

Competing contextual processes rely on the infralimbic and prelimbic medial prefrontal cortices in the rat

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Abstract

Ambiguous relationships between events may be established using interference procedures such as latent inhibition, extinction or counterconditioning. Under these conditions, the retrieval of individual associations between a stimulus and outcome is affected by contextual cues. To examine the roles of the dorsal (prelimbic) and ventral (infralimbic) medial prefrontal cortex in the contextual modulation of such associations, we investigated the context specificity of latent inhibition. Male Lister hooded rats were pre-exposed to two separate stimuli, one in each of two distinct contexts. Both stimuli were then paired with the delivery of mild foot-shock in the same one of these contexts. Finally, the strength of the resultant conditioned emotional response (CER) to each stimulus was assessed in each context. For the sham-operated control rats, the CER was attenuated for each stimulus when it was tested in the context in which it had been pre-exposed. Rats who had received lesions to the infralimbic cortex showed this effect only in the conditioning context, whereas rats with lesions to the prelimbic cortex showed the effect only in the context in which conditioning had not taken place. These findings indicate that infralimbic and prelimbic cortices play distinct, and competing, roles in the contextual modulation of initial and later learning.

Keywords: rat, prelimbic cortex, latent inhibition, interference, infralimbic cortex, context

Introduction

During Pavlovian conditioning, an initially neutral stimulus (conditioned stimulus [CS]) may be repeatedly paired with an outcome (unconditioned stimulus [US]) to establish a predictive relationship between the two. As a result, the CS will come to evoke a conditioned response (CR), the nature of which will depend on the properties of the US. If the CS is then repeatedly presented alone, the CR will weaken. This extinction of the CR does not reflect unlearning of the CS–US association acquired during conditioning, but is believed to occur due to the development of a second, inhibitory (CS– \overline{US}), association [1, 2]. Furthermore, expression of this inhibitory association is dependent upon the context in which the stimulus is presented. If there is a change in context between extinction and test, the CR returns [3, 4]. To establish *latent inhibition*, these two phases of training are reversed, and the CS is pre-exposed alone prior to the CS–US pairings [28, 29]. During the conditioning phase, acquisition of the CR to a pre-exposed CS is retarded relative to a non-pre-exposed CS. Latent inhibition may be explained in terms of a similar associative structure to extinction: a CS–no event association is learned during pre-exposure and this association then competes for expression with a CS–US association learned during later conditioning trials [1, 14]. Like extinction, latent inhibition exhibits some degree of context specificity [6, 47], resulting in a reinstatement of conditioned responding to the pre-exposed stimulus. In both extinction and latent inhibition, expression of

the association acquired when the CS is presented alone (CS– \overline{US} or CS–no event, respectively) is particularly affected by a change in context.

In rats, different regions of the medial prefrontal cortex (mPFC) are involved in the expression of conditioned fear, and the suppression of fear following extinction (e.g. [43]). The prelimbic cortex (PrL) is implicated in the expression of conditioned fear. Inactivation of PrL attenuates a conditioned fear response to discrete or contextual cues but does not affect expression of innate fear [7, 24], and sustained excitatory responses of PrL neurons are correlated with behavioral fear responses [5]. *Suppression of fear* following extinction, however, involves the infralimbic cortex (IL) [35]. Lesion or inactivation of IL does not affect acquisition of extinction but impairs consolidation or retrieval of extinction learning the next day (e.g. [24, 25, 36]).

Research employing latent inhibition paradigms has yielded a less clear picture. Lingawi et al. [26] reported equivalent effects of pharmacological manipulation of IL mPFC on extinction and latent inhibition and concluded that in both cases an inhibitory CS– \overline{US} (or CS–no event) association is stored in and retrieved from the IL. Stimulation of IL during retrieval of the inhibitory memory enhanced both effects, whereas NMDA blockade in the IL disrupted them. But there is evidence that the PrL plays different roles in latent inhibition and extinction. Following limited pre-exposure, which was not sufficient to produce latent inhibition in sham-operated control animals, Nelson et al [33] found that dopamine depletion of the PrL enhanced latent inhibition,

Table 1. Simplified design of the experiment

	Pre-exposure days 1–6	Unconditioned suppression day 7	Conditioning days 8–13	Test days 15–18
Context A	X → ∅ (6 x 30s)	X → ∅ (4 x 30s) and Y → ∅ (2 x 30s)	X → shock (2 x 30s) and Y → shock (2 x 30s)	X → ∅ (2 x 30s) and Y → ∅ (2 x 30s)
Context B	Y → ∅ (6 x 30s)	Y → ∅ (4 x 30s) and X → ∅ (2 x 30s)		X → ∅ (2 x 30s) and Y → ∅ (2 x 30s)

We employed a within-subject version of a CER design described by Lovibond, Preston and Mackintosh [27]. Rats received pre-training lesions to either the IL, or to the PrL, or they underwent sham surgery before being trained to press a lever to earn food rewards on an RI-30s schedule. This instrumental baseline remained in place throughout the experiment. Two auditory stimuli (X and Y; a 4-kHz tone and a 20-Hz clicker, counterbalanced) were then pre-exposed in different contexts (A and B). Stimulus X was pre-exposed in Context A, and Stimulus Y was pre-exposed in Context B. Both stimuli were then individual paired with the delivery of foot-shock in Context A. Finally, test trials were given in which each stimulus was presented in each context. Hence, Stimulus X received pre-exposure and conditioning in the same context before being tested in both that context (AAA) and the alternative context (AAB). For Stimulus Y there was a context change between pre-exposure and conditioning, and it was tested in both the pre-exposure (BAB) and the conditioning (BAA) contexts. During the pre-exposure phase, the unconditioned suppression sessions, and the test sessions, presentations of either stimulus were without consequence (∅). Figures in parentheses indicate the number and duration of trials with each stimulus presented in each session. Rats were given familiarization sessions on day 14 (not shown) in which they were exposed to each context, but no stimuli were presented.

whereas dopamine depletion of the IL had no effect. In contrast, experiments employing pre-training excitotoxic lesions found no effect of lesions to the mPFC encompassing both the IL and PrL [17, 23, 40] when latent inhibition was assessed on a single-test trial following an off-baseline conditioned emotional response (CER) procedure. Using a more sensitive on-baseline CER procedure, however, George et al [9] observed enhanced latent inhibition during conditioning in rats with lesions to both PrL and IL, and in those with lesions restricted to the IL mPFC.

