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The Contribution of Medial Prefrontal Cortical Regions to Conditioned Inhibition

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Abstract

Few studies have considered the process by which individuals learn to omit a response, which is an essential aspect of adaptive behavior. Several lines of evidence indicate that two regions of the medial prefrontal cortex have disparate roles in behavioral flexibility. In particular, the prelimbic cortex (PL) is thought to facilitate the generation of a strategy to inhibit a pre-potent response, while the infralimbic cortex (IL) appears to be more important for maintaining extensively trained inhibitory behaviors. The present experiments were designed to elucidate the contributions of PL and IL to the acquisition and maintenance of Pavlovian conditioned inhibition. In Experiment 1, damage to PL prior to training in a compound feature negative discrimination task impaired inhibitory learning. By comparison, lesions of the IL had little effect. In Experiment 2, lesions of PL or IL occurred after over-training, and damage to IL significantly impaired subsequent performance in the task, suggesting that this region is involved in the continued expression of Pavlovian conditioned inhibition after thorough training. PL may also be involved in maintaining inhibition, as evidenced by a marginally significant lesion-induced performance deficit. These data support the notion that PL and IL have distinguishable roles in modulating inhibition, while contributing important information about the specific role for PL in acquisition of an inhibitory response and IL in performance.

Keywords

prelimbic; infralimbic; associative learning

INTRODUCTION

Although inhibitory behavior has been the focus of substantial prior research, most studies have emphasized motor inhibition by measuring performance variables such as stop-signal reaction time or levels of premature responding (Logan, 1994; Eagle et al., 2008; Robbins, 2002). Far fewer studies have considered the process by which individuals learn to *omit* a response. Yet, learning to withhold a behavioral response when signaled by a cue in the environment is an essential aspect of adaptive behavior. For example, cue-directed inhibition has the utility of regulating energy expenditure and in some cases increasing awareness and avoidance of potentially dangerous situations. This behavior depends on learning about

environmental stimuli and evaluating which stimuli are significant for goal-directed behavior. Considerable evidence implicates the prefrontal cortex (PFC) in a number of tasks in which responding is based on discriminating between stimuli, including the five choice serial reaction time task (Muir, Everitt & Robbins, 1996), signal discrimination (McGaughy, Kaiser & Sarter, 1996) and attentional set shifting procedures (Birrell & Brown, 2000; McGaughy, Ross & Eichenbaum, 2008). Once the precedent for behavioral inhibition has been established, the processes involved in withholding a behavior must be consistently executed. The execution of behavioral inhibition has also been associated with activity in PFC (Neill 1976; Rieger, Gauggel, & Burmeister, 2003; Rubia, Smith, Brammer, & Taylor, 2003; Aron, Fletcher, Bullmore, Sahakian, & Robbins, 2003; Aron, Robbins, & Poldrack, 2004; Cardinal, Winstanley, Robbins, & Everitt, 2004; Bari & Robbins 2011). Specifically, PFC has an essential role in suppressing dominant response tendencies in favor of more appropriate goal-directed behaviors (Iversen & Mishkin, 1970; Guitton, Buchtel, & Douglas, 1985; Diamond 1990; Sweeney et al., 1996; Dias, Robbins & Roberts, 1997; Monchi, Petrides, Petre, Worsley & Dagher, 2001).

Several lines of evidence indicate that sub-regions of PFC have distinct roles in controlling behavior. In particular, two regions of medial PFC have been identified as having disparate, and in some cases, opposing roles in behavioral flexibility. The prelimbic cortex (PL) is thought to facilitate the generation of a strategy to inhibit a prepotent response (Ragozzino, 2007) and to support goal-directed behaviors over habits (Balleine & Dickinson, 1998; Killcross & Coutureau, 2003). These roles are reflected by neuronal activity in PL during a rule-switching task. Indeed, PL neurons are active during the early stages of learning a response strategy and PL activity also predicts strategy switching (Rich & Shapiro, 2009). In addition, lesions of PL result in perseveration of a previously reinforced response and impair the ability to learn a new rule for obtaining reinforcement (Ragozzino, 2007). In contrast, the infralimbic cortex (IL) appears to be important for maintaining extensively trained inhibitory behaviors (Ragozzino, 2007). Furthermore, it has been suggested that IL mediates retrieval mechanisms that indicate the requirement for inhibition (Herry & Garcia, 2002; Milad & Quirk, 2002; Laurent & Westbrook, 2009). Indeed, there is evidence of impulsive responding following IL lesions, which most likely results from the decoupling of a behavior from stimulus control (Chudasama et al., 2003). In addition, IL contributes to establishing the persistence of a response strategy as reflected by activation of IL neurons increasing only as a response strategy becomes consistently associated with its expected outcome (Rich & Shapiro, 2009).

