.4.6. Renewal test

The renewal test comprised two sessions, an AM session on experimental day 6 and a PM session on experimental day 7. On day 6, half the animals in each group received the renewal test in context A and the other half received the renewal test in context

B. This counterbalancing was reversed on day 7. The parameters were the same in these test sessions as in extinction. No shock was delivered during test sessions in either contexts A or B.

### 2.4.7. Data analysis

Freezing was used to assess fear conditioning to the clicker CS. Freezing was defined as the absence of all movement except respiration. Each rat was scored as 'freezing' or 'not freezing' by a blind, trained observer, every 2 s during presentation of the CS and a 30 s baseline period that immediately preceded CS presentation (i.e. pre-CS period). A percentage was calculated for the proportion of total observations a rat spent freezing. We also measured orienting towards the CS as an index of an attentional response during the test phase of each experiment (Hall & Channel, 1985; Holtzman, Siette, Holmes, & Westbrook, 2010; Swan & Pearce, 1988). Orienting was defined as head jerking towards the top of the experimental chamber, directed towards the auditory stimulus. Head jerking was characterised by short rapid horizontal and/or vertical movements of the head (Holland, 1977). Rats were scores as 'head jerking' or 'not head jerking' by a trained, blind observer every 2 s during CS presentation. The number of orienting responses made was calculated as a proportion of total observations to create a percentage of time spent orienting. Head jerking was chosen as it is often used as an index of an attentional response towards an auditory cue (Hall & Channel, 1985; Holtzman et al., 2010; Swan & Pearce, 1988), and was the most common behaviour recorded other than freezing during test sessions, consistent with other experiments observing attentional responding in aversive paradigms (Holtzman et al., 2010). Though some researchers have taken an orienting response as indicative of conditioned responding (Holland, 1977), others have argued that the degree of attention directed towards a stimulus can be defined by the ability of the stimulus to elicit an orienting response (Hall & Channel, 1985; Holtzman et al., 2010; Swan & Pearce, 1988; Wilson, Boumphrey, & Pearce, 1992). Given theories of selective attention in associative learning posit that attention determines the rate of learning, discriminating between an attentional and conditioned response is inherently difficult. However, a dissociation between orienting and conditioned responding, where an orienting response precedes magazine approach (Wilson et al., 1992), suggests head jerking may be a reliable measure of attentional responding.

## 3. Results

### 3.1. Pre-training lesions of the PL cortex

#### 3.1.1. Histology

All rats recovered from surgery and no significant weight loss or behavioural problems were observed. Three rats from the PL-lesioned group received lesions considered outside the boundaries of the PL cortex and were excluded from all analyses. One rat from the sham-lesioned group exhibited extensive needle tract damage to the adjacent anterior cingulate cortex and so was also excluded from all analyses. These exclusions yielded the following final group sizes: sham $n = 15$, PL $n = 13$. Fig. 1 illustrates the maximal and minimal lesions accepted into analyses.

#### 3.1.2. Conditioning

All rats acquired the conditioned freezing response to the clicker CS by the end of the second session of conditioning. A one-way ANOVA revealed no differences in the mean level of freezing in the final presentation of the CS in the second session of conditioning (mean% (±SEM): sham 82.67 (3.97); PL 83.08 (3.94), $F < 1$). A mixed-design repeated-measures ANOVA on the data from the first conditioning session revealed a main effect of trial
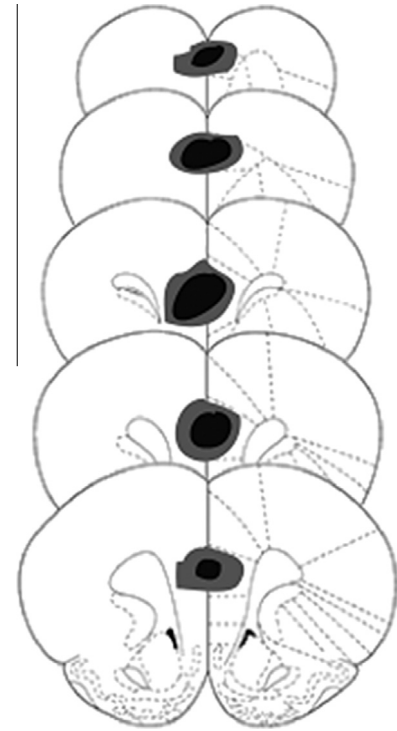


**Fig. 1.** Schematic representations of excitotoxic lesions of the PL cortex for experiment 1. Shaded areas represent the maximum (dark gray) and minimum (black) extent of the lesions for the animals that were included in behavioural analyses. Coronal sections are taken from the following points on the anteroposterior plane beginning at top: +5.20, +4.70, +4.20, +3.70, +3.20, and +2.70 mm anterior to bregma (Paxinos and Watson, 1998). Damage typically extended from +4.70 to +2.70 mm anterior to bregma. Subjects with significant damage to the adjacent anterior cingulate or infralimbic cortices were excluded from all analyses.

