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The Prelimbic Cortex Contributes to the Down-Regulation of Attention Toward Redundant Cues

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Previous research suggests disruption of activity in the prelimbic (PL) cortex produces deficits in tasks requiring preferential attention toward cues that are good predictors of an event. By manipulating cue predictive power, we clarify this role using Pavlovian conditioning. Experiment 1a showed pretraining excitotoxic lesions of the PL cortex disrupted the ability of animals to distribute attention across stimuli conditioned in compound. Experiment 1b demonstrated that these lesions did not affect the ability to block learning about a stimulus when it was presented simultaneously with another stimulus that was previously paired with the outcome. However, in a subsequent test, PL-lesioned animals learnt about this blocked cue faster than sham-lesioned animals when this stimulus alone was paired with reinforcement, suggesting these animals did not down-regulate attention toward the redundant cue during blocking. Experiment 2 tested this hypothesis using an unblocking procedure designed to explicitly reveal a down-regulation of attention during blocking. In this, sham-lesioned animals were shown to down-regulate attention during blocking. PL-lesioned animals did not exhibit this effect. We propose that observed deficits are the result of a specific deficit in down-regulating attention toward redundant cues, indicating the disruption of an attentional process described in Mackintosh's (Mackintosh NJ. 1975. *Psychol Review*. 82:276) attentional theory.

Keywords: associative learning, attention, Mackintosh, prelimbic cortex, rats.

Introduction

An organism's survival is dependent on the ability to predict motivationally significant events on the basis of the environmental cues that consistently precede their occurrence. In a rich environment where multiple cues predict the same or many outcomes, an animal must select which cues will engage the learning process. Despite the complexity of this process, 2 classes of learning theories have been relatively successful in characterizing the mechanisms that underlie stimulus selection. On one hand, error-correction models state that the degree to which a cue will be learnt about is dependent on the discrepancy between the outcome predicted by the presence of a cue and the actual outcome that occurs when the stimulus is presented, referred to as prediction error (PE) (Bush and Mostellar 1951; Rescorla and Wagner 1972). If there is an error in outcome prediction when a cue is presented, learning about that cue will take place. Hence, these models focus on the importance of changes in the processing of the outcome, i.e. the unconditioned stimulus (US). On the other hand, attentional theories argue that learning about a cue does not take place simply because a PE is induced. Rather, the extent to which PE will become attributed to a certain cue is dependent on how much attention is paid toward that to-be conditioned stimulus (CS) (Mackintosh 1975; Pearce and Hall 1980). The degree of attention is determined by how well that stimulus, or other present stimuli, has predicted the outcome in the past. Hence, these theories focus on the importance of changes in processing of the CS determining how well a cue will attract learning. Given the existence of evidence which supports each of these contrary views, it is currently thought that the stimulus selection is a result of both changes in processing of the outcome and changes in the amount of attention paid toward the CS (Le Pelley 2004).

It is well established that dopaminergic neurons in the midbrain send error signals as a result of an unexpected occurrence or the absence of a rewarding event (Schultz et al. 1997; Schultz 1998). Essentially, these signals contain information regarding the degree and direction of PE. In this manner, these signals may support learning processes akin to error-correction models (Schultz 1998). In terms of a neural locus for CS processing, research has predominantly focused on a role for the hippocampus and amygdala in changing the degree of attention toward cues on the basis of how well they have predicted motivationally significant events (Holland and Gallagher 1993; Han et al. 1995; Esber et al. 2012). For example, Han et al. (1995) reported that lesions of the hippocampus disrupt the ability of animals to decrease attention toward stimuli that signal no change in the outcome. Conversely, Holland and Gallagher (1993) argued that lesions of the central nucleus specifically prevented animals from increasing the degree of attention directed toward cues that signaled an unexpected outcome, while leaving intact the ability of animals to decrease attention to cues which do not signal any change in reinforcement. More recently, Esber et al. (2012) have argued that activity in neurons of the basolateral Amygdala (BLA) seems to mirror the more general process whereby attention declines toward a cue that already fully predicts the absence or presence of an outcome and increases when an outcome is unexpected (Esber et al. 2012). That is, the BLA seems to play a role when an unexpected outcome occurs which maintains attention toward that stimulus so that it is learnt about and decreases when the CS fully predicts the outcome and it does not need to be learnt about (Esber et al. 2012). Interestingly, Esber et al. (2012) found 6-hydroxydopamine lesions of the amygdala prevent this type of activity, prompting the authors to suggest that dopaminergic innervation is integral to facilitating this form of attentional processing. Further, the effect of the hippocampus on decrements in attention has been observed by

removing cholinergic input to this structure, which suggests that acetylcholine may play a role in these types of attentional processes as well (Baxter et al. 1999). Thus, while CS and US processing may be discretely located within the brain, these findings suggest that these mechanisms may interact to create a unified learning process.

Despite the extant literature on the role of subcortical structures in facilitating attentional processing, there is relatively little research attempting to investigate candidate sites for this sort of processing in cortical areas. However, research has suggested that damage or inactivation of the rodent PL cortex affects performance on tasks that require behavioral flexibility supported by changes in the degree of attention paid toward different sets of stimuli. For example, lesions centered on the PL cortex produce impairments in extradimensional set shifting and a response strategy set shifting (Birrell and Brown 2000; Floresco et al. 2008). Further, specific inactivation of the PL cortex has been found to disrupt performance when animals are required to utilize previously redundant contextual stimuli to resolve response conflict, a task recruiting some of the cognitive strategies inherent in the Stroop task (Marquis et al. 2007). The common element of these tasks is that they require a preferential degree of attention toward a stimulus or set of stimuli, supporting a role for the PL cortex in modulating attention toward cues during learning.