The enhancement of latent inhibition observed by George et al [9] suggests that lesions to the IL result in an unusually strong influence of first-learned associations over behavior due to impaired retrieval of second-learned associations. Using an appetitive procedure, Rhodes and Killcross [37, 38] found that similar lesions to the IL increased the magnitude of spontaneous recovery, context renewal and reinstatement of conditioned responding following extinction. All of these effects may be explained by an increase in the context specificity of second-learned associations (CS–US in the case of latent inhibition, CS–US for extinction). There is additional evidence from other preparations that the mPFC is involved in learning about contextual cues [16], and that the PrL has a role in the hierarchical control of behavior by contextual cues [11, 30, 39, 42]. Importantly, the IL and PrL may operate in competition with each other for control of behavior [12, 30].

Despite the parallels between extinction and latent inhibition, investigation of the role of the IL and PrL in the contextual control of behavior in a latent inhibition paradigm has been neglected. The aim of the experiment reported here was to determine what effect pre-training lesions to either PrL or IL mPFC would have on the context-specificity of latent inhibition using a within-subject version of a CER design described by Lovibond et al [27] (see Table 1).

MATERIALS AND METHODS

Subjects

A total of 32 male Lister hooded rats (Harlan UK, Bicester, UK) were used in this study (mean *ad libitum* weight, 376 g; range, 345–420 g). Twelve rats received pre-training excitotoxic lesions to the dorsal mPFC centered on the PrL cortex, and 12 rats received bilateral excitotoxic lesions to the ventral mPFC centered on

the IL cortex. The remaining eight rats served as sham-operated controls. After surgery, rats were maintained at 85% of their age-matched *ad libitum* weights. The rats had free access to water in their home cages. They were housed in pairs in a light-proof holding room maintained on a 14-hour light/dark cycle (06:00 to 20:00), at a temperature of $21 \pm 1^\circ\text{C}$ and a humidity of $55 \pm 5\%$. The subjects were tested on successive days, at the same time, during the period that the lights were on in their holding room. All experimental procedures involving animals and their care were performed in accordance with the United Kingdom Animals Scientific Procedures Act (1986) and were subject to Home Office approval (Project License PPL 30/2158).

Surgery

Rats were first anesthetized with isoflurane, their heads were shaved, and they were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). An incision was made in the scalp, the skull was exposed, and a skull flap overlying the prefrontal cortex was drilled out. Lesions to the IL were produced by giving automated injections of 0.15 μL of ibotenic acid at a rate of 0.1 $\mu\text{L}/\text{min}$ at two sites: anteroposterior (AP), +2.6; mediolateral (ML), ± 0.6 ; dorsoventral (DV), -5.4 . For lesions to the PrL, 0.2 μL of ibotenic acid was injected at a rate of 0.1 $\mu\text{L}/\text{min}$ (AP, +3.2; ML, ± 0.6 ; DV, -4.0). Sham-operated controls underwent an identical procedure ($n = 4$ with IL coordinates, and $n = 4$ with PrL coordinates), with the exception that no toxin was infused. After a minimum of 1 week of postoperative recovery, rats were gradually reduced to 85% of age-matched free-feeding weights.

Histology

Following completion of testing, rats were given a lethal overdose of sodium pentobarbitone (Euthatal) and were perfused with saline (0.9%) followed by formal saline (10% w/v). Brains were removed and postfixed in formal saline. Before slicing, they were transferred to a 25% sucrose solution, in which they remained for 24 hours. Coronal slices (40 μm) were cut using a cryostat (Leica Microsystems GmbH, Wetzlar, Germany), and were mounted on gelatin-coated slides. These were dried at room temperature for 24 hours before being stained with cresyl violet, and this was followed by the addition of a coverslip in DPX. The extent and location of cell loss were verified using a light microscope and the brain atlas of Paxinos and Watson [34].

Apparatus

Sixteen standard conditioning chambers (Med Associates, St Albans, VT) were used, each housed within a sound-attenuating, ventilated enclosure. Each chamber measured 30.5 × 24.1 × 21.0 cm. The left and right walls of the chamber were aluminium, whereas the rear wall, ceiling and a door that served as the front wall were made of clear Plexiglas. The grid floor of the chamber consisted of 19 steel rods, 4.8 mm in diameter, spaced 1.6 cm apart. A recessed food magazine (5.1 × 5.1 cm), into which 45 mg food pellets (Sandown Scientific, UK) could be delivered, was located in the middle of the right-hand wall, with its base 0.5 cm above the grid floor. Food reward always consisted of a pair of food pellets, delivery of which was separated by a 200-ms interval. Access to the magazine was recorded by means of infrared detectors mounted across the mouth of the recess. Two flat-panel retractable levers were fitted to the left and right of the food magazine; the right-hand lever remained withdrawn throughout the experiments. The house-light was illuminated throughout the experimental sessions. An 8-Ω speaker mounted on the rear wall of the chamber delivered a 4-kHz tone produced by a programmable tone generator (ANL-926B; Med Associates). A heavy-duty relay, also mounted on the rear wall, was used to generate a 20-Hz train of clicks. All stimuli were presented at an intensity of approximately 78 dB. Experimental events were controlled, and responses were recorded, by a PC running Med-PC IV software (Med Associates).