($F(2, 52) = 75.69$, $p < 0.05$), and no interaction by groups and trial ($F < 1$). Further, this analysis demonstrated that there was no overall group difference in levels of freezing during CS presentations across this session ($F < 1$). Similarly, a mixed-design repeated-measures ANOVA on the data from the second conditioning session again revealed an effect of trial ($F(2, 52) = 4.49$, $p < 0.05$), and no groups by trial interaction ($F < 1$). Again, there was no overall difference in the average levels of freezing to the CS during this session ($F < 1$). Similarly, a one-way ANOVA showed that there was no difference in responding during the baseline 30-s pre-CS period between groups (mean% (±SEM): sham 28.22 (3.37); PL: 20.77 (3.78), $F(1, 26) = 1.90$, $p > 0.05$). These data demonstrate that the PL cortex is not necessary for the acquisition of a conditioned freezing response.

#### 3.1.3. Extinction

All rats expressed high levels of fear on the first presentation of the clicker CS in the first extinction session with no difference between groups (mean (±SEM): sham 90.22 (2.26); PL 91.28 (1.85), $F < 1$). During the first extinction session, all rats reduced responding to the CS in the absence of shock. This was confirmed by a mixed-design repeated measures ANOVA which revealed a significant main effect of trial ($F(4, 104) = 17.41$, $p < 0.01$). There was no significant trial by group interaction ($F(4, 104) = 2.38$, $p > 0.05$), suggesting the rates of extinction did not differ between groups, and no between-group differences in overall levels of freezing in the session ($F < 1$). The second extinction session yielded similar results with a mixed-design repeated-measures ANOVA revealing a main effect of trial ($F(4, 104) = 12.13$, $p < 0.05$), and no significant interaction between trial and group suggesting there was no difference in the rates of extinction during the second
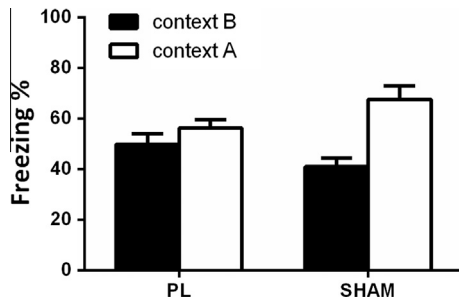
**Fig. 2.** Animals with lesions of the PL cortex fail to exhibit the renewal effect. Rates of responding are represented as percentage of total observations where rats exhibited freezing during CS presentation (±SEM). Sham-lesioned animals exhibited a selective increase in freezing to the CS when presented in the conditioning context (context A), whilst maintaining lower levels of responding in the extinction context (context B). PL-lesioned animals failed to demonstrate this effect, responding to the CS similarly in both contexts.

session ($F < 1$). Further, a one-way ANOVA also demonstrated there was no difference in the level of freezing at the end of the second extinction session (mean (±SEM): sham 55.11 (8.57); PL 61.03 (3.87), $F < 1$). There were no differences in pre-CS rates of freezing during the extinction session (mean% (±SEM): sham 2.27 (1.03); PL 1.64 (0.60), $F < 1$). These data demonstrate that the PL cortex is not necessary to express conditioned fear to a CS.

### 3.1.4. Renewal test

Fig. 2 illustrates the results across both test sessions. The freezing data show that sham-lesioned rats exhibited the renewal effect, as indexed by a selective increase in conditioned responding when the CS was presented back in the conditioning context (context A), relative to lower rates of freezing when the CS was presented in the extinction context (context B). PL-lesioned animals, however, failed to demonstrate this effect, showing similar levels of freezing

to the CS in both contexts. This was confirmed with statistical analyses. A mixed-design repeated-measures ANOVA revealed a significant main effect of context ($F_{(1,26)} = 12.77$, $p < 0.01$), and a significant interaction between group and context ($F_{(1,26)} = 5.23$, $p < 0.05$). Further analyses of simple main effects revealed a significant difference between levels of freezing to the CS presented in context A relative to context B for the sham-lesioned animals ($F_{(1,26)} = 18.49$, $p < 0.05$), but not for the PL-lesioned animals ($F < 1$). Levels of freezing during the pre-CS period did not differ between groups ($F < 1$). There was no difference in responding between groups to the CS in context B ($F_{(1,26)} = 2.63$, $p > 0.05$) or in context A ($F_{(1,26)} = 2.43$, $p > 0.05$). Overall Levels of freezing during the pre-CS period did not differ between groups (mean% (±SEM): sham 0.0 (0.0); PL 0 (0.0), $F < 1$).