Most research investigating the roles of PL and IL in behavioral flexibility and inhibition has involved learning about a cue that indicates the necessity for behavioral *cessation* (e.g. a 'stop-signal'). Comparatively less research has determined whether these regions are required for mediating cue-directed behavioral *omission*. Furthermore, selective lesions of PL and IL have previously been used to probe the process of learning to inhibit a previously trained response. However, there is a dearth of research focusing on the role of these two regions in the *acquisition* phase of a task demanding inhibition of a response. Thus, the present experiments tested the effects of PL or IL damage using a compound feature negative discrimination paradigm, which produces conditioned inhibition (Pavlov, 1927;

Holland, 1984). Rats were exposed to two types of training trials: During one trial type, a tone (the ‘target’ stimulus) was presented for 10 seconds and immediately followed by food; during the other type of trial, a light (the ‘feature’ stimulus) was simultaneously presented along with the tone and not reinforced. Normal adult rats learn to discriminate between these two trial types after ~6 training sessions (Meyer & Bucci, 2014), as indicated by approaching the food cup in anticipation of receiving a reward when the tone is presented by itself, but inhibiting that behavior during light-tone simultaneous compound trials. The result is that the light acts as a conditioned inhibitor, and modulates conditioned behavior by entering into a direct inhibitory association with the unconditioned stimulus (US; Rescorla, 1969; Holland & Lamarre, 1984). Successful discrimination between the trial types results from excitatory conditioning to the tone and conditioned responding, which in turn is reduced on compound trials by the presence of the conditioned inhibitor (Holland, 1984; Bouton & Nelson, 1994).

The present experiments were designed to elucidate the contributions of PL and IL to the acquisition and maintenance of conditioned inhibition. In Experiment 1, rats with lesions of either PL or IL underwent discrimination training. In Experiment 2, another set of rats received extensive training prior to being lesioned and were then tested on their ability to perform the previously acquired task. Given the disparate functions of PL and IL in acquisition and maintenance of task requirements, it was predicted that lesions of PL would disrupt acquisition of the discrimination but have no effect after rats reached stable performance levels. Conversely, IL lesions were expected to have no effect on acquisition of the task, while impairing responding in rats that were lesioned and tested after thorough training.

MATERIALS AND METHODS

Subjects

The subjects were 62 naïve, adult male Long Evans rats (30 in Experiment 1 and 32 in Experiment 2), initially weighing ~250 g (Harlan Laboratories, Indianapolis, IN). Rats were housed individually and allowed at least seven days to acclimate to the colony room prior to beginning surgery (Experiment 1) or food restriction and behavioral testing (Experiment 2). Water and food (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Laboratories) were available ad libitum during this acclimation period. All rats were ~3 months old at the start of the behavioral training. During the week prior to the behavioral training, rats were handled daily and body weight was gradually reduced to 85% of free-feeding baseline. Food restriction was maintained for all groups until completion of behavioral training, with supplemental rat chow given to each rat after the daily session to maintain the target weight. The colony room was maintained on a 14:10 light:dark cycle and monitored and cared for in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines and the Dartmouth College Institutional Animal Care and Use Committee.

Surgery

All surgeries took place over the course of a three-day period that immediately followed the acclimation period (Experiment 1) or initial behavioral training (Experiment 2). Subjects

were anesthetized with isoflurane gas (1.5-3% in oxygen) and placed in a Kopf stereotaxic apparatus. The skin was retracted and holes were drilled in the skull bilaterally over the intended lesion sites. The lesion target was either the PL or IL region defined by the Paxinos and Watson (2007) rat brain atlas. Electrodes insulated to within 1 mm of the tip were lowered to the following coordinates: for PL, 2.7 and 4.0 mm anterior to bregma at ± 0.7 mm from midline at each site and -4.1 and -3.8 mm from the surface of the skull, respectively; for IL, 2.5 and 3.0 mm anterior to bregma at ± 0.6 mm from the midline and -5.4 mm from the surface of the skull. Electrolytic lesions consisted of 2.5 mA delivered for 10 s at each site. Electrolytic lesions were used in this study to provide control over the timing of damage to the target sites (Ross & Eichenbaum, 2006). In particular, immediate damage was crucial for the post-training evaluation in Experiment 2. Electrolytic lesions also provided more control over the extent of damage, which was an important factor in this study given the close proximity of PL and IL (Ross & Eichenbaum, 2006). Control rats received sham lesions consisting of a craniotomy and shallow, non-puncturing burr holes to minimize damage to underlying cortex. Following the lesions, the wound was sutured and an analgesic was administered. In Experiment 1, surgery was carried out before any behavioral training; in Experiment 2, rats were trained in the task for 16 days before receiving surgery. In both experiments, rats were allowed 10 days to recover and return to baseline weight before beginning food restriction and subsequent behavioral training.