Eight of the conditioning chambers, housed in one room, served as Context A; and the remaining eight chambers, housed in a different room, served as Context B. The two contexts were made distinct by decoration of the walls. Clear Plexiglas panels were inserted into the chambers and covered the left and right walls. Holes were cut out of these panels to accommodate the retractable levers, food magazine and the house light. Cardboard sheets decorated with either a black and white checkerboard pattern (Context A; squares measured 2.0 × 2.0 cm), or black circles on a white background (Context B; diameter of the circles was 1.5 cm and distance between the centers of adjacent circles measured 2.5 cm) were mounted on the reverse of the Plexiglas panels, facing into the chambers. Similar cardboard sheets were also attached to the outside of the two Plexiglas walls and the ceiling of the chambers. A perforated Plexiglas sheet (perforation diameter 0.3 cm; distance between adjacent perforations 1.0 cm) was placed on top of the grid floors of the Context B chambers. The grid floor of each chamber that served as Context A was connected to a shock generator that, when appropriate, delivered a scrambled shock (0.4 mA) for 0.5 s. The delivery and intensity of shocks used were controlled via Med-PC software.

For half of the rats in each lesion group, the 4-kHz tone served as Stimulus X, and the 20-Hz clicker as Stimulus Y. For the remaining rats, these designations were reversed.

Procedure

Lever-press pre-training

Over three days prior to the start of the experiment, rats were trained to press a lever to earn food reward. On each day, they received two sessions of training, one in each context. For each rat there was a delay of four hours between completion of the first session each day and the start of the second. The timing of the session in each context was constant across days for each rat, but the order in which rats experienced Context A and Context B was counterbalanced within lesion groups. Hence, half of the rats in each group was trained in Context A in the morning and in

Context B in the afternoon, whereas the other rats in each group were trained in Context B in the morning and in Context A in the afternoon. These timing remained the same throughout all phases of the experiment.

The first two sessions in each context began with the delivery of 20 food rewards according to a random-time 60-second schedule: each second, there was a 1/60 chance that a reward would be delivered. After the 20th reward, the left-hand response lever was extended into the chamber and responses on it were reinforced on a progressive-interval schedule, starting with a fixed-interval 1-second schedule. After 10 rewards were earned, the schedule progressed through a random-interval (RI) 2-second schedule to RI 15 seconds with an increment of 1 second for every additional two rewards earned. The RI schedule was implemented by making food available with a 1/t probability each second, where t was the value of the schedule. The first response after food was made available was rewarded. On the third session in each context, the lever was inserted into the chamber at the beginning of the session and lever-press responses were reinforced according to an RI 15 s schedule throughout the session. Each session lasted for 48 minutes.

Pre-exposure

The rats received two sessions of stimulus pre-exposure on each of seven consecutive days: one in Context A and one in Context B. Each session lasted for 48 minutes, the left-hand lever was inserted into the box throughout the session and responses on the lever were reinforced by the delivery of a food reward according to an RI 30-second schedule. The first six sessions of pre-exposure in each context consisted of six 30-second presentations of a single stimulus: X in Context A, and Y in Context B. The mean inter-trial interval, measured from the onset of one trial to the onset of the next, was 480 seconds (range ± 15%). On the seventh day, the first four trials in each session followed the same pattern. These trials were followed by two 30-second presentations of the stimulus that had been pre-exposed in the alternative context: Y in Context A, and X in Context B. This unconditioned suppression session was included to reduce the initial disruption of lever pressing and magazine approaching observed during presentation of a novel stimulus [19–21] in each context.

Conditioning

Each of the following six daily sessions was conducted in Context A, lasted for 48 minutes, and consisted of two 30-second presentations of each stimulus (X and Y), which co-terminated with the delivery of a mild foot-shock. The stimuli were presented in a pseudo-random sequence, with the constraint that each stimulus was presented in each consecutive block of two trials. The mean inter-trial interval was 720 s (range ± 15%). Following the last trial of each session, the rats were left in the conditioning chamber for 6 minutes before being returned to their home cage. The lever was inserted into the box throughout the conditioning sessions, and responses were reinforced according to the same schedule as during the pre-exposure sessions. For each rat, conditioning sessions were conducted at the same time of day that they had received pre-exposure sessions in Context A.

To re-familiarize the rats with Context B, all animals received one session of exposure to each of the two contexts on the day following the final conditioning session. All details of these sessions were the same as for the pre-exposure sessions with the exception that no stimuli were presented.

Test

Testing commenced on the day immediately after the re-familiarization sessions. A total of four test sessions (two in Context A and two in Context B) were conducted, one per day at the time that pre-exposure had been received in that context. The order of the sessions was counterbalanced within the different groups and followed the sequence ABBA or BAAB for each rat. All other details of the test sessions were the same as for the conditioning sessions with the exception that no foot-shocks were delivered.

Statistical methods

The rates of lever-press responding during presentations of Stimulus X and Stimulus Y and during the inter-trial interval were recorded for the pre-exposure, conditioning, and test sessions.

To check that there were no differences in the baseline rates of responding across groups or contexts, the data for the inter-trial intervals averaged across the seven days of pre-exposure were subjected to a two-way analysis of variance (ANOVA) with the between-subject factor of lesion group (sham, IL and PrL) and the within-subject factor of context (A and B).