### 3.2. Functional inactivation of the PL cortex across extinction sessions

#### 3.2.1. Histology

All rats recovered from surgery and no significant weight loss or behavioural problems were observed. Three animals had cannulae tips outside of the boundaries of the PL cortex, yielding the final group sizes: saline $n = 11$, muscimol $n = 8$. Fig. 3a illustrates the approximate location of the cannula tips.

#### 3.2.2. Conditioning

All rats acquired the conditioned freezing response to the clicker CS by the end of conditioning. There was no difference in the intended groups during the last presentation of the CS in the second conditioning session (mean% of intended groups (±SEM): saline 83.64 (3.29), muscimol 87.50 (3.94), $F < 1$). A mixed-design repeated-measures ANOVA on responding to the CS during the first conditioning session revealed a main effect of trial ($F_{(2,34)} = 124.02$, $p < 0.05$), and no significant difference in the intended groups in the rates of change across trials ($F < 1$). Further,
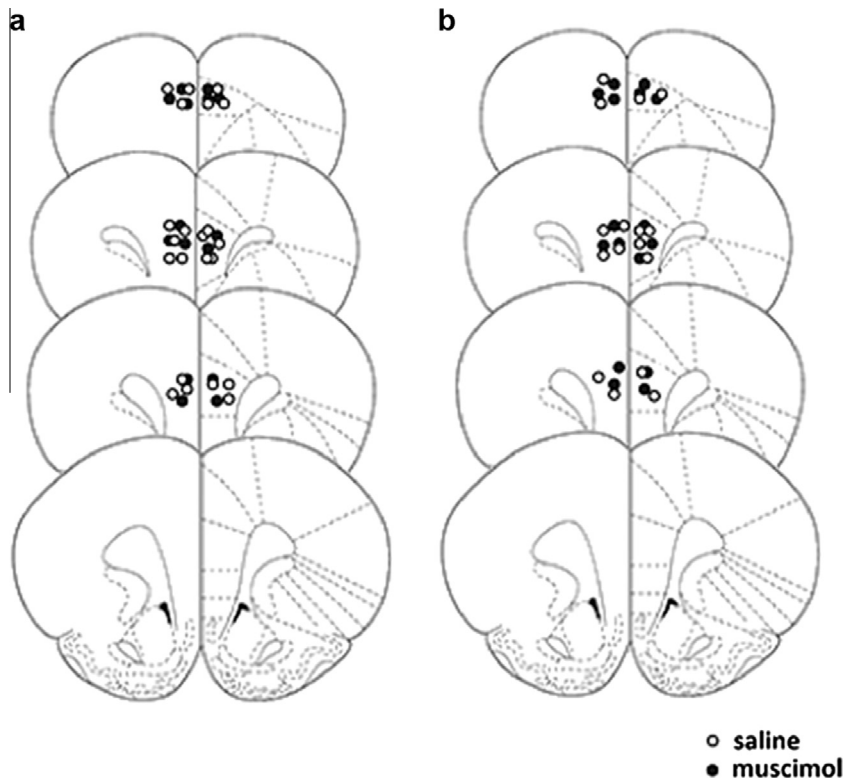


**Fig. 3.** Schematic representation of the placement of the cannula tips for experiment 2. Placement for cannula tips in experiment 2a represented on the left (a), and cannula placement for experiment 2b represented on the right (b). Coronal sections are taken from the following points on the anteroposterior plane beginning at top: +5.20, +4.70, +4.20, +3.70, +3.20, and +2.70 mm anterior to bregma (Paxinos and Watson, 1998).
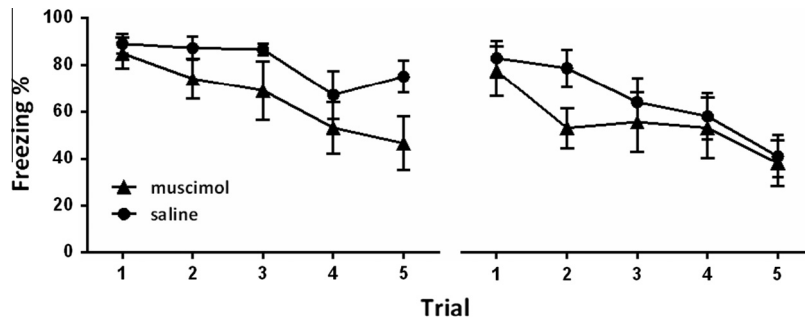
**Fig. 4.** Responding to the CS across extinction. Animals infused with muscimol during extinction exhibited a trend towards lower levels of responding in the final presentation of the CS in the first extinction session. However, there were no significant between-group differences in overall levels of responding to the CS.

this analysis also revealed that there was no difference between the intended groups in overall rates of freezing during CS presentations ($F < 1$). A mixed-design repeated-measures ANOVA on the data from the second conditioning session demonstrated that there was no main effect of trial (F(2, 34) = 2.78, $p < 0.05$), and no interaction between the intended groups and trial ($F < 1$). Again, there was no difference between overall levels of freezing during this session ($F < 1$). Similarly, there was no difference between average levels of pre-CS rates of freezing between groups across these sessions (mean% (±SEM): saline 25.43 (3.45); muscimol 27.51 (4.3), $F < 1$).