Given that CS-processing theories argue that the degree of attention paid toward a stimulus is determined by how well that cue predicts an outcome, by manipulating a cue's predictive power we can change the degree of attention that is directed toward it. Two paradigms commonly used to do this are the overshadowing and blocking procedures. Overshadowing involves presenting 2 cues in compound with an outcome. When each stimulus is presented alone under extinction, responding to the compound stimuli is reduced compared with a stimulus that was paired with the outcome alone. Likewise, blocking involves pairing a compound with reinforcement. However, in this case, one element of the compound has been previously trained with the same outcome. When the novel cue is presented alone at test, responding is low and learning is said to be blocked by prior training with the other cue. According to attentional models, overshadowing occurs because each element of the compound is rendered partially redundant by the presence of the other and so attention declines toward both (Mackintosh 1975). Similarly, in the blocking preparation, the introduction of the novel cue signals no change in reinforcement, causing a rapid decline in attention toward the blocked cue and little learning about its relationship with the outcome (Mackintosh 1975; Pearce and Hall 1980).

We conducted 3 experiments to examine the impact of pretraining lesions of the PL cortex on the ability of animals to modulate attention directed toward stimuli on the basis of their predictive value. Experiment 1a used an overshadowing procedure to assess the ability of animals to distribute attention across multiple cues. Experiment 1b explored the impact of these lesions on exhibition of the blocking effect, while a subsequent test compared rates of learning about the blocked cue when it was subsequently paired alone with reinforcement. Finally, Experiment 2 explicitly assessed the ability of animals with PL lesions to down-regulate attention toward the blocked cue during the blocking phase using an unblocking design.

Materials and Methods

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 behavioral 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: 09/39B).

Apparatus

Training and testing took place in 8 operant chambers (30 cm × 24 cm × 22 cm; Med Associates, VT) which were individually housed in light- and sound-attenuating compartments. Each chamber was equipped with a pellet dispenser that delivered one 45-mg pellet into a recessed magazine when activated. Access and duration of time spent in the magazine was detected by means of infrared detectors mounted across the mouth of the recess. Two panel lights (2 cm in diameter), were located on the right hand wall of the chamber above the magazine. A 3W house light was located on the upper left hand wall of the chambers. The chambers contained a white noise generator and a heavy duty relay that delivered a 5 kHz clicker stimulus. A computer equipped with MED-PC software (Med Associates, VT) controlled the equipment and recorded responses.

Surgery

Rats received excitotoxic lesions of the PL cortex or sham 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. 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.

Rats were given 10 days to recover from surgery, after which they were placed on a food restriction schedule where they received 100 g of food pellets per cage, per day. Throughout the duration of the experiment, animals had free access to water in their home cages and were weighed 3 times per week to ensure they maintained at least 85% of their free-feeding weight.

Behavioral Procedures

All conditioned stimuli were 10 s in duration, separated by an ITI that varied about a 2-min mean. Four stimuli were used in all experiments (click, noise, flashing panel light, and the house light), with the exception of Experiment 1a where steady panel lights were used instead of the house light. The physical identity of all stimuli was counterbalanced across rats within modality (where, in Table 1, stimulus A and C are flashing panel lights and a house light, respectively, for half the rats, and a house light and flashing panel lights for the other half. For half the rats in each of these subgroups, stimuli B and D are a clicker and a white noise, respectively, and a white noise and a clicker for

Table 1
Design of Experiments 1, 2, and 3

Experiment	Stage-1	Stage-2	Stage-3	Test	Test 2
1a	AB+ C+ D+			A B C D	
1b	A+	AB+ CD+		B D	B+
2	A+ C+	AB+	AB++ CD++	B D	

Experiment 1a, during stage-1 conditioning rats received presentations of an audio-visual compound (AB) and 2 elemental stimuli (C and D) paired with reinforcement. At test, animals received an extinction test where all stimuli were presented alone without reinforcement. Experiment 1b, rats were initially trained with visual stimulus (A) paired with reinforcement. In stage-2, rats were presented with 2 audio-visual compounds, one comprising the previously trained stimulus (AB) and another novel compound (CD). At test, animals were presented with stimuli B and D under extinction. Experiment 2, animals were initially trained with 2 visual stimuli paired with reinforcement (A and C). In stage-2, compound AB was presented with the same magnitude of reinforcement as stage-1. In stage-3, stimulus AB was again presented with another novel compound CD. During this stage, rats received an increased magnitude of reinforcement. At test, rats were presented with stimuli B and D alone under extinction.

the other half of these subgroups). All experiments were conducted in darkness. Prior to conditioning, all rats received 2 30-min sessions of magazine training, where a pellet was delivered according to a 60-s random interval schedule. The unconditioned stimuli used in all experiments were 40-mg grain pellets (dustless precision grain-based pellets, Bio-serv, NJ, USA). Refer to Table 1 for design of experiments 1, 2, and 3.

Experiment 1a: Overshadowing

Conditioning
Rats received 16 conditioning sessions. During these sessions, 2 stimuli were presented simultaneously to form an audio-visual compound (stimulus AB) and 2 were presented as elemental stimuli (stimulus C and D). Each session consisted of 18 reinforced trials (6 trials with the stimulus AB, 6 with both stimulus C and D), where presentation of any stimulus was coterminated by delivery of a single-food pellet. These sessions approximated 40 min in length.