For the conditioning and test sessions, the response rates were also used to calculate suppression ratios for both stimuli during each session for individual rats. Suppression ratios were of the form $CS/(CS + ITI)$, where CS was the rate of responding during the stimulus presentation, and ITI was the rate of responding during the inter-trial interval. This ratio provides a measure of the strength of the CER evoked by a stimulus as a consequence of being paired with foot-shock. A ratio of 0.5 indicates that the rate of responding during the stimulus was the same as during the inter-trial interval (no CER), whereas a ratio of 0.0 would reflect complete suppression of lever press responding by the stimulus presentation (strong CER).

For the conditioning sessions, the resulting data were analyzed using a three-way ANOVA with the between-subjects factor of lesion group (sham, IL, and PrL), and the within-subject factors of stimulus (X and Y) and session (1 to 6). Test data were analyzed using another three-way ANOVA with the between-subjects factor of lesion group (sham, IL, and PrL), and the within-subject factors of stimulus (X and Y) and context (A or B). Because the identities of the stimuli were fully counterbalanced within each group, and each stimulus was pre-exposed in a different context (which were not counterbalanced), the factors of stimulus and context in these ANOVAs might otherwise be described as pre-exposure context and test context, respectively.

RESULTS

Histology

The aim of this experiment was to investigate the effects of focal lesions to either the IL cortex or PrL cortex. Rats in the IL group were excluded from further analysis if they showed less than 50% damage to the IL cortex bilaterally, or if they suffered bilateral damage to the PrL cortex. Similarly, rats in the PrL group were excluded if they showed <50% damage to the PrL cortex bilaterally, or if they suffered bilateral damage to the IL cortex. Consequently, two rats were removed from the IL group, and two rats were removed from the PrL group. Figure 1 depicts, for the remaining rats, the minimum (black region) and maximum (black + grey region) extent and location of damage in the IL ($n = 10$), and PrL ($n = 10$), and photomicrographs showing a representative lesion in each group.

Behavior

Baseline

Mean rates of lever-press responding in Context A during the inter-trial interval across the seven sessions of pre-exposure were 6.08 responses/min ($SD = 0.72$) for the sham-operated control group, 6.96 (1.85) for the IL group and 6.62 (1.63) for the PrL group. Corresponding figures for Context B were 5.68 (1.27), 6.81 (2.23) and 5.80 (1.64), respectively. There was no effect on response rate of lesion group ($F_{2,25} = 1.05$; $P = 0.365$, $MSE = 4.57$), or of context ($F_{1,25} = 2.98$; $P = 0.097$, $MSE = 0.964$), and no Group–Context interaction ($F < 1$).

Conditioning

Panels A–C of Figure 2 show group mean suppression ratios for each stimulus (X and Y) for each of the six sessions of conditioning conducted in Context A. There was no main effect of group ($F < 1$), but there was a main effect of stimulus ($F_{1,25} = 7.47$; $P = 0.011$, $MSE = 0.035$, $\eta_p^2 = 0.23$, 90%CI [0.03 0.43]); conditioning was more effective to Stimulus Y (which underwent a change in context between pre-exposure and conditioning) than to Stimulus X (which did not). There was, however, no difference in suppression to the two stimuli on the final session of conditioning ($t < 1$). Although the effect of stimulus appears to be present for rats in the IL and Sham-operated control groups, but not the PrL group, there was no significant interaction of stimulus and group ($F_{2,25} = 1.30$; $P = 0.291$, $MSE = 0.035$), nor of stimulus and session ($F_{5,125} = 1.59$; $P = 0.166$, $MSE = 0.015$), and no significant three-way interaction ($F < 1$). There was a significant effect of session ($F_{5,125} = 58.22$; $P < 0.001$, $MSE = 0.019$, $\eta_p^2 = 0.70$, 90% CI [0.62 0.74]) and a significant interaction of session and group ($F_{10,125} = 2.12$; $P = 0.027$, $MSE = 0.019$, $\eta_p^2 = 0.15$, 90% CI [0.01 0.18]). Simple effects analysis, however, revealed no significant effect of group on any session (largest $F_{2,25} = 2.75$; $P = 0.083$, $MSE = 0.019$), but a significant effect of session for all three groups (smallest $F_{5,25} = 10.97$, $P < 0.001$, $MSE = 0.019$, $\eta_p^2 = 0.69$, 90% CI [0.46 0.78]).

Test

Sham-operated control rats displayed clear context-specificity of latent inhibition in each context (Figure 2, panel D); they showed less suppression of lever-press responding in the presence of the stimulus that had been pre-exposed in each context (X in Context A, and Y in Context B) than to the stimulus that had been pre-exposed in the other context (Y in Context A, and X in Context B). Rats with lesions to the IL (Figure 2, panel E) showed the same pattern of results as the sham-operated control rats in Context A (less suppression to X than to Y), but showed comparable (low) levels of conditioned suppression to the two stimuli in Context B. Conversely, rats with lesions to the PrL (Figure 2, panel F) behaved similarly to the sham-operated controls in Context B (less suppression to Y than to X), but lever-press responding was suppressed to an equal amount to the two stimuli in Context A. The test data are replotted in Figure 3 to facilitate comparison between groups, along with data from individual rats.