### 3.2.3. Extinction

Fig. 4 shows the rates of conditioned responding across the two extinction sessions. Rats receiving infusions of either saline or muscimol across both these sessions exhibited high levels of freezing during the first presentation of the CS, with an ANOVA confirming no differences between levels of responding during the first presentation of the CS in the first extinction session (mean% (±SEM): saline 89.09 (4.24); muscimol 85.00 (6.60), $F < 1$). These data again demonstrate that animals without PL function are capable of expressing conditioned fear, inconsistent with a role for the PL cortex in fear expression. During the first extinction session, all animals reduced levels of responding across presentations of the CS in the absence of shock. A mixed-design repeated-measures ANOVA revealed a main effect of trial ($F(4, 68) = 5.43$, $p < 0.05$), and no interaction between group and trial suggesting the rate of extinction did not differ across groups ($F < 1$). There was no significant between-group difference in overall levels of freezing ($F(1, 17) = 3.04$, $p > 0.05$). Further, this analysis also revealed that there no overall difference in the level of freezing during CS presentations ($F < 1$). However, an exploratory one-way ANOVA revealed a non-significant trend in muscimol-infused animals exhibiting lower levels of freezing in the final presentation of the CS which may suggest they had extinguished more to the CS by the end of the first extinction session ($F(1, 17) = 4.18$, $p = 0.06$). A mixed-design repeated-measures ANOVA on the data from the second session of extinction revealed a main effect of trial ($F(4, 68) = 5.85$, $p < 0.05$), and no significant interaction between group and trial ($F < 1$). This analysis also revealed that there was no significant between-group differences in overall rates of freezing during this session ($F < 1$). A one-way ANOVA revealed that there was no difference in freezing during the final CS presentation of the second extinction session (mean (±SEM): saline 41.21 (9.03); muscimol 38.33 (9.89). Similarly, there were no differences in pre-CS rates of freezing during the extinction sessions (mean% (±SEM): saline 3.39 (2.1); muscimol 2.39 (1.9), $F < 1$).

### 3.2.4. Renewal test

The data in Fig. 5 shows that animals infused with either muscimol or saline into the PL cortex during extinction in context B,

exhibited the renewal effect when presented with the extinguished CS in the context A. This was confirmed in statistical analyses. A mixed-design repeated-measures ANOVA of the data from the two test sessions revealed a significant main effect when comparing freezing to the CS in context A relative to context B ($F(1, 17) = 17.97$, $p < 0.05$), and no significant between-group interaction in the difference in responding to the CS in context A relative to context B ($F < 1$). This suggested that all animals exhibited a significant renewal effect and the magnitude of the difference in responding to the CS in the different contexts did not differ across groups. There was no significant between-group difference in levels of freezing across these test sessions ($F(1, 17) = 3.21$, $p > 0.05$). Further, there was no difference in pre-CS levels of responding during these sessions (mean (±SEM): saline 1.4 (0.5); muscimol 1.2 (0.6), $F < 1$).

## 3.3. Functional inactivation of the PL cortex at test

### 3.3.1. Histology

All rats recovered from surgery and no significant weight loss or behavioural problems were observed. All cannulae tips were considered within the boundaries of the PL cortex, yielding the following final group sizes: saline $n = 8$, muscimol $n = 8$. Fig. 3b illustrates the approximate location of the cannula tips.

### 3.3.2. Conditioning

All rats acquired the conditioned freezing response towards the clicker CS across the conditioning session. There were no differences in the rates of conditioning in the intended groups or in levels of freezing at the last presentation of the CS in the second conditioning session (mean% of intended groups (±SEM): saline
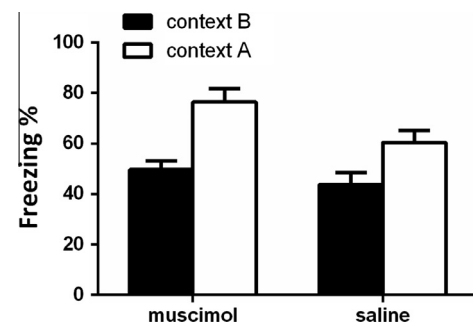


**Fig. 5.** Infusing muscimol into the PL cortex during extinction does not disrupt the renewal effect. Rates of responding are represented as the percentage of total observations spent freezing during presentation of the CS (±SEM). Animals infused with either saline or muscimol during CS extinction in context B exhibited a significant renewal effect when the CS was subsequently presented back in the conditioning context (context A).