Extinction Test
Following conditioning, rats received 2 extinction test sessions. During this session, animals received 2 presentations of each of the 4 stimuli alone (A, B, C, and D) without reinforcement. The order of stimulus presentation was fully counterbalanced.

Experiment 1b: Blocking

Stage-1 Conditioning
Rats received 12-stage-1 conditioning sessions. These sessions involved presentation of 1 visual stimulus (stimulus A), followed by delivery of a single pellet. Each session comprised 14 trials, creating a session of around 30 min in duration. In the last 4 sessions, rats also received an additional 2 non-reinforced presentations of the alternate visual stimulus (stimulus C). This was to facilitate discrimination between the 2 visual stimuli.

Stage-2 Conditioning
Rats received 6 sessions of stage-2 conditioning. During this stage, 2 compound stimuli were formed. One comprised stimulus A and a novel auditory stimulus (click or noise; stimulus AB) and the other comprised stimulus C and another novel auditory stimulus (noise or click; stimulus CD). Rats received 6 compound training sessions consisting of 12 reinforced trials, 6 with each compound. Each compound presentation was coterminated by presentation of a single-food pellet, as in stage-1. These sessions approximated 25 min in length.

Extinction Test
Rats received a single extinction test where both auditory cues (stimulus B and D) were presented alone without reinforcement. The order of stimulus presentation was fully counterbalanced. The session contained 12 trials, 6 presentations of each stimulus.

Post-Extinction Acquisition Test
Following extinction, rats were given 2 sessions of conditioning to stimulus B. Each conditioning session comprised 12 reinforced trials, with each session approximating 25 min.

Experiment 2: Blocking of Unblocking

Stage-1 Conditioning
All rats received 14 sessions of stage-1 conditioning where 2 visual stimuli were followed by the delivery of a single pellet (stimuli A and C). Each session comprised 16 trials, with 8 presentations of each stimulus.

Stage-2 Conditioning
Animals then received 5 sessions of stage-2 conditioning, where stimulus A was paired with an auditory stimulus (click or noise) to create an audio-visual compound (stimulus AB). Each session comprised 16 presentations of stimulus AB, followed by delivery of a single pellet, as in stage-1.

Stage-3 Conditioning
Rats received 5 sessions of stage-3 conditioning. In this stage another audio-visual compound was formed with stimulus C and a novel auditory stimulus (noise or click) to create stimulus CD. Animals were given 8 presentations of stimulus AB and 8 of stimulus CD. Presentations of both stimuli were followed by the delivery of 3 food pellets, where the last 2 pellets were delivered 5 s after the first pellet. This constituted an increase in reinforcer magnitude for this third stage of conditioning.

Extinction Test
Rats received 2 extinction test sessions where auditory stimuli B and D were presented without reinforcement. Each session comprised 2 trials. The order of presentation of stimuli was fully counterbalanced, and all rats received the opposite counterbalancing in the second session.

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 subsequently assessed for the extent and placement of lesions microscopically by a trained observer. The PL region was defined by the boundaries specified in the atlas of Paxinos and Watson (1998). Rats with incomplete damage to the PL cortex, or with extensive damage to surrounding areas, were excluded from all analyses.

Results

Experiment 1a: Overshadowing

Histology
All rats recovered from surgery and no significant weight loss or behavioral problems were observed. One PL-lesioned rat exhibited a unilateral lesion and so was excluded from all analyses, yielding the following final group sizes: sham $n = 10$, PL $n = 13$. Figure 1 illustrates the maximal and minimal lesions accepted into analyses for all experiments.

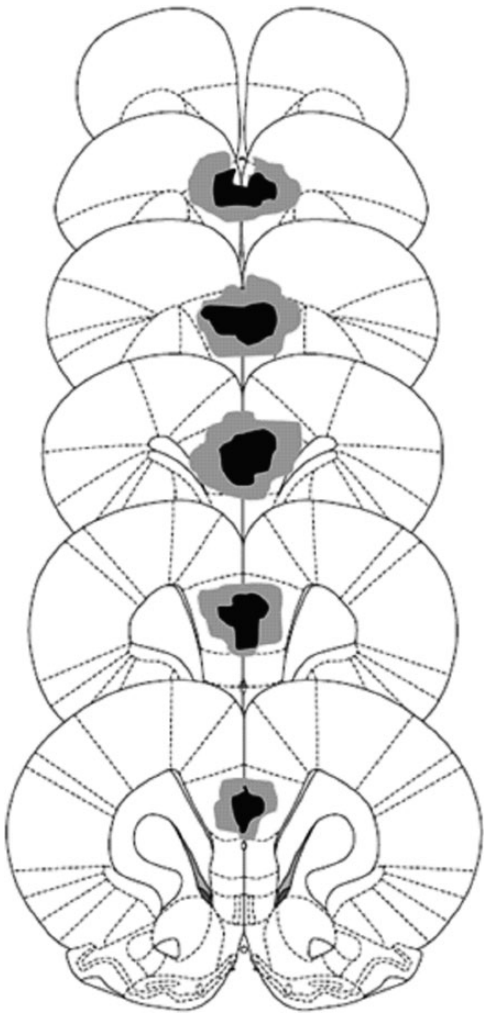

Figure 1. Schematic representations of excitotoxic lesions of the PL cortex for Experiments 1, 2, and 3. Coronal sections are taken from the following points on the anteroposterior plane beginning at top: +5.64, +5.16, +4.68, +4.20, +3.24, and +2.52 mm anterior to bregma (Paxinos and Watson 1998).

