Perirhinal Damage Produces Modality-Dependent Deficits in Fear Learning

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Abstract

The perirhinal cortex (PER) receives multimodal and unimodal sensory information from all modalities. In addition, the PER is anatomically connected with several brain regions that support fear learning. Several studies suggest that the PER is involved in fear conditioning to discontinuous auditory cues but not to continuous auditory cues. To date, studies examining the role of the PER in fear conditioning has largely focused on auditory and contextual stimuli. The present study assessed whether the role of the PER in fear conditioning would extend to visual modalities. Rodents were randomly assigned to one of four conditioned stimuli, which consisted of either a tone or a light stimulus that was either continuous or discontinuous. Pre-training excitotoxic lesions to the PER significantly reduced freezing to auditory and visual cues during the acquisition phase regardless of stimulus continuity. During subsequent testing, perirhinal lesions produced significant decreases in freezing levels to both continuous and discontinuous tones but not to either of the light CS groups. These results suggest that the PER is involved in the acquisition of fear across multiple cue modalities. However, the PER may have a more limited role in the retrieval of the fear memory dependent upon the cue modality.

Keywords: fear conditioning, emotional learning, stimulus unitization

1. Introduction

Appropriate defensive behaviors to environmental stimuli are evolutionarily important for survival. Acquired fear responses, which are among the most studied defensive behaviors, typically are examined using an associative learning paradigm, called Pavlovian Fear Conditioning. During fear conditioning a neutral stimulus (conditioned stimulus, CS) is presented with an aversive stimulus (unconditioned stimulus, US). Subsequent to paired presentations the CS elicits a conditioned response (CR) even without the presence of the US. Decades of research employing this paradigm suggest congruency between rodent and human behavior as well as largely conserved biological routes among species (LeDoux, 2000; Milad & Quirk, 2012).

The brain structures that most prominently underly fear learning consist of three interconnected brain regions: the amygdala (AM), the hippocampal formation (HF), and the medial prefrontal cortex (mPFC). The AM has been shown to be involved in all aspects of fear learning (acquisition, consolidation, and expression; Asok et al., 2018; Kapp, Whalen, Supple, & Pascoe, 1992; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990). The HF has been shown to be important in the contextual modulation of fear learning during acquisition, consolidation and extinction (Anagnostaras, Gale, & Fanselow, 2001; Canteras & Swanson, 1992; Maren & Fanselow, 1995). The two divisions of the mPFC, the prelimbic cortex and the infralimbic cortex, have been shown to be differentially involved in fear expression and fear extinction, respectively (Giustino & Maren, 2015; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011).

In recent years, additional brain structures have been implicated in fear learning in a cue-dependent manor. For example, several studies have suggested the perirhinal cortex (PER) is necessary for several types of fear learning. Lesions of the PER have produced deficits in fear

conditioning to pre-recorded rat ultrasonic vocalization CSs and contexts (Bang & Brown, 2009; Bucci, Phillips, & Burwell, 2000; Bucci, Saddoris, & Burwell, 2002; Kent & Brown, 2012; Kholodar-Smith, Allen, & Brown, 2008; Lindquist, Jarrard, & Brown, 2004). The PER also has been implicated in fear learning to discontinuous, but not continuous, tone CSs (Bang & Brown, 2009; Kholodar-Smith, Allen, et al., 2008; Lindquist et al., 2004). This cue-specific role of the PER has recently been extended to include involvement in the retrieval of the fear extinction memory to a discontinuous light CS (Potter, Calub, & Furtak, 2020). To date, the role of the PER in fear acquisition has been mostly limited to auditory cues and contexts. Although, there have been a number of studies that have explored its role in fear learning to olfactory stimuli (see Herzog & Otto, 1997, 1998; Otto & Giardino, 2001) and more recently in taste neophobia (see Ramos