Behavioral Apparatus

Behavioral procedures were carried out in standard conditioning chambers (Med Associates, St. Albans, VT). The chambers ($24 \times 30.5 \times 29$ cm) consisted of aluminum front and back walls, clear acrylic sides and top, and grid floors. Each chamber was outfitted with a dimly illuminated food cup (recessed in the center of the front wall), a 2.8-W white panel light located 5 cm above the opening to the food cup (serving as the visual stimulus), and a speaker located 15 cm above and to the right of the food cup (used to present the 1,500 Hz, 78 dB auditory stimulus). Delivery of two 45-mg food pellets (Bioserv, Frenchtown, NJ) served as the US. Each chamber was equipped with a pair of infrared photocells located across the entrance to the food cup to monitor entries into the cup and connected to a PC-clone computer. Each chamber was enclosed in a sound-attenuating cubicle ($62 \times 56 \times 56$ cm) with an exhaust fan to provide airflow and background noise (~ 68 dB) and a red house-light (mounted on the ceiling) to provide background illumination. The cubicles also contained surveillance cameras used to monitor the rats during behavioral training.

Behavioral Procedures

Each day, rats were placed in plastic transporters and moved from the colony room to the conditioning chambers. One day prior to behavioral training rats were trained to eat from the food cup during a single 64 min session in which two food pellets were randomly delivered 16 times (average intertrial interval (ITI) of 4 min, ranging from 2.5 to 5.5 min). Behavioral training consisted of daily 68 min sessions with 4 reinforced and 12 non-reinforced trials (average ITI of 4 min, ranging from 2.5 to 5.5 min). During reinforced trials, the tone was presented for 10-sec and followed immediately by the delivery of two food pellets. On non-reinforced trials, the panel light and tone were presented simultaneously for 10 sec, after which no food was delivered. The two trial types occurred randomly during each session and

the presentation order was varied daily. In Experiment 1, animals received lesions before any behavioral training. In Experiment 2, rats were lesioned after 16 training sessions and then tested with the same trial and stimulus arrangement for 6 additional sessions.

Lesion Verification

After the behavioral procedures were completed, rats were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline followed by 10% buffered formalin. The brains were removed and stored for at least 24 hours in 10% buffered formalin followed by at least 24 hours in a 20% glycerol solution. Brains were sectioned on a freezing microtome (75 μ m) and Nissl-stained using thionin. The amount of gross tissue damage was assessed using StereoInvestigator software (version 6; Microbrightfield, Inc., Williston, VT) and a compound microscope (Axioskop I, Zeiss, Inc., Thornwood, NY). Areal measurements were obtained using the StereoInvestigator Cavalieri estimator probe with 100 μ m grid spacing at 2.5, 3.0, and 3.7 mm anterior to bregma. Measurements included the total area of the target region and the area of the target region that exhibited gross tissue damage. In addition, the number of sections on which PL or IL damage was present along the rostrocaudal extent of the region was recorded. The number of sections containing damage to regions outside of the target area was also noted. Subjects were removed from the study if the damage to the target site was not bilateral or was two standard deviations below the mean tissue damage for the lesion group.

Data Analysis

The amount of time that the photobeam in front of the food cup was broken during presentation of the tone or the light-tone compound conditioned stimulus (CS), during the 5-sec period immediately preceding any stimulus presentation (the pre-CS period), and during the 5-sec period following food delivery on reinforced trials (the post-CS period) was recorded during each trial. Elevation scores were used as the primary measure of conditioned food-cup behavior and were calculated by subtracting the amount of time spent in the food cup during the pre-CS period from the amount time spent in the food cup during presentation of the CS (e.g., Baxter, Holland & Gallagher, 1997). Elevation scores were adopted because of the variability observed in the baseline amount of food cup behavior exhibited by individual rats. Elevation scores were averaged across rats in each group for each trial type and the difference in responding between trial types (referred to as the discrimination magnitude) was obtained by subtracting the elevation score on non-reinforced trials from the elevation score on reinforced trials. Z-scores were calculated by dividing that result by the standard error of the mean (SEM) of the difference in responding across all rats in a group.

Difference scores served as the primary dependent variable of interest. Successful discrimination between the two trial types was defined as a greater amount of time spent in the food cup during presentation of the tone on reinforced trials than during presentation of the light-food stimulus on non-reinforced trials (a Z-score of at least 2.325; $p < 0.01$; Meyer & Bucci, 2014). In Experiment 2, rats were trained until the learning curves reached asymptote prior to receiving lesions. Changes in the ability to discriminate between trial types after surgery were assessed by calculating the average difference in elevation score

between reinforced and non-reinforced trials before and after surgery. Specifically, an asymptotic performance baseline was calculated by averaging the difference scores of the last two days of training before surgery. This value was compared to the first two-session block of testing after surgery, allowing for an analysis of changes in the rats' ability to discriminate after experimental manipulation. Group differences in discrimination magnitude across training (Experiment 1) and upon reaching asymptotic performance (Experiment 2) were analyzed with a univariate ANOVA. Changes in discrimination magnitude before and after lesioning in Experiment 2 were analyzed with a repeated measures ANOVA.