80.00 (6.42); muscimol 83.33 (6.04), $F < 1$). A mixed-design repeated-measures ANOVA on the data from the first conditioning session revealed a main effect of trial ($F(2,28) = 37.10$, $p < 0.05$), and no interaction between trial and the intended groups ($F < 1$). Further, this analysis also revealed that there was no overall difference in the levels of freezing between the intended groups ($F(1,14) = 1.02$, $p > 0.05$). A mixed-design repeated-measures ANOVA of the second conditioning session demonstrated that there was no main effect of trial ($F < 1$), and no interaction between the intended groups and trial ($F < 1$). Further, this analysis also showed that there was no difference in overall levels of freezing during this session ($F < 1$). Finally, there was no difference in average levels of pre-CS freezing during these sessions (mean% (±SEM): saline 15. 26 (3.8); muscimol 17.01 (4.31), $F < 1$).

### 3.3.3. Extinction

All animals initially exhibited high levels of freezing to the CS, with a one-way ANOVA confirming no difference between the intended groups in levels of conditioned responding during the first presentation of the CS (mean% (±SEM): saline 91.67 (3.73) muscimol 83.33 (6.42), $F(1,14) = 1.26$, $p > 0.05$). During the first extinction session, all animals reduced their responding to the CS in the absence of shock. A mixed-design repeated-measures ANOVA revealed a non-significant trend towards animals reducing levels of responding across successive presentations of the CS ($F(4,56) = 2.21$, $p = 0.08$). There was no significant trial by group interaction ($F(4, 56) = 1.40$, $p > 0.05$), suggesting there was no difference in the rates of extinction across the first session between the intended groups. The second extinction session yielded a similar set of results, with a mixed-design repeated-measures ANOVA revealing a significant main effect of trial ($F(4, 56) = 4.95$, $p < 0.05$) and no significant group by trial interaction ($F < 1$). There was no differences in intended groups levels of responding during the final CS presentation in the second extinction session (mean% (±SEM): saline 60.83(10.50); muscimol 44.17(11.54), $F(1, 14) = 1.14$, $p > 0.05$) There were no differences between the intended groups in rates of freezing during the pre-CS baseline period (mean% (±SEM): saline 3.21 (1.3); muscimol 2.56 (0.9), $F < 1$).

### 3.3.4. Renewal test

Fig. 6 represents the responding during the two test sessions. Animals receiving infusions of saline into the PL cortex exhibited a selective increase in conditioned responding when the CS was presented back in the conditioning context (context A). However, animals infused with muscimol during the test session failed to exhibit the renewal effect, exhibiting low levels of freezing in both contexts A and B. This was confirmed by statistical analyses. A mixed-design repeated-measures ANOVA showed that there was
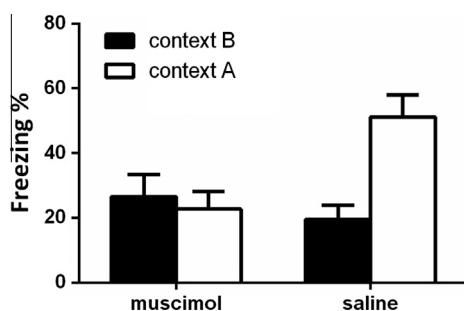
a significant main effect of context when comparing differences in responding to the CS in context A relative to context B ($F(1, 14) = 9.57$, $p < 0.05$), and a significant interaction between groups in the amount of difference between responding to the CS in contexts A and B ($F(1, 14) = 15.57$, $p < 0.05$). This suggested the magnitude of the difference in responding to the CS in the two contexts was different between groups. This analysis also revealed that there was no difference in the overall levels of freezing to the CS between groups ($F(1, 14) = 2.25$, $p > 0.05$). Follow up simple-effects analyses demonstrated that the group by context interaction was due to a significant difference in saline-infused animals responding to the CS in context A relative to context B ($F(1, 14) = 24.28$, $p < 0.05$) which was not present in muscimol-infused animals ($F < 1$). Further analysis of simple main effects demonstrated a significant difference between levels of responding to the CS in context A between groups ($F(1, 14) = 10.50$, $p < 0.05$), suggesting muscimol-infused animals exhibited lower levels of freezing to the CS in context A. There was no simple main effect between groups when comparing levels of responding to the CS in context B ($F < 1$). Again, there was no difference in levels of pre-CS freezing during these sessions (mean (±SEM): saline 2.31 (0.3); muscimol 2.10 (0.4), $F < 1$).