These observations were supported by a significant three-way interaction between group, context and stimulus ($F_{2,25} = 4.91$; $P = 0.016$, $MSE = 0.011$, $\eta_p^2 = 0.28$, 90% CI [0.03 0.45]). Simple effects analysis revealed that sham-operated control rats showed significantly less suppression to X than to Y in Context A ($F_{1,25} = 18.44$; $P < 0.001$, $MSE = 0.011$, $\eta_p^2 = 0.43$, 90% CI [0.18 0.60]), and significantly less suppression to Y than to X in Context B ($F_{1,25} = 9.69$; $P = 0.005$, $MSE = 0.011$, $\eta_p^2 = 0.28$, 90% CI [0.06 0.49]). They also showed less suppression to X in Context A than in Context B

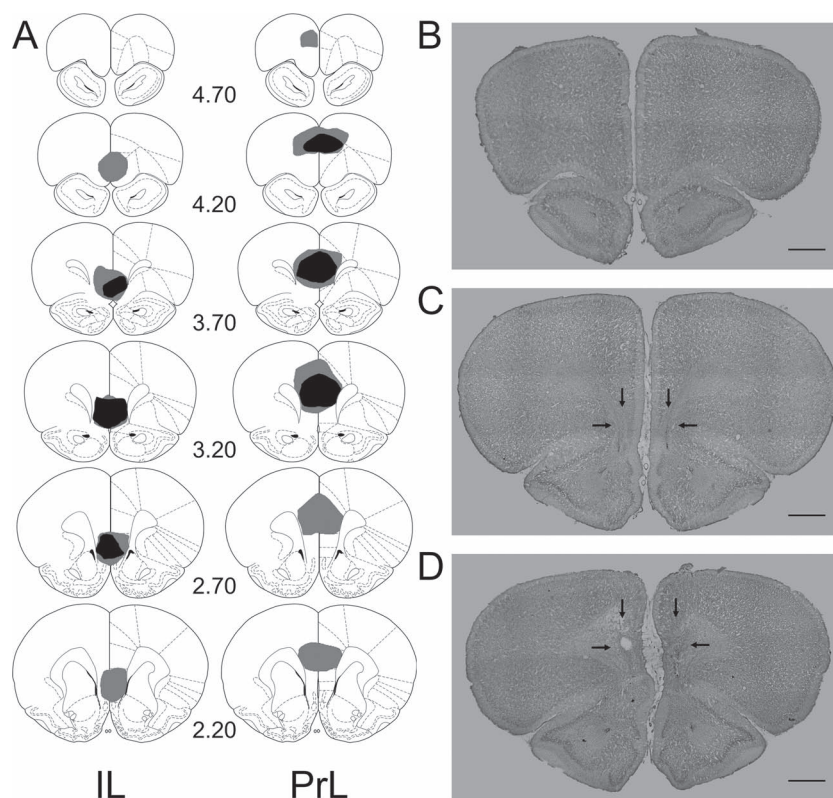


Figure 1. Histological evaluation of the selective prefrontal subregion lesions. Panel A: Reconstructions of the smallest (black) and largest (black + grey) lesions to the infralimbic (IL) and prelimbic (PrL) medial prefrontal cortex are shown for the cases included in the behavioral analyses. The numbers (in millimeters relative to bregma) denote the anterior–posterior level of the illustrated sections, in correspondence to the stereotaxic rat brain atlas by Paxinos and Watson [34]. Panels B–D: Photomicrographs showing a coronal section from a sham-operated control subject (B), and representative lesions to IL cortex (C) and PrL cortex (D). Scale bars in the bottom right corner of each panel represent 1 mm.

($F_{1,25} = 8.96$; $P = 0.006$, $MSE = 0.011$, $\eta_p^2 = 0.26$, 90% CI [0.05 0.47]) and more suppression to Y in Context A than in Context B ($F_{1,25} = 20.76$; $P < 0.001$, $MSE = 0.011$, $\eta_p^2 = 0.45$, 90% CI [0.21 0.63]).

Rats with lesions to the IL showed significantly less suppression to X than to Y in Context A ($F_{1,25} = 7.48$; $P = 0.011$, $MSE = 0.011$, $\eta_p^2 = 0.23$, 90% CI [0.03 0.44]), but not in Context B ($F < 1$), and a significant effect of context for Stimulus Y ($F_{1,25} = 15.63$; $P < 0.001$, $MSE = 0.011$, $\eta_p^2 = 0.39$, 90% CI [0.14 0.57]), but not for X ($F < 1$).

There was no significant difference in suppression to X and Y in Context A for rats with lesions to the PrL ($F < 1$), but these rats showed less suppression to Y than to X in Context B ($F_{1,25} = 22.90$; $P < 0.001$, $MSE = 0.011$, $\eta_p^2 = 0.48$, 90% CI [0.23 0.64]). For this group of rats, the effect of context was not significant for Stimulus X ($F_{1,25} = 3.81$; $P = 0.062$, $MSE = 0.011$), and just failed to achieve significance for Stimulus Y ($F_{1,25} = 4.19$; $P = 0.051$, $MSE = 0.011$).

The main effect of context was also significant ($F_{1,25} = 4.65$; $P = 0.041$, $MSE = 0.020$, $\eta_p^2 = 0.16$, 90% CI [0.00 0.36]), with greater suppression of responding in the conditioning context (A), than in Context B. There was no main effect of either group or stimulus [$F_s < 1$]. There were significant interactions of stimulus with group ($F_{2,25} = 6.06$; $P = 0.008$, $MSE = 0.020$, $\eta_p^2 = 0.33$, 90% CI [0.06 0.49]) and with context ($F_{1,25} = 53.94$; $P < 0.001$, $MSE = 0.011$, $\eta_p^2 = 0.68$, 90% CI [0.47 0.78]), but no significant interaction of group and context ($F_{2,25} = 2.05$; $P = 0.149$, $MSE = 0.020$).

DISCUSSION

In this experiment we examined the effects of selective lesions to either the IL or PrL mPFC on the context specificity of latent

inhibition. Rats were first pre-exposed to Stimulus X in Context A and Stimulus Y in Context B. Both stimuli were then paired with foot-shock in Context A. Finally, CER to each stimulus was assessed in each context.