There was no difference in the extent or variability of lesions across experiments.

Conditioning

All rats acquired the conditioned response to all stimuli by the end of training. There was no difference in responding during the baseline 10 s preCS period between groups [mean (±SEM): sham 1.4 (±0.3); PL 1.8 (±0.4), $F < 1$]. Similarly, there were no differences between the rates of learning or the number of entries during CS presentations in the final session of training between groups (mean [± SEM]: sham 7.9 [±1.2]; PL 7.6 [±0.7], $F < 1$). There was no difference in rates of responding during presentations of the compound stimulus (AB) between groups (mean [±SEM]: sham 8.3 [±0.9]; PL 8.7 [±1.4], $F < 1$). Further, there were no differences in responding between groups during presentations of the visual stimulus (C) (mean [±SEM]: sham 5.8 [±0.9]; PL 5.6 [±1.0], $F < 1$) or the auditory stimulus (D) (mean [±SEM]: sham 9.7 [±0.9]; PL 8.4 [±1.4], $F < 1$). There was a significant difference between responding during presentation of the auditory stimulus compared with the visual stimulus ($F_{1,21} = 41.40$, $P < 0.05$), which

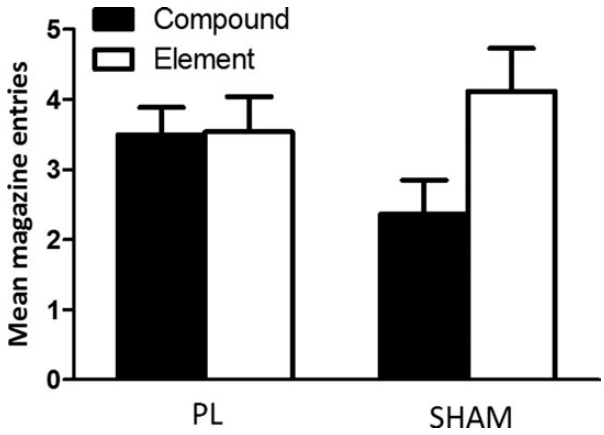

Figure 2. Effects of lesions of the PL cortex on overshadowing. Rates of responding are represented as the antilog of the mean number of magazine entries during CS presentations under extinction (±SEM). Sham-lesioned animals demonstrated lower levels of responding to stimuli conditioned in compound compared with those conditioned alone, indicative of an overshadowing effect. PL-lesioned animals did not show this effect, responding at a similar level to stimuli conditioning in compound and alone.

did not differ between groups ($F < 1$). This is unsurprising given the difference in salience, or the capacity to control behavior, of visual and auditory stimuli to rodents. Given that the test results show responding averaged across auditory and visual stimuli presentations, according to whether they were trained in compound or as an element, a bias toward auditory stimuli would be equal across both compound and elemental stimuli.

Extinction Test

Figure 2 shows the data from the critical extinction test averaged across stimuli trained in compound (*A* and *B*) and those trained elementally (*C* and *D*). The data show that sham-lesioned rats demonstrated an overshadowing effect, whereas PL-lesioned animals failed to exhibit this effect. This was confirmed by statistical analyses. Analyses were conducted using the transformation $\log(a + 1)$ as the standard deviation was found to increase with the mean in the raw data (Howell 2007). ANOVA revealed a significant main effect of stimulus ($F_{1,21} = 5.83$, $P < 0.05$), and a significant interaction between group and stimulus ($F_{1,21} = 5.37$, $P < 0.05$), indicating that the magnitude of the difference between responding to these stimuli was greater in sham-lesioned animals relative to PL-lesioned animals. Further analysis of simple main effects revealed a significant difference between responding for stimuli trained in compound relative to those trained alone for sham-lesioned animals ($P < 0.05$), but not for PL-lesioned animals ($P > 0.05$). A one-way ANOVA of responding during the preCS period did not reveal any significant differences between groups (mean [±SEM]: sham 0.6 [0.2]; PL 0.7 [±0.2], $F < 1$).

Experiment 1b: Blocking

The results obtained from Experiment 1a indicate that rats with lesions of the PL cortex did not distribute learning across 2 cues trained in compound. These animals responded to cues presented in compound in the same way as cues trained individually. According to an attentional theory, attention

declines to each stimulus that forms part of the compound as the presence of the other stimulus renders it partially redundant (e.g. Mackintosh 1975). Thus, PL-lesioned animals may not modulate their attention toward these cues and their responses may condition more toward the stimuli trained in compound on this basis. Experiment 1b aimed to explore this hypothesis further by assessing whether rats with lesions of the PL cortex are capable of blocking learning about a stimulus when it is presented simultaneously with another cue that has already been established as a predictor of the outcome. Given that attentional theories argue that blocking is the consequence of a decrement in attention toward the blocked cue as it signals no change in reinforcement (Mackintosh 1975; Pearce and Hall 1980), if animals with lesions of the PL cortex do not change the degree of attention directed toward cues they will maintain attention to the blocked cue and fail to exhibit the blocking effect.

Histology

All the rats recovered from surgery and no significant weight loss or behavioral problems were observed. One PL-lesioned animal received damage to the adjacent infralimbic (IL) cortex and so was excluded from all analyses. Further, 1 sham-lesioned animal failed to acquire the Pavlovian response and so was excluded leaving the final group sizes: sham $n = 9$, PL $n = 13$. Refer to Figures 1 and 3.