## 3.4. Assessment of orienting responses

The data gathered from these experiments suggested that pre-training lesions and infusions of muscimol which rendered the PL cortex non-functional at test prevented animals from exhibiting the ABA renewal effect. Given previous research which has suggested that PL lesions produce deficits in modulating attention towards predictive cues (Birrell and Brown, 2000; Floresco et al., 2008; Sharpe & Killcross, 2014), we wanted to investigate whether there was a change in the degree of attention directed towards cues in our renewal procedure. In order to investigate this possibility, we examined whether there would be differences in the level of orienting responses made in animals with a functional or non-functional PL cortex during the renewal test sessions. These data are pooled across experiments to represent orienting in animals with a functional PL cortex or a non-functional PL cortex at the time of test and shown in Fig. 7. These data suggest that animals with a functional PL cortex at test show higher levels of orienting towards the extinguished CS in the conditioning context, mirrored by their reinstatement of freezing in this context. However, animals with a non-functional PL cortex at test fail to use the contextual cues to modulate orienting responses towards the CS and exhibit a similar level of orienting in both the conditioning and
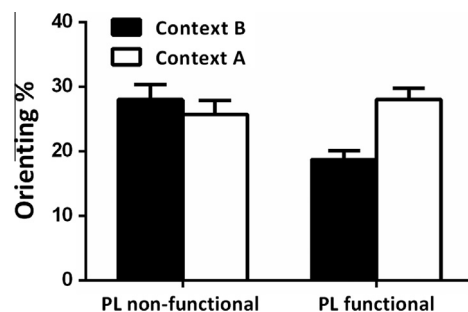
**Fig. 6.** Infusion of muscimol into the PL cortex at test disrupts the renewal effect. Rates of responding are represented as the percentage of total observations that rats spent freezing during CS presentations (±SEM). Animals receiving saline infusions at test exhibited higher levels of responding to the CS in context A relative to context B. However, animals infused with muscimol failed to demonstrate the renewal effect, exhibiting low levels of responding to the CS in both contexts.

**Fig. 7.** Animals with the PL cortex rendered inactive at test failed to use contextual cues to modulate attention towards the CS. Rates of responding are represented as percentage of total observations spent orienting towards the CS (±SEM). Animals receiving saline infusions at test, infusions across extinction, or sham lesioned exhibited a selective increase in attention towards the extinguished CS in context A relative to context B. Animals with pre-training lesions or those receiving muscimol infusions at test failed to demonstrate a difference in orienting responses across contexts.

extinction contexts. This was confirmed by statistical analyses. A mixed-design repeated-measures ANOVA revealed a significant main effect of context ($F(1, 61) = 5.05$, $p < 0.05$), and a significant group by context interaction ($F(1, 61) = 13.56$, $p < 0.01$).

## 4. Discussion

Experiment 1 demonstrated that pre-training lesions of the PL cortex disrupted exhibition of the renewal effect. Significantly, animals with PL lesions expressed levels of fear to a CS comparable with sham-lesioned animals. Further, they extinguished responding to the CS in context B at the same rate as sham-lesioned animals. These data demonstrate that the PL cortex is not necessary for the expression or extinction of conditioned fear. However, when the extinguished CS was presented back in the conditioning context animals with PL lesions failed to exhibit a renewal of conditioned fear. Experiments 2a and 2b assessed the impact of PL inactivation at different time points during ABA renewal. Experiment 2a showed that PL inactivation across extinction did not disrupt renewal when animals were tested drug free. Further, we found that these animals exhibited normal levels of fear towards the CS during extinction, again demonstrating that the PL cortex is not necessary for the expression of conditioned fear. However, PL inactivation during the renewal test abolished the effect. Taken together, these data suggest that the PL cortex is involved in using contextual cues to modulate performance, but not in the learning process that allows the context to play a role in the association developed in extinction.

Bouton's (1993) influential model of extinction argues that the context comes to modulate the memory developed in extinction. That is, when the CS is presented in the absence of shock during extinction, animals come to use the contextual cues present in extinction to disambiguate the significance of the CS as the CS now has multiple meanings (Bouton, 1993; Bouton, 1994; Harris et al., 2000). There are many associative forms that the contextually-mediated memory may take. For example, theories have argues that the extinction context comes to exert control over an inhibitory S–R that is developed during extinction or a CS-no US association (Bouton, 1994; Delamater, 1996; Rescorla, 1997). Further, there is also evidence that the original excitatory association developed during conditioning is also modulated by the contextual cues present during acquisition (Harris et al., 2000), though the associative structure of this influence has been less explored. Though the specifics are not pertinent here, the important aspect of this idea is that the contextual cues will exert control over the behavioural response elicited by the CS. Thus, the present context will signal which association is in effect and inform the animals of whether the CS is predictive of shock. We found that animals without PL function were not capable of renewing levels of fear towards the extinguished CS, suggesting they could not use contextual cues to modulate responding towards the CS.