Consistent with previous reports, sham-operated control rats expressed less fear towards each stimulus (higher suppression ratio) when it was tested in the context in which it had been pre-exposed. Hence, a latent inhibition effect was observed to Stimulus X in Context A, and Stimulus Y in Context B, simultaneously highlighting and demonstrating the context-sensitivity of latent inhibition. The symmetry of this effect was affected by either lesion, but in different ways. Rats with lesions to IL mPFC only showed this latent inhibition effect in Context A, the conditioning context. In contrast, they showed equivalent, low, levels of fear (high suppression ratio) to both stimuli in the context in which conditioning did not take place (Context B). Rats with lesions to PrL mPFC only showed the latent inhibition effect in Context B, and instead showed equivalent, relatively high, levels of fear (low suppression ratio) to both stimuli when tested in the conditioning context (Context A).

These results provide important insights into the involvement of these two regions of the mPFC in the contextual control of associations.

Context dependency of associations

Latent inhibition has been shown to be sensitive to a change in context between pre-exposure and conditioning (e.g. [6]) or between pre-exposure and test [47]. Such a context change results

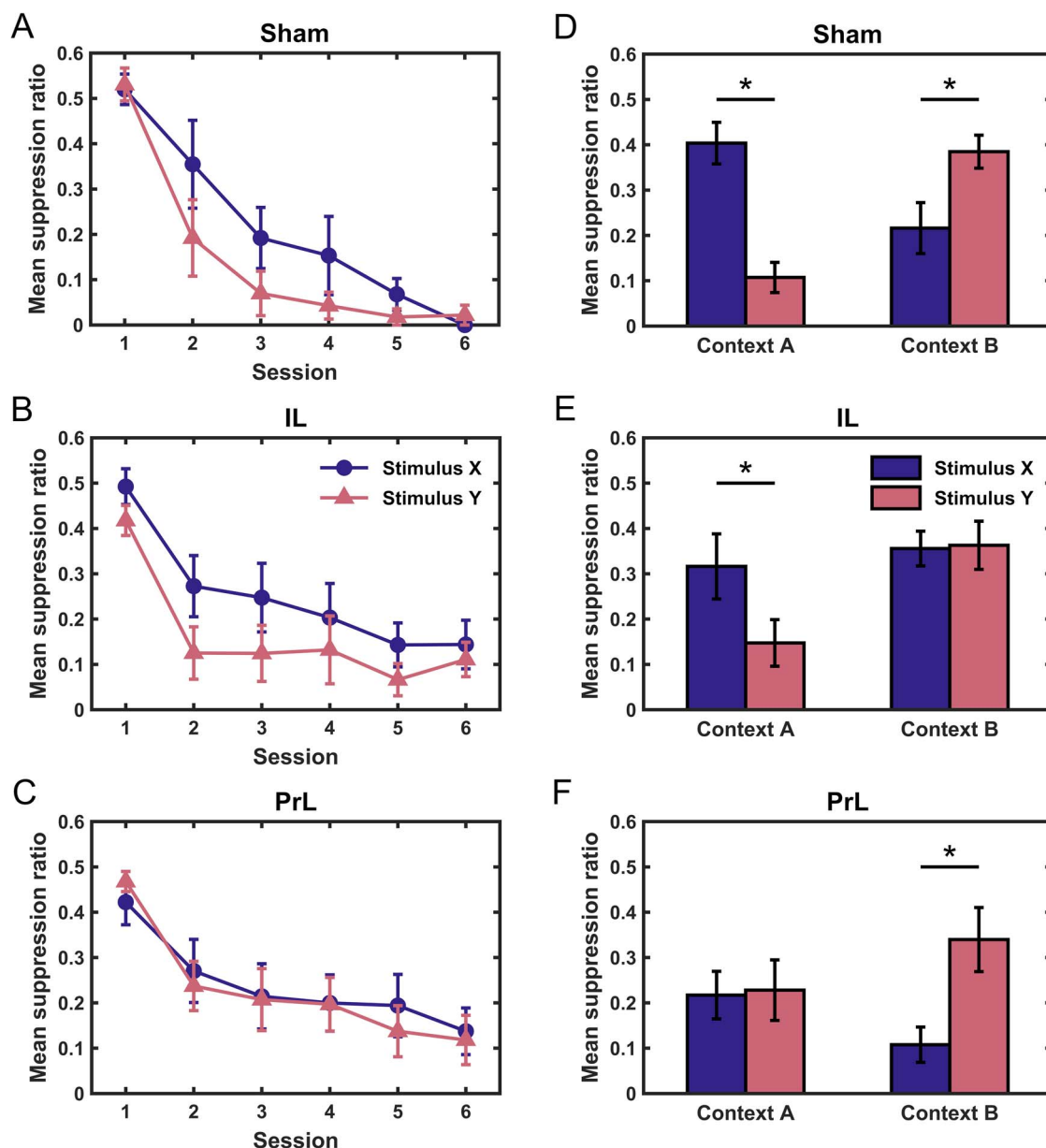


Figure 2. Group mean suppression ratios for lever press responding in the presence of the two stimuli (X and Y) during each of the six conditioning sessions that took place in Context A (panels A-C), and during the test sessions conducted in both contexts (panels D-F). A suppression ratio of 0.5 indicates that rates of lever pressing were the same during stimulus presentation and the period immediately preceding it. A ratio of 0.0 indicates total suppression of responding during stimulus presentation consistent with a strong conditioned emotional (fear) response. Lever-press responding declined over the six sessions of conditioning for both stimuli for all three groups of rats. At the end of the conditioning phase there was no difference in suppression ratios for the two stimuli, and no difference between the three groups. (D) During the test sessions in each context, sham-operated control animals showed less suppression of responding (i.e. latent inhibition) to the stimulus that had been pre-exposed in that context (X in Context A; Y in Context B) than to the other stimulus. (E) Rats with lesions to the IL only showed this latent inhibition effect in Context A, the conditioning context. (F) Rats with lesions to the PrL only showed the latent inhibition effect in Context B. Error bars show one standard error of the mean. (* difference significant at $P < 0.05$).