Conditioning

All rats acquired the conditioned response to visual stimulus A, with no difference in rates of learning or responding between groups at the end of training (mean [±SEM]: sham 3.9 [±0.6]; PL 4.7 [±1.3], $F_{1,20} = 1.29$, $P > 0.05$). Further, no significant difference was found in baseline preCS entry rates between groups during stage-1 conditioning (mean [±SEM]: sham 0.5 [±0.1]; PL 0.5 [±0.1], $F < 1$). During stage-2 conditioning, all rats acquired the conditioned magazine approach to both compounds AB and CD. In the last session of training, there was no difference in responding to AB or CD for sham-lesioned animals (mean [±SEM]: AB 7.9 [±1.1]; CD 6.5 [±0.9], $F < 1$) or PL-lesioned animals (mean [±SEM]: AB 8.7 [±1.2]; CD 8.6 [±0.6], $F < 1$). Again, no difference was found in baseline responding during the preCS period between groups (mean [±SEM]: sham 0.7 [±0.2]; PL 1.1 [±0.4], $F < 1$). No other main effects or interactions were significant.

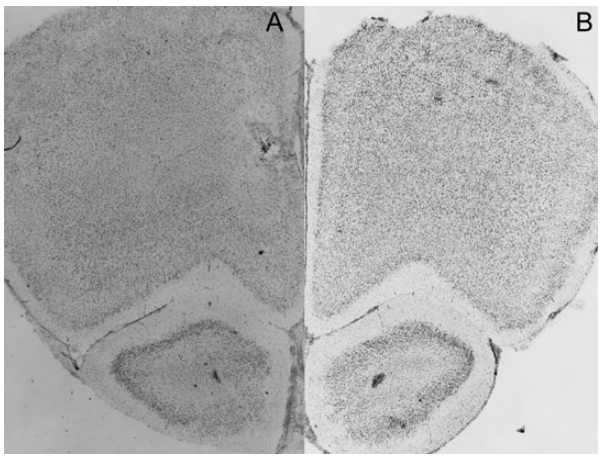

Figure 3. Photomicrographs showing cresyl violet-stained coronal sections through the PL cortex (approximately +4.20 mm anterior to bregma) (*A*) PL lesion (*B*) Sham lesion.

Extinction Test

Figure 4*A* shows data from the critical extinction test session averaged across 2 presentations of both *B* and *D*. Both groups exhibited greater levels of responding to D relative to B, indicating the prior training with A successfully blocked responding to B. As in Experiment 1a, logarithmic transformations were applied to these data as the standard deviation was again found to increase with the mean (Howell 2007). A significant blocking effect was confirmed with ANOVA which revealed a significant effect of stimulus ($F_{1,20} = 7.03$, $P < 0.05$), with no significant stimulus by group interaction ($F < 1$), suggesting the magnitude of this effect was similar between groups. Analysis of simple main effects revealed an effect of stimulus type for each group (Ps < 0.05), suggesting animals in both groups demonstrated a significant blocking effect. There was no difference in responding during baseline preCS period between groups (mean [±SEM]: sham 0.2 [±0.2]; PL 1.0 [±0.6], $F_{1,20} = 2.84$, $P > 0.05$).

Post-Test Acquisition to the Blocked cue

The results from Experiment 1b show that animals with lesions of the PL cortex exhibit the blocking effect. This may suggest that rats with lesions of the PL cortex are capable of changing the degree of attention directed toward a cue when it is paired with an outcome that has already been predicted by another cue. However, past research has suggested that the blocking phenomenon has multiple origins (Rescorla and Holland 1982). While attentional theories argue that blocking occurs due to a reduction in attention toward the blocked stimulus, blocking has also been described as resulting from changes in the processing of the outcome rather than the cue. According to US-processing theories, blocking occurs as there is no change in reinforcement at stage-2, so no PE is generated when this cue is introduced and so learning does not take place. Even if animals with lesions of the PL cortex have deficits in attentional modulation during conditioning, blocking in these animals may be brought about by the absence of PE (i.e. a US-processing mechanism). One way to test whether an animal has down-regulated attention to the blocked cue during the blocking phase is to pair the blocked cue alone with the outcome in an additional conditioning phase. If an animal's attention toward the blocked cue is low, conditioning will proceed slowly when compared with an animal that has not down-regulated attention toward the blocked cue.

Post-Test Acquisition to the Blocked cue

Figure 4*B* shows responding across the 2 sessions in which stimulus B alone was paired with reinforcement. While responding to the blocked cue was similar between groups in Session 1, by Session 2 PL-lesioned animals were exhibiting faster acquisition to the blocked cue. This was confirmed by statistical analyses. ANOVA revealed a significant interaction between session and group ($F_{1,20} = 6.08$, $P < 0.05$), showing that animals with lesions of the PL cortex demonstrated faster acquisition to the blocked cue. There was no significant difference between baseline preCS responding rates between groups (mean [±SEM]: sham 1.4 [±0.2]; PL 1.7 [±0.2], $F < 1$).