Thus, the present experiments demonstrate that the PL cortex is involved in allowing the context to actively modulate the response made towards the CS. We interpret these data as a role for the PL cortex in the ability of the context to exert higher-order control over the responding towards the CS. In line with this view, animals without a functioning PL cortex appear to respond in a context-independent manner. That is, we found that animals without PL function during the renewal test were not capable of using contextual cues to govern behaviour and instead behaved in the same manner to the CS across contexts. Though the specific mechanism here is unclear, these data may be interpreted as a lack of contextual modulation which results in animals responding on the basis of the associative strength of the CS, independent of contextual cues. Such findings suggest that the PL cortex contributes to the

ability of animals to use contexts to modulate responding towards a CS in ambiguous circumstances. This is consistent with the appetitive literature, which has demonstrated that the PL cortex is necessary for animals to use contextual cues to modulate performance when there is conflict between multiple responses (Haddon and Killcross, 2006; Marquis et al., 2007).

The finding that PL inactivation during extinction did not disrupt the subsequent exhibition of renewal is in some senses counterintuitive. That is, if the PL cortex is involved in using contextual cues to modulate responding towards a CS, it may be expected that it is also involved in allowing the context to become integrated into the association developed in extinction. However, other theories account for renewal without appealing to a role for the context in exerting top–down modulation over responding towards the CS. For example, the Rescorla and Wagner (1972) model argues that the source of the renewal in an ABA procedure is due to the extinction context acquiring negative associative strength (i.e. the context becomes a conditioned inhibitor; Delamater & Westbrook, 2014). That is, when the CS undergoes extinction both the CS and the extinction context lose associative strength due to the presence of the summed error term in the Rescorla and Wagner (1972) model. This effectively protects the CS from losing all of its associative strength. Thus, when the CS is presented back in the conditioning context, responding is reinstated as the inhibitory influence of the extinction context is no longer present. As we have demonstrated previously, animals without PL function are capable of using as a summed error term to govern learning in the absence of a modulatory mechanism to demonstrate the blocking effect (Sharpe & Killcross, 2014). Further, the trend towards a difference in extinction at the end of the second session in PL-inactivated animals in the present study is also predicted by a Rescorla and Wagner (1972) account of renewal, where the loss of associative strength of a compound (in this case the context and the discrete CS) will be accelerated relative to extinction to the CS alone (Delamater & Westbrook, 2014). Thus, while a modulatory mechanism may usually dominate learning in an ABA procedure, in the absence of this mechanism, animals may be able to rely on other mechanisms to exhibit a renewal of fear. Under conditions where a Rescorla and Wagner (1972) mechanism is not available, we would expect that the PL cortex contributes to the learning process as suggested by previous research (Furlong, Cole, Hamlin, & McNally, 2010; Sharpe & Killcross, 2014).

The present results also demonstrated that the PL cortex is not necessary for the expression of conditioned fear. That is, we found that lesions of the PL cortex did not disrupt the ability of animals to acquire the conditioned response or extinguish that response. Further, specific inactivation of the PL cortex during the extinction session did not disrupt the exhibition of conditioned fear or the rate of extinction across this session. This is in contrast to findings which have reported that PL inactivation reduces the expression of conditioned fear towards a CS (Corcoran & Quirk, 2007; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011). A potential source of this difference is the parameters used in previous studies. For example, Corcoran and Quirk (2007) gave rats seven tone–shock parings across a single conditioning session with an inter-trial interval (ITI) which varied about a 3-min ITI, without any pre-training exposure to the conditioning session. These parameters have previously been found to promote competition between contextual and discrete cues (Holland, 2000; Phillips & LeDoux, 1994; Rescorla, 1984). Under normal circumstances, rats will still learn that the CS is the temporally accurate predictor of the US and will attribute a greater level of associative strength towards the discrete CS. However, the ability to resolve this competition is dependent of the ability of animals to direct attention towards predictive cues in line with a process described by Mackintosh (1975), found to be disrupted in animals with PL lesions (Sharpe & Killcross,

2014). In line with a role for the PL cortex in resolving any such competition, studies in which animals have received considerable pre-training exposure to the context (where there is little evidence of competition) have found no effect of PL lesions on fear conditioning (Holson, 1986). In contrast, studies which have used less pre-exposure to the context and shorter ITIs (which promotes competition between contextual and discrete cues) have found that animals with PL lesions exhibit enhanced learning about a context (Lacroix, Spinelli, Heidbreder, & Feldon, 2000; Morgan & LeDoux, 1995), in one instance at the expense of learning about a CS, relative to sham-lesioned animals (Lacroix et al., 2000). In the current study, we used lengthy ITIs and considerable pre-training exposure to the context to reduce such competition and ensure learning and responding was directed solely to the CS. Thus, attentional modulation was not necessary to direct a response towards the CS in the present design. This is an important aspect of the data that may be due a difference in parameters used across these studies which warrants further investigation.