in stronger conditioned responding than when no change in context occurs. Furthermore, conditioning does not appear to erase the effects of pre-exposure. If an animal is pre-exposed in one context and then conditioned in a second, latent inhibition re-emerges if the animal is later returned to the pre-exposure context for testing ([47]; Figure 2D Context B test). Bouton [1] proposed that retrieval of CS–no event associations formed during pre-exposure (and inhibitory CS– \overline{US} associations learning during extinction) is dependent on the pre-exposure context, whereas retrieval of CS–US associations formed during conditioning is

relatively independent of the context. There is, however, evidence that expression of first-learned excitatory CS–US associations can be influenced by contextual cues [13, 15]. Hence, all associations may be affected by context to some extent.

We found here that the magnitude of the CER was greater, overall, in the context in which conditioning took place (A) than in the other context (B). This finding is consistent with the idea that the retrieval of excitatory CS–US associations is also somewhat dependent upon context in latent inhibition preparations.

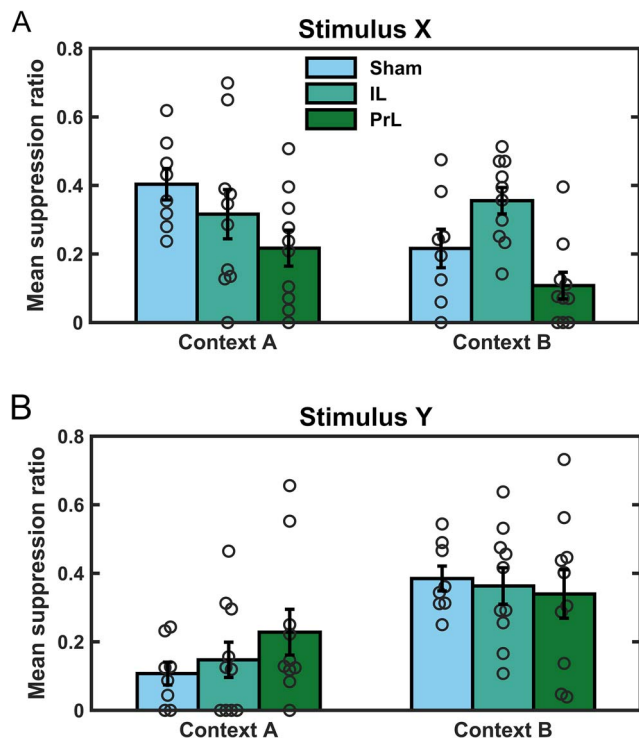


Figure 3. Group mean suppression ratios in the presence of each of the two stimuli (X and Y) during the test sessions in each of the two contexts (A and B). The same data are shown as in Figure 2 (panels D–F), replotted to facilitate comparison between groups. Circles show the performance of individual animals. Error bars show one standard error of the mean.

Lesions to the IL result in second-learned associations that are unusually sensitive to a change of context

Lesions to the IL cortex affect the consolidation and retrieval of extinction learning and conditioned responding following extinction. These effects may all be attributed to failures to retrieve second-learned inhibitory CS–US associations acquired during extinction training. Although initial extinction learning in rats with IL lesions proceeds normally, later retrieval of that learning is impaired [24, 25, 36], and conditioned responding is reacquired more rapidly when a stimulus is again paired with an outcome [31]. Spontaneous recovery, reinstatement and context renewal effects are all greater in rats with IL lesions than in normal control animals [36–38].

George et al [9] reported that lesions to the IL cortex resulted in an enhanced latent inhibition effect. Rats with these lesions were unusually slow to acquire a CER to a pre-exposed stimulus but showed normal acquisition of the response to a novel stimulus. They argued that this effect may also be attributed to a failure to retrieve second-learned contingencies—in this case, excitatory CS–US associations. Hence, in both extinction and latent inhibition paradigms, retrieval of second-learned associations may be unusually context bound in rats with IL lesions. In the current experiment, the results from rats with lesions to the IL cortex are consistent with this interpretation. When the two stimuli were tested in the conditioning context (A), a normal context-specific latent inhibition effect was observed. Stimulus X, which had been pre-exposed in that context, produced a smaller CER than Stimulus Y which had been pre-exposed in the other context.

This indicates that, at the least, expression of the initial CS–no event association was context-specific in these animals.

When the stimuli were tested in the context in which conditioning had not taken place (B), equivalent low levels of fear (i.e. suppression ratios close to 0.5) were observed to each. Importantly, there was an effect of context on conditioned suppression to Stimulus Y, but no effect of context for Stimulus X. This suggests neither stimulus was able to retrieve the second-learned excitatory association with the foot-shock when they were presented outside the conditioning context. That is, in addition to the context-specificity of the CS–no event association, following IL lesions retrieval of the second-learned CS–US association is also context sensitive.