To test for possible lesion-induced changes in baseline responding, we measured the amount of time spent with the head in the food cup during the 5-sec period immediately prior to the onset of the CS (i.e., pre-CS period). Similarly, potential changes in the motivation to seek food reward were assessed by analyzing the amount of time spent with the head in the food cup during the 5-sec period immediately after food was delivered (i.e., post-CS period). The mean pre-CS and mean post-CS food cup behavior was averaged across sessions and analyzed with univariate ANOVAs. All analyses were conducted using an alpha level of 0.05.

EXPERIMENT 1

Results

Histology—Three PL-lesioned rats and two IL-lesioned rats exhibited damage that was 2 standard deviations lower than the average tissue damage for the lesion group and hence the data from these rats were excluded from further analyses. Three PL-lesioned rats exhibited low food cup responding during the tone (<0.05 sec during the 10-sec stimulus) and were also excluded. Following these exclusions, the remaining sample sizes were 10 rats per lesion group. Representative PL and IL lesions are illustrated in Figure 1. Damage extended throughout the rostro-caudal extent of both PL and IL (Figure 1B). Lesions of PL extended from +2.8 to +5.1 mm relative to bregma with $68 \pm 6\%$ of the target region damaged for each rat. Damage outside PL was limited to the most rostral regions of the medial orbital cortex and minor damage of the cingulate cortex. In IL-lesioned brains, damage extended from +2.6 to +4.0 mm relative to bregma, with $53 \pm 4\%$ of the target region damaged. Damage outside IL included the dorsal peduncular cortex and the most ventral portions of PL. There were no differences in the histology or behavior (see below) of sham PL-lesioned rats or sham-IL lesioned rats, so their data were combined into one control group. In order to maintain comparable sample sizes the data from a randomly selected subset of these rats was used for subsequent analyses ($n = 10$).

Behavior—Rats that received sham PL lesions and rats that received sham IL lesions displayed comparable levels of responding during reinforced trials (3.7 ± 0.6 sec for PL-sham rats, 3.8 ± 0.5 sec for IL-sham rats) and during non-reinforced trials (2.7 ± 0.5 sec for PL-sham rats, 2.9 ± 0.4 sec for IL-sham rats) across the ten training sessions. There were no significant group differences for either reinforced ($p > 0.9$) or non-reinforced ($p > 0.8$) trials; thus, data from these two sets of rats were pooled into a single sham-lesion control group.

The elevation scores are shown in Figure 2. Rats in both the sham-lesioned (top panel) and IL-lesioned (bottom panel) groups began to discriminate between reinforced and non-reinforced trials on session 6 ($Z = 2.54$, $p < 0.01$ and $Z = 2.33$, $p < 0.01$, respectively). In contrast, rats in the PL-lesioned group did not reach the discrimination criterion by the end of 10 sessions (middle panel).

The average difference scores across the ten training sessions for each group are shown in Figure 3. A one-way ANOVA indicated a significant main effect of group [$F(2,27) = 4.6$, $p < 0.05$]. Post-hoc comparisons using the Fisher LSD test revealed that sham-lesioned rats discriminated between trial types to a greater degree than PL-lesioned rats ($p < 0.01$), but did not differ from rats in the IL-lesioned group ($p > 0.1$). There were also no significant differences in discrimination magnitude between IL-lesioned and PL-lesioned rats ($p > 0.2$).

Baseline food cup behavior was considered by analyzing responding during the pre-CS period. The mean amount of time spent in the food cup during the pre-CS period was very low for all three groups (0.5 ± 0.1 sec for sham-lesioned rats, 0.2 ± 0.04 sec for PL-lesioned rats, and 0.6 ± 0.1 sec for IL-lesioned rats). Yet a one-way ANOVA revealed a small, but statistically significant difference between groups [$F(2,27) = 4.7$, $p < 0.05$]. Post-hoc comparisons using the Fisher LSD indicated that pre-CS responding was significantly lower for PL-lesioned rats compared to sham-lesioned rats ($p < 0.05$) or IL-lesioned rats ($p < 0.01$). No differences were observed between sham-lesioned rats and IL-lesioned rats ($p > 0.4$). However, since these differences were very small (on the order of tenths of a second), it is unlikely that they contributed significantly to the differences observed in the elevation scores.