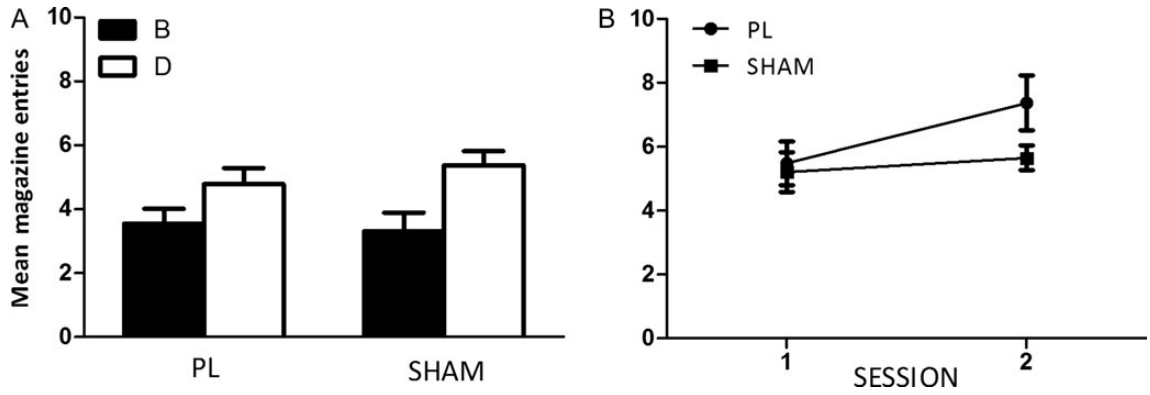

Figure 4. Effects of lesions of the PL cortex on the exhibition of blocking and the down-regulation of attention toward the blocked cue. (*A*) Rates of responding are represented as the antilog of the mean number of magazine entries (±SEM) made across 2 presentations of both (*B* and *D*). Animals in both groups demonstrated greater levels of responding to stimulus D relative to stimulus B. (*B*) mean number of magazine entries (±SEM) made across 2 sessions of post-test conditioning with the blocked cue. PL-lesioned animals demonstrated faster acquisition to the blocked cue relative to sham-lesioned animals.

Experiment 2: Blocking of Unblocking

The results of Experiment 1b showed that animals with lesions of the PL cortex exhibit blocking but subsequently demonstrate faster acquisition to the blocked cue following extinction. This is consistent with the idea that PL-lesioned animals may have a deficit in down-regulating attention toward cues, and that the presence of the blocking effect in these animals was likely to be the result of changes in processing of the outcome rather than changes in attention directed toward the cue. However, given the potential confounds associated with conducting a post-extinction acquisition test, Experiment 2 used an unblocking design to explicitly test this hypothesis (refer to Table 1). Following a blocking phase, animals will again learn about a blocked cue if the blocking compound is subsequently paired with a greater magnitude of reinforcement (i.e. a more rewarding outcome). This is because the added reinforcement is unexpected and, therefore, PE is again induced. However, according to an attentional theory, animals will have down-regulated attention to the blocked cue during the blocking phase (Mackintosh 1975; Pearce and Hall 1980). Hence, learning about the blocked cue will still proceed more slowly in comparison with another cue that has not received a blocking phase prior to its introduction and an increase in reinforcement magnitude. This effect is not predicted by theories which argue that the effects of stimulus competition are related to changes in the processing of the outcome or the presence of PE (Rescorla and Wagner 1972). These theories do not allow for changes in attention to the CS, and so the blocking phase will have no impact on attention directed toward the cue. Rather, if animals with lesions of the PL cortex are relying on an outcome-processing mechanism, we might anticipate greater levels of responding to the blocked cue at test. This would reflect any additional learning that may have taken place in the blocking phase, as a consequence of any residual PE from the initial conditioning phase in stage-1, but masked in sham-lesioned animals by a down-regulation of attention slowing learning in the unblocking stage. As this experiment involved an increase in reinforcer magnitude, we measured the duration that animals spent in the magazine during CSs relative to the baseline preCS period. This is standard practice within this literature (Holland and Gallagher 1993; Holland and Kenmuir 2005), as animals tend to spend more time in the magazine when the CS predicts multiple food reinforcers, rather than entering the magazine multiple times.

Histology

All animals recovered from surgery and no significant weight loss or behavioral problems were observed. One PL-lesioned rat had incomplete bilateral damage to the PL cortex and was excluded from all analyses. This exclusion left the following final group sizes: PL $n = 12$, sham controls $n = 19$.

Stage-1 Conditioning

Both groups acquired the conditioned response to visual stimuli A and C. There was no difference in the time spent in the magazine during presentation of A or C, relative to the preCS baseline, for sham-lesioned animals (mean [±SEM]: A 3.3 [±0.3]; C 3.3 [±0.3], $F < 1$) or PL-lesioned animals (mean [±SEM]: A 4.4 [±0.3]; C 4.5 [±0.5], $F < 1$). Further, there was no difference in time spent in the magazine during the baseline preCS period between groups (mean [±SEM]: sham1.1 [±0.2]; PL 0.7 [±0.3], $F_{1,29} = 1.56$, $P > 0.05$).

Stage-2 Conditioning

Both groups slightly elevated responding during stage-2 conditioning. There were no differences between groups in time spent in the magazine during presentations of the compound stimulus AB at the end of training, relative to baseline (mean [±SEM]: sham 6.2 [±0.3]; PL 5.6 [±0.3], $F_{1,29} = 3.25$, $P > 0.05$). Again, there were no differences in time spent in the magazine during the baseline preCS period (mean [±SEM]: sham 0.9 [±0.3]; PL 0.4 [±0.2], $F_{1,29} = 1.44$, $P > 0.05$).

Stage-3 Conditioning

Both groups maintained responding during presentation of compound stimuli AB and CD during stage-3 conditioning. In the last session of training, there was no difference between time spent in the magazine during the compound stimuli for sham-lesioned animals, relative to the baseline preCS period (mean [±SEM]: AB 5.4 [±0.2]; CD 5.1 [±0.3], $F < 1$) or PL-lesioned animals (mean [±SEM]: AB 5.1 [±0.7]; CD 5.0 [±0.4], $F < 1$). Again, there were no differences in time spent in the magazine during the baseline preCS period between groups (mean [±SEM]: sham 1.1 [±0.3]; PL 1.1 [±0.3], $F < 1$).

Extinction Test

Figure 5 displays results from the 2 extinction test sessions. Sham-lesioned animals exhibited lower levels of responding to stimulus B relative to stimulus D, consistent with the idea that they had down-regulated attention to stimulus B during stage-2 conditioning. However, animals with PL lesions did not demonstrate this effect. Rather, they tended to exhibit greater levels of responding to stimulus B relative to stimulus D. This was confirmed by statistical analyses. As the standard deviation did not increase with the mean, we did not transform raw scores for analyses. There was a significant interaction between group and stimulus ($F_{1,29} = 7.74$, $P < 0.05$). Further, analyses of simple main effects for the sham-lesioned animals revealed more responding to D than to B ($F_{1,29} = 4.33$, $P < 0.05$). In contrast, there was a non-significant trend toward PL-lesioned rats responding more to B relative to D ($F_{1,29} = 3.60$, $P = 0.068$). There were no differences in time spent in the magazine during the baseline preCS period between groups (mean [±SEM]: sham 0.8 [±0.2]; PL 0.5 [±0.2], $F_{1,29} = 1.92$, $P > 0.05$).

Discussion

The results of the present experiments support a role for the PL cortex in modulating attention toward cues during learning. Findings from Experiment 1a showed that PL-lesioned animals demonstrate an attenuated overshadowing effect, showing that the PL cortex is involved in distributing learning across multiple cues. Experiment 1b demonstrated that animals with lesions of the PL cortex are capable of blocking learning about a stimulus when it is paired with another cue that has previously been established as a predictor of reinforcement. This suggested PL-lesioned animals' fail to learn in the absence of PE (i.e. according to a US-processing mechanism; Rescorla and Wagner 1972). However, these animals subsequently exhibited faster acquisition to this blocked cue, suggesting the attention paid toward the blocked cue remained high comparative to sham-lesioned animals. These data specifically implicate the PL cortex in the down-regulation of attention toward cues. This was confirmed in Experiment 2, where animals with lesions of the PL cortex demonstrated greater levels of responding to a cue that received a blocking stage before being paired with a greater level of reinforcement, relative to a novel cue whose introduction signaled an immediate increase in reinforcement magnitude. This effect was opposite to sham-lesioned animals who exhibited the blocking of unblocking effect, demonstrating they had down-regulated attention toward the blocked cue in the blocking phase which slowed learning about this cue when paired with a greater degree of reinforcement. This finding is consistent with the idea that PL-lesioned animals cannot down-regulate attention toward cues but can modulate learning based on the presence of PE.

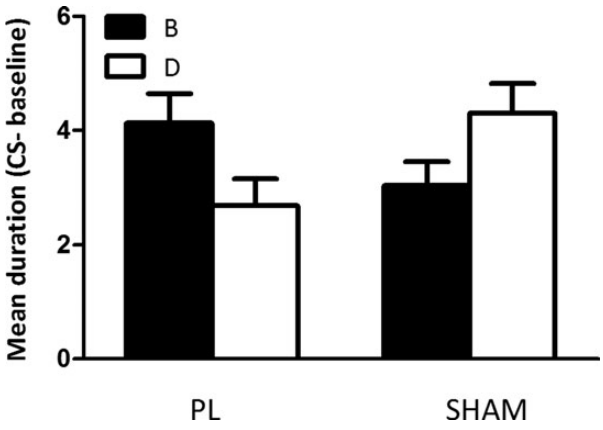

Figure 5. Effects of lesions of the PL cortex on unblocking. Data are represented as the mean duration spent in the magzine relative to the baseline preCS period across the 2 test sessions. Sham-lesioned animals responded significantly more to stimulus D relative to stimulus B, demonstrating a down-regulation of attention toward the blocked cue during blocking. PL-lesioned animals did not demonstrate this effect, trending toward responding more to stimulus B relative to stimulus D.

A hypothesized role for the PL cortex in down-regulating attention toward cues is also compatible with the wider literature. For example, Birrell and Brown (2000) reported that lesions of the medial prefrontal cortex (mPFC), centered on the PL cortex, produced deficits in extradimensional set shifting. This task involved training rats to dig in bowls to obtain a pellet. On any 1 trial, 2 bowls were presented and only one was baited. Rats had to learn to discriminate between baited bowls on the basis their odor, the medium that filled the bowl, or the texture that covered the bowls surface. Lesions of the mPFC did not impact on the ability of animals to perform compound discriminations, reversal discriminations, or an intra-dimensional shift. This suggested that lesions did not disrupt the ability of animals to form an attentional set or manipulate responding within this set. However, lesioned rats demonstrated a specific deficit when required to shift between basing responding on contingencies established within 1 stimulus dimension (e.g. medium) toward those of another stimulus dimension (e.g. odor), referred to as an extradimensional shift. Our findings expand on those reported by Birrell and Brown (2000), and allow us to interpret this result as lesions encompassing the PL cortex disrupting the ability of animals to down-regulate attention toward the previously relevant attentional set in order to learn the new contingencies associated with the other stimulus dimension.

This interpretation can also account for the effects that have been reported using specific inactivation of the PL cortex. Marquis et al. (2007) reported that inactivating the PL cortex disrupted the use of previously redundant contextual cues to resolve response conflict in a rodent version of the Stroop task. Rats were trained on 2 bi-conditional discriminations, 1 auditory and 1 visual, in 2 distinct contexts. In one context, the 2 visual cues dictated pressing either the left or right lever, and in the other context the auditory cues would dictate the correct lever press. The PL cortex was inactivated before a test session where animals were presented with 2 types of novel audio-visual compounds, congruent and incongruent, in both contexts. Congruent compounds comprised 2 stimuli that dictated the same lever press during training, whereas incongruent compounds comprised stimuli that dictated opposing lever presses during training. On incongruent compound trials, animals needed to use the task-setting contextual cues in order to disambiguate response conflict and perform the correct lever press. Inactivation of the PL cortex before test specifically disrupted performance on the incongruent trials. This effect could be interpreted as inactivation of the PL cortex disrupting a contextually regulated downshift in attention toward the irrelevant cue not trained in the testing context. This supports a role for the PL cortex in the

down-regulation of attention toward redundant cues, and further generalizes this effect to specific inactivation of the PL cortex.

Interestingly, George et al. (2010) recently reported data from an optional set-shifting procedure which supports the notion that PL-lesioned animals do not have a deficit unless required to down-regulate attention toward stimuli and switch from 1 stimulus dimension to another. Further, they implicate the adjacent IL cortex in an opposing up-regulatory role in attention. During stage-1 training, rats learn that 2 auditory cues (A1 and A2) consistently predict that 1 of 2 lever presses will be reinforced (R1 or R2), whereas 2 visual cues (V1 and V2) are irrelevant and predict neither response. In stage-2, animals are given a shift discrimination where 2 novel audio-visual compounds (A3V3 and A4V4) consistently predict which lever press will be reinforced (R1 or R2). In a test phase, animals are given incongruent compounds which dictate opposing lever presses from stage-2 (A3V4 and A4V3). If rats have formed an attentional set of the relevant auditory cues during stage-1, and maintained that set during the stage-2 shift discrimination, they will respond on the basis of the auditory cues (A3 and A4) and press the lever that was associated with that cue in stage-2. However, if they have not formed or maintained that attentional set, they will respond equivalently on both levers when presented with these compounds. During the test phase, PL-lesioned rats exhibited a lever bias dependent on the auditory cue, suggesting they has formed an attentional set and maintained it across the stage-2 discrimination. However, rats with lesions of the IL cortex exhibited equivalent responding on both levers, regardless of which compound was presented. This suggested that they did not form an attentional set, or did not maintain that set across stage-2. Taken together, these results suggest that the IL and PL regions of the mPFC act together to establish a balance between an appropriate maintenance of attention toward a stimulus, or set of stimuli, and an appropriate shift away from these stimuli when environmental contingencies change, promoting behavioral flexibility.

To this point, a distinction has not been drawn between disruption to a Mackintosh (1975) or a Pearce and Hall (1980) CS-processing mechanism underlying the deficit in down-regulating attention exhibited by animals with lesions of the PL cortex. However, these data might favor a Mackintosh (1975) interpretation. First, the results from Experiment 1a are more readily explained by Mackintosh's (1975) theory that predicts dynamic shifts in attention during overshadowing, as opposed to Pearce and Hall's (1980) model which relies on the distribution of learning determined by the fixed level of salience of each cue to produce this effect. Secondly, Pearce and Hall's (1980) attentional mechanism does not readily account for the attentional set-shifting or optional set-shifting effects that have been reported following PL lesions. Thus, these data may specifically implicate the PL cortex as a region involved in down-regulating attention toward poorer predictors as described by Mackintosh's (1975) theory, as opposed to a Pearce and Hall (1980) mechanism which predicts that attention declines toward cues that do not predict changes in anticipated outcomes. Further, the results reported by George et al. (2010) may suggest that that we can conceptualize the mPFC more generally as a Mackintosh (1975) attentional system which down-regulates attention toward to poor predictors of reinforcement, while up-regulating attention toward cues that are good predictors of reinforcement.

Interestingly, the deficits in attentional set shifting following lesions of mPFC activity can also be produced with dopamine depletion in the prefrontal cortex (Crofts et al. 2001), and dopamine agonists can modulate normal rats' ability to use redundant contextual stimuli to disambiguate response conflict when specifically targeting distinct regions of the mPFC (Haddon and Killcross 2011). This suggests that dopaminergic innervation of the PFC may be integral to supporting the ability of the mPFC to modulate attention toward environmental stimuli (though acetylcholine may also play an important role as suggested by Baxter et al. (1999) findings). This parallels the idea recently proposed by Esber et al. (2012), where dopaminergic systems in the midbrain send information regarding the degree and direction of PE to support subsequent attentional processing in the amygdala of the kind described in Pearce and Hall's (1980) attentional theory. Taken together, this research lends support for hybrid theories that have been recently developed (Le Pelley 2004; Pearce and Mackintosh 2010), whereby distinct CS- and US-processing mechanisms interact to create a flexible and unified learning system.

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Notes

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