Lesions to the PrL result in first-learned associations that are unusually sensitive to a change of context

The PrL mPFC also plays a role in contextual processing. Previous research investigating the region’s involvement in the hierarchical control of behavior has made use of bi-conditional discrimination tasks in which two stimuli are paired with different outcomes in one context, and these contingencies are reversed in a second context (e.g. A: X→shock, Y→∅; B: X→∅, Y→shock). Sharpe and Killcross [42] found that temporary inactivation of the PrL impaired both acquisition and expression of this bi-conditional discrimination learning. In a related task designed to model aspects of the Stroop task [44], both lesions to [11] and temporary inactivation of [30] the PrL affected the ability of rats to select context-appropriate responses. Furthermore, lesions or temporary inactivation of the PrL abolishes context renewal of conditioned responding following extinction [22, 41].

Here, we observed that when rats with lesions to the PrL mPFC were tested in the conditioning context (A) they showed equivalent high levels of fear (suppression ratios closer to 0 than to 0.5) to stimuli regardless of whether they had been pre-exposed in that context or another (Context B). These results are consistent with a failure to retrieve a memory of the first-learned CS–no event association acquired during pre-exposure. One explanation for this failure is that Context A at test was different to Context A during pre-exposure. During pre-exposure, Context A was simply a place in which rats could press a lever in order to earn food pellets and where a single stimulus was occasionally presented. At test, however, it was a context in which numerous foot-shocks had been delivered. Bouton [1] has suggested that a reinforcer may be incorporated into the representation of a context, and that this explains reinstatement effects following extinction training. Similarly, Killcross and Dickinson [18] observed that occasional non-contingent presentations of the reinforcer during pre-exposure resulted in an enhanced latent inhibition effect, reflecting an increase in the similarity between the pre-exposure and conditioning contexts. Whilst this context shift is not enough to prevent the observation of latent inhibition in standard procedures in intact animals (cf. responding to Stimulus X in Context A in sham-operated rats in Figure 2D), this is not the case in animals with lesions of the PrL mPFC. Hence, when tested in Context A, rats with lesions to the PrL failed to retrieve CS–no event associations learned during pre-exposure in either context but successfully retrieved stimulus–shock associations learned during conditioning. In contrast, when tested in Context B, the CS–no event association is fully supported by the unchanged contextual cues, and normal latent inhibition is observed.

Competing contextual processes

It has previously been suggested [8, 9] that the IL and PrL mPFC play complementary, and competing roles in a number of different learning situations. A striking example is context renewal of conditioned responding following extinction. Lesions to the IL enhance this effect [38], whereas lesions to the PrL abolish it [22, 41].

We propose that there are at least two mechanisms through which the expression of learning may come under the control of contextual cues, and that interaction of the IL and PrL mPFC influences the relative contributions of these mechanisms to the control of behavior. The first system is involved in the processing of incidental contextual cues and is dependent upon the IL mPFC. This involves the integration of information across repeated events to detect regularities across the long-term. For example, in situations in which a stimulus is consistently paired with the same event (or, in the case of pre-exposure, no event), we would expect the contextual control of the association between that stimulus and event to be mediated by this automatic, incidental process. When the situation changes or is ambiguous then a second process is required to resolve this ambiguity by engaging in controlled, context-specific, learning to allow changes in behavior to occur more rapidly. This process is dependent upon PrL mPFC. Under normal circumstances, interaction between these automatic (IL mPFC) and controlled (PrL mPFC) processes will limit the context-dependency of associations to allow behavior to generalize somewhat across different situations. If, however, one or other structure is compromised then the other structure will operate unfettered and will exert an unusual degree of contextual control upon its target associations. The effect of this unregulated activity is what we have observed in the experiment reported here: an extreme sensitivity to context change in the retrieval of either first- or second-learned associations dependent upon the precise locus of the brain lesion.

There is some similarity between these proposals, and those made recently by Green and Bouton [10] about the roles of IL and PrL mPFC in instrumental behavior. They suggested that the IL is important in the regulation of well-trained behaviors and switching between conflicting behavioral states or strategies (such as goal-directed actions and habits). The PrL act as a hub to consolidate different types of contextual information (e.g. physical contexts, discrete stimuli, interoceptive states and behaviors), and is also important for the expression of behaviors in their conditioning context. For example, Trask et al. [45] conditioned an instrumental response in one context (A) before extinguishing it in a second (B). They found that inactivation of the PrL attenuated renewal of responding in the conditioning context (i.e. reduced ABA renewal), but had no effect on responding in a novel context (i.e. ABC renewal was preserved). These results, however, are not entirely consistent with ours and it remains to be seen how models of mPFC function in latent inhibition and instrumental behavior may be reconciled. Finally, there may be limitations in our use of neurotoxin lesions (e.g. [32, 46]), compared to the reversible techniques employed by Trask et al. Although excitotoxic lesions spare fiber of passage and can help to determine whether a brain structure is necessary for a specific psychological function, there are problems with drawing strong inferences about the operation of an intact system from observations of a damaged one. There might be non-specific effects of lesions and lost function might recover or be compensated for by other brain regions or changes in cognitive strategy. The dissociation of the effects of IL and PrL lesions and sham surgery that we

observed, however, strengthens our findings and reduces the potential that they might be explained in terms of non-specific effects.

Funding

This work was supported by a Royal Society University Research Fellowship to D.N.G. and a Wellcome Trust 'Value in People' award to J.E.H.

Author contribution

D.N.G., S.K., J.E.H. participated in the conceptualization. D.N.G., S.K. participated in the methodology. D.N.G. and J.E.H. participated in the investigation. D.N.G. participated in the formal analysis. D.N.G. participated in the writing—original draft. D.N.G., S.K., J.E.H. participated in the writing—review and editing. D.N.G. and J.E.H. participated in the funding acquisition.

Conflict of interest

None.

Data availability

The data underlying this article are available at <https://osf.io/vjx9/>

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