No significant differences were observed in responding during the 5-sec period following the tone (post-CS period) during reinforced trials ($p > 0.1$). The post-CS behavior on reinforced trials was comparably high in all three groups (4.2 ± 0.2 sec for sham-lesioned rats, 4.0 ± 0.1 sec for PL-lesioned rats, and 3.6 ± 0.2 sec for IL-lesioned rats). This suggests that food retrieval behavior was not affected by damage to either region.

EXPERIMENT 2

In this experiment, a new set of rats were trained in the compound feature negative discrimination task for 16 sessions. Previous results have shown that naïve adult rats are able to learn the discrimination between reinforced and non-reinforced trial types in six sessions (Meyer & Bucci, 2014). Thus, rats in this experiment received ten extra sessions in order to allow responding to stabilize and reach asymptotic levels. Rats were then randomly assigned to groups that received sham, PL, or IL lesions and were subsequently exposed to six more days of the discrimination paradigm. In this way, the involvement of PL and IL in the maintenance of learned inhibition could be examined.

Results

Histology—PL lesions extended from +2.5 to +5.3 mm relative to bregma, with minor damage the medial orbital and cingulate cortices. All rats in the PL ($n = 11$) lesion group had bilateral damage, affecting $45 \pm 2\%$ of the target region. Damage was highly similar to that

observed in Experiment 1. One IL-lesioned rat exhibited damage that was 2 standard deviations lower than the average and hence the data from this rat were excluded from further analyses. Following this exclusion, the remaining sample size was 11 rats. Lesions of IL extended from +2.4 to +4.0 mm relative to bregma with $54 \pm 6\%$ of the target region damaged for each rat. IL lesions included minor damage to the dorsal peduncular cortex and ventral portions of PL. As in Experiment 1, the data from sham PL-lesioned rats and sham-IL lesioned rats were combined into one control group, and the data from a randomly selected subset of these rats was used for subsequent analyses ($n = 10$).

Behavior—After surgery, the rats that received sham PL lesions and the rats that received sham IL lesions exhibited comparable levels of responding on reinforced trials (5.2 ± 0.9 sec for PL-sham rats, 4.7 ± 0.5 sec for IL-sham rats) and on non-reinforced trials (2.5 ± 0.7 sec for PL-sham rats, 2.0 ± 0.4 sec for IL-sham rats) across the six testing sessions. There were no significant differences between groups for either reinforced ($p > 0.6$) or non-reinforced ($p > 0.6$) trials, thus their data were subsequently pooled.

Prior to surgery, asymptotic responding during reinforced trials was similarly high for all three groups (5.0 ± 0.6 sec for sham-lesioned rats, 5.1 ± 0.7 sec for PL-lesioned rats and 5.3 ± 0.6 sec for IL-lesioned rats). Responding during the non-reinforced trials was similarly low for all three groups (2.6 ± 0.4 sec for sham-lesioned rats, 2.3 ± 0.6 for PL-lesioned rats and 2.7 ± 0.5 for IL-lesioned rats). A one-way ANOVA did not reveal a difference in asymptotic responding on reinforced ($p > 0.9$) or non-reinforced trials ($p > 0.8$). Furthermore, there was no significant difference between groups in discrimination magnitude during the last two days of training ($ps > 0.9$). The mean discrimination magnitude was 2.4 ± 0.4 sec for sham-lesioned rats, 2.8 ± 0.5 sec for PL-lesioned rats, and 2.6 ± 0.5 sec for IL-lesioned rats. These results indicate that no differences in conditioned behavior existed prior to lesioning.

Because the primary measure of interest was a deviation from asymptotic responding after damage to PL or IL (Figure 4), a repeated-measures ANOVA was performed using the discrimination magnitude for the last block of pre-lesion training and the three two-day blocks of post-lesion testing. This was followed by pair-wise comparisons to test the a priori hypothesis that lesion damage would change the level of responding observed during training compared to testing. The result of the ANOVA was a main effect of Session [$F(3,27) = 4.0$, $p < 0.05$] suggesting that there was a difference in discrimination magnitude in at least one of the blocks. Conversely, there was no main effect of Surgery ($p > 0.8$) or an interaction effect between Session and Surgery ($p > 0.3$). Post hoc contrasts indicated the main effect of Session to be a result of a significantly decreased discrimination magnitude on the first block of testing, compared to the last block of training [$F(1,29) = 9.7$, $p < 0.005$]. Follow-up pairwise comparisons between these two blocks were conducted using Bonferroni adjusted alpha levels of 0.017. The results indicated that IL-lesioned rats exhibited significantly less discrimination between trial types following surgery [$t(10) = 3.0$, $p < 0.02$]. Interestingly, there was also a marginally significant difference in discrimination magnitude in PL-lesioned rats following surgery [$t(10) = 2.2$, $p = 0.05$]. No change in discrimination magnitude was observed for sham-lesioned rats ($p > 0.9$). Because no significant differences were observed between the final block of training and the second and

third blocks of testing, it is likely that although damage impaired performance early in the testing phase, rats recovered the ability to modulate responding in accordance with the trial type.