A role for the PL cortex in modulating attention towards cues to influence fear expression may be supported by the analysis of orienting responses during the renewal test session in the current study. This analysis demonstrated that rats without PL function did not change the degree of attention that was directed towards the CS in the different contexts. We found that rats with a functional PL cortex at test exhibited a selective increase in the attentional response directed towards a stimulus when placed back in the conditioning context following extinction. This change in the degree of attention directed towards the CS in the conditioning context also mirrored their renewal of fear. Though a role for attentional responses driving fear renewal has not been well explored, there is evidence from other paradigms that contexts can modulate attention towards CSs (Hall & Channel, 1985; Nadel & Willner, 1980). For example, Hall and Channel (1985) presented a cue to animals repeatedly without reinforcement until their orienting towards the cue declined. When they presented the cue in a different context, animals' orienting responses towards the cue increased relative to animals who received the cue in the same context. This increase in orienting responses was also accompanied by faster rates of leaning when the cue was paired with reinforcement, in line with theories of selective attention that argue that degree of attention determines the rate of learning (Mackintosh, 1975; Pearce & Hall, 1980). This suggests that, just as the associative history of a cue can influence the degree of attention directed towards it, contextual cues can also change the degree of attention directed towards a cue. Though the PL cortex may also likely influences the renewal of fear by directly modulating the behavioural response, the analysis of orienting responses suggests the PL cortex may be contributing to the ability of the context to modulate the degree of an attentional response made towards a CS, where the behavioural response to the CS may partly be a function of this attention. However, this observation requires further investigation as the pattern of data, where animals without PL function exhibit greater orienting responses towards the CS in both contexts, does not allow us to conclude that an attentional deficit underlies the impairment in renewal.

Finally, it is worth noting here that a role for the PL cortex in modulating attention towards cues is an extension of our previous research which specifically implicated the PL cortex in the down-regulation of attention towards cues (Sharpe & Killcross, 2014). More specifically, we have previously found that pre-training lesions of the PL cortex produced a deficit in the ability of animals to exhibit the overshadowing effect. This was interpreted as an inability of these animals to down-regulate attention towards the less salient element of the compound as the irrelevant predictor of the outcome (Mackintosh, 1975). An attentional interpretation of this effect was supported by the fact that animals with PL lesions

were still capable of demonstrating the blocking effect and, thus, using a summed error term to govern learning. However, these animals failed to down-regulate attention to the blocked cue, exhibiting faster acquisition to this cue when it was paired with the outcome following the extinction test session. This was confirmed in a subsequent experiment which demonstrated that animals with PL lesions failed to exhibit the blocking of unblocking effect, explicitly testing the ability of these animals to down-regulate attention. Although these experiments tested whether PL lesions disrupted the ability of animals to down-regulate attention towards cues, we argued that these data implicated the PL region in the ability of animals to change the degree of attention directed towards cues on the basis of their associative history. That is, we would argue that the PL cortex is involved in modulating attention towards cues on the basis of how well they predict an outcome whether that change involved an up- or down-regulation of attention towards the cue, in line with an attentional process described by Mackintosh's (1975) theory of attention. However, this distinction may warrant further investigation.

The present study has demonstrated that the PL cortex is involved in using contextual cues to influence responding in an ABA renewal procedure. A role for the PL cortex in ABA renewal is consistent with a general role for the PL cortex in promoting behavioural flexibility. The ability to express and suppress fear is essential to survival and contextual cues in the environment provide an animal with the information needed to respond adaptively. The exhibition of ABA renewal demonstrates an animal can recognise when danger may again be present and respond adaptively. The PL cortex has also been implicated in tasks requiring response conflict resolution, promotion of goal-directed behaviours over habits, strategy switching, response monitoring, an modulating attention towards predictive cues (Floresco et al., 2008; Gisquet-Verrier & Delatour, 2006; Killcross & Coutureau, 2003; Marquis et al., 2007; Sharpe & Killcross, 2014). These tasks all require the use of higher-order cues such as goal value, task demands, contextual stimuli, and associative history to influence the way an animal responds to ambiguity. Such research suggests the PL cortex utilises information in the environment to endow an animal with a level of behavioural complexity that affords it flexibility in uncertain situations.

## Acknowledgment

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