The mean amount of time spent in the food cup during the pre-CS period was low for all three groups during training (0.7 ± 0.2 sec for sham-lesioned rats, 1.0 ± 0.2 sec for PL-lesioned rats, and 0.6 ± 0.2 sec for IL-lesioned rats) as well as testing (0.6 ± 0.1 sec for sham-lesioned rats, 0.9 ± 0.2 sec for PL-lesioned rats, and 0.5 ± 0.1 sec for IL-lesioned rats). A one-way ANOVA showed no differences in baseline responding between any of the groups during training ($p > 0.3$) or testing ($p > 0.1$).

Finally, the post-CS responding on reinforced trials was comparably high in all three groups during training (4.4 ± 0.2 sec for sham rats, 4.4 ± 0.3 sec for PL-lesioned rats, and 4.8 ± 0.1 sec for IL-lesioned rats) as well as testing (4.1 ± 0.2 sec for sham rats, 4.3 ± 0.3 sec for PL-lesioned rats, and 4.6 ± 0.3 sec for IL-lesioned rats) and did not differ significantly during training ($p > 0.3$) or testing ($p > 0.4$). As in Experiment 1, these results suggest that lesions of PL or IL did not affect food retrieval behavior.

DISCUSSION

The present experiments tested the involvement of PL and IL in the acquisition and maintenance of a compound feature negative discrimination. In Experiment 1, rats received lesions of PL, IL, or sham-lesions prior to training. Within six sessions, sham-lesioned rats learned to approach the food cup in anticipation of receiving a food reward during presentation of the tone alone and to inhibit responding when the tone was presented in compound with the light. In contrast, rats with PL lesions did not learn to discriminate between the two trials types, even after ten training sessions. Furthermore, the magnitude of the difference in responding during reinforced and non-reinforced trials was reduced in PL-lesioned rats compared to those with sham lesions. These findings provide further support for the role of PL in the acquisition of excitatory conditioning (Ashwell & Ito, 2014; Balleine & Dickinson, 1998; Killcross & Coutureau, 2003) and the ability to inhibit a behavior (Ragozzino, 2007; MacLeod & Bucci, 2010; Bari et al., 2011).

Like rats in the sham-lesioned group, rats with IL lesions in Experiment 1 also learned to discriminate between the two types of trials in just six sessions. However, the magnitude of the discrimination observed in IL-lesioned rats did not differ from that of the PL-lesioned rats. Thus, IL-lesions may have had a mild effect on acquisition of the discrimination. If so, this result would differ from prior findings indicating that IL damage did not affect the acquisition of behavioral strategies (Quirk, Russo, Barron, & Lebron, 2000; Chudasama & Robbins, 2003; Boulougouris, Dalley & Robbins, 2007, MacLeod & Bucci, 2010). However, the paradigms used in these studies (extinction, autoshaping, spatial reversal learning, and occasion setting, respectively) differ from the conditioned inhibition procedure used here, suggesting that the acquisition of conditioned inhibitory behavior may differentially recruit the IL compared to other forms of learning or inhibition.

In Experiment 2, rats were over-trained in the discrimination procedure and then received sham surgery or PL- or IL-lesions. After recovering from surgery, rats received six test sessions to determine if PL and IL damage affected performance of the previously-learned inhibition. Damage to IL significantly decreased discrimination between trial types, suggesting that it is involved in the continued expression of inhibitory learning after thorough training. Importantly, lesion-induced changes in discrimination cannot be attributed to a loss of memory resulting from the 12 day lapse in exposure to the task, because sham-lesioned rats showed no differences in discrimination magnitude after this time. These results are consistent with literature suggesting that IL is involved with sustaining (Ragozzino, 2007; Rhodes & Killcross, 2007) and retrieving (Laurent & Westbrook, 2009; Milad & Quirk, 2002; Santini, Quirk, & Porter, 2008) well-trained or habitual inhibitory response patterns.

It is important to note, however, that the effects of IL were transient. This may be due to compensatory activity in alternate brain regions that were involved during training. Indeed, there is evidence that the expression of behavioral inhibition requires fronto-striatal circuitry (Fuster, 1988; Band & van Boxtel, 1999; Aron et al., 2007; Rieger et al., 2003; Rubia et al., 2003; Eagle & Robbins, 2003a,b; van den Wildenberg et al., 2006). In particular, interactions between PL and dorsomedial striatum have been shown to underlie behavioral control (Christakou, Robbins, & Everitt, 2001) and PL or dorsomedial striatal inactivation both lead to perseveration of a previously reinforced response and impaired ability to learn a new rule for obtaining reinforcement (Ragozzino, Ragozzino, Mizumori, & Kesner, 2002). In contrast, IL is linked to the dorsolateral striatum through the central amygdala (Lingawi & Balleine, 2012) and there is evidence that habit formation requires concurrent activity in these two regions (Smith & Graybiel, 2013).

Interestingly, PL damage also produced a marginally significant decrease in the discrimination between trial types in Experiment 2. Further research is needed to conclusively determine the effects of PL-lesions after extensive training, however the results suggest that PL may be involved in both establishing and *maintaining* an inhibitory response pattern. Although this may seem to conflict with prior research that attributes the contributions of PL only to the development and initiation of a behavioral strategy, there is actually very little evidence that conclusively shows that PL is not involved once the strategy has been established. Furthermore, evidence that does exist consists of observations of more perseverative errors than regressive errors following an extradimensional shift, a type of strategy switching requiring the inhibition of a pre-potent response (Ragozzino, Detrick, & Kesner, 1999; Ragozzino, Wilcox, Raso, & Kesner, 1999; Ragozzino et al., 2003; Rich & Shapiro, 2009). In contrast, the present experiments retained the same inhibitory contingency while testing the effects of damage to the PL on maintaining performance levels of an established behavior.

Importantly, the use of the compound feature negative discrimination task in the present experiments extends the literature in two key ways. First, rats must acquire knowledge about an inhibition paradigm with no previous exposure to the context or cues. Conversely, prior research has focused primarily on learning that requires inhibition of a previously-relevant strategy while acquiring a new strategy, based on cues that were previously presented

(Nonneman, Voigt, & Kolb, 1974; Dias et al., 1997; Boulougouris et al., 2007; Ragozzino, 2007; Ashwell & Ito, 2014). Second, rats learn about a cue that signals omission of reinforcement and thus do not approach the food cup. In contrast, previous findings have tested an animal's ability to learn about a cue that signals the *cessation* of a response that has already been initiated (i.e. motor inhibition; Aron et al., 2003, 2007; Eagle et al., 2008; Bari et al., 2011).

To our knowledge, few studies have examined the involvement of prefrontal regions in learning to omit a behavior when signaled by a cue in the environment. MacLeod and Bucci (2010) determined that PL but not IL is required for learning a negative occasion setting task (serial feature negative discrimination), and that neither region is required once the rat reaches asymptotic performance levels. As with the present procedure, serial feature negative discrimination requires learning about the presence of a feature cue that indicates the non-reinforcement of a target cue. However, in contrast to the task used here, the feature is presented prior to the target and the two cues are separated by a feature-target interval of five seconds. Importantly, it has been shown that the temporal relationship of the feature and target cues can critically influence what is learned (Holland, 1992). With the negative occasion setting procedure it has been suggested that the target CS acquires both excitatory and inhibitory associations with the US. Importantly, the negative feature does not act upon the US itself, but instead modulates or “gates” the inhibitory association between the target and the US (Bouton & Nelson, 1994). In contrast, when the feature is presented in simultaneous compound with the target (present study), the feature acquires a direct inhibitory association with the US, and the learning can be described as “conditioned inhibition” (Rescorla & Holland, 1977). Thus, the present results expand on Macleod and Bucci's findings to indicate a role for PL in the acquisition of conditioned inhibition as well as negative occasion setting. This might suggest that PL is critical to the formation of an inhibitory association between the feature stimulus and a US as well as a modulatory relationship between the feature stimulus and the target-US association. In addition, these results suggest that IL may be specifically involved in learning about the inhibitory feature-US association but *not* the gating properties of the feature.

Notably, the results of Experiment 2 differ from those of MacLeod and Bucci (2010), which suggested no effect of either type of lesion on withholding responding after thorough training. A potential explanation for this difference is that negative occasion setting includes a working memory component. Indeed, rats must maintain the meaning of the light over the inter-stimulus interval to apply that meaning to the tone. Thus, after thorough training the inhibitory behavior may be under the control of an alternate region, such as the hippocampus. Indeed, the hippocampus is required for learning a serial feature negative discrimination (Holland, Lamoureux, Han, & Gallagher, 1999) but is not required for learning a compound feature negative discrimination (Chan, Jarrard, & Davidson, 2003). Alternatively, the different findings could reflect use of a more sensitive statistical measure in the present study. Indeed, MacLeod & Bucci (2010) used a 7-session average after lesioning instead of the 2-session blocks used here.

Previous research using compound feature negative discriminations suggests that the contingency results in a classically testable form of inhibition, in which the feature can delay

excitatory conditioning of a novel cue (retardation) and suppress responding to another excitatory CS when introduced into a compound with that CS (summation) (*cf.* Holland, 1992). Although we did not directly test the effects of PL or IL damage on these inhibitory properties of the feature, it is unlikely that the present results were due to cue-generalization, or working memory deficits. For example, rats with either lesion were eventually able to discriminate between trial types in Experiment 2, suggesting that the damage to PL or IL was not simply causing rats to generalize between the two stimuli. This is consistent with prior research suggesting that the acquisition of a conditional discrimination is not influenced by damage to regions of medial PFC (Chudasama, Bussey, & Muir, 2001; Delatour & Gisquet-Verrier, 1999; Boulougouris et al., 2007). Conditional discriminations generally include trials in which the stimuli to be discriminated are presented simultaneously and a response to one stimulus by default results in omission of a response to the other stimulus. Conversely, in the compound feature negative discrimination, rats must withhold responding to a solitary stimulus and learn that a subset of trials will not be reinforced, regardless of the response pattern displayed. Thus, the decrease in discrimination magnitude observed in Experiment 2 likely reflects a deficit in the ability to express the inhibition, rather than the ability to discriminate between trial types.

In summary, the present results support a distinction between the roles of PL and IL in learned inhibition. Specifically, PL is critical for the acquisition of a Pavlovian inhibitory behavior and has less of a role in maintaining the behavior, while IL is required more for maintaining the performance of learned behavioral omission and has less of a role in acquiring inhibitory behavior. Learning to reduce responding when a feature in the environment signals an absence of reward can conserve energy or attentional resources when not required. Furthermore, this learning can prepare the animal to allocate such resources elsewhere, thus, it is a key aspect of adaptive behavior. Finally, the compound feature negative discrimination task used here has a similar structure to the go/no-go and stop-signal tasks, which are commonly used to assess inhibition in patients with schizophrenia and ADHD (Potter & Newhouse, 2008; Rubia et al., 2001; Vaidya et al., 1998). Thus, identifying the neural substrates of inhibitory behavior has important implications for understanding and treating these and other disorders in which the ability suppress inappropriate thoughts and actions is impaired.

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A **B** **Figure 1.**

(A) Photomicrographs of representative prelimbic (left) and infralimbic (right) damage at 3.7 mm and 3.0 mm anterior to bregma (respectively). (B) Schematic diagrams indicating the rostro-caudal extent and the largest (gray) and smallest (black) lesions of prelimbic (left) and infralimbic (right). Abbreviations: Cg1, cingulate cortex area 1; DP, dorsal peduncular cortex; fmi, forceps minor corpus callosum; IL, infralimbic cortex; PL, prelimbic cortex.

Figure 1B is adapted from *The Rat Brain in Stereotaxic Coordinates* (6th ed.), plates 8,9,11, & 13, by G. Paxinos and C. Watson, 2007, New York, NY: Academic Press. Copyright 2007 by Elsevier Academic Press. Adapted with permission.

Figure 2.

Damage to prelimbic cortex impaired conditioned responding to the tone and the tone/light compound CS in Experiment 1. Data are mean amount of time spent in food cup during the tone minus food cup behavior during the pre-CS period. **(A)** Sham-lesioned rats discriminated between trial types by session 6 ($Z = 2.54$, $p < 0.01$). **(B)** Prelimbic-lesioned rats did not discriminate between trial types by the end of 10 sessions. **(C)** Infralimbic-lesioned rats discriminated between trial types by session 6 ($Z = 2.33$, $p < 0.01$). Abbreviations: PL, prelimbic cortex; IL, infralimbic cortex. Data are mean $\pm$ SEM.

Figure 3.

Damage to prelimbic cortex significantly decreased discrimination magnitude. Data are the average difference between mean amount of time spent in food cup during the tone minus food cup behavior during the pre-CS period on reinforced and non-reinforced trials over the ten sessions of compound feature negative discrimination in Experiment 1. Sham-lesioned rats discriminated between trial types to a greater extent than prelimbic-lesioned rats but did not differ from rats in the infralimbic-lesioned group. Infralimbic-lesioned and sham-lesioned rats exhibited similar amounts of conditioned food cup behavior. Abbreviations: PL, prelimbic cortex; IL, infralimbic cortex. ** $p < 0.01$.

Figure 4.

Damage to infralimbic cortex significantly decreased discrimination magnitude during the first block of testing, compared to the last block of training. Damage to PL resulted in a marginally significant difference in discrimination magnitude. No change in discrimination magnitude was observed for sham-lesioned rats. Data are the average difference between elevations cores on reinforced and non-reinforced trials during presentation of the tone over the last two days of training and the three two-session blocks of testing in Experiment 2. Data are $\pm$ SEM. * $p < 0.05$, $^{\dagger}p < 0.05$ on a Bonferroni corrected p-value of 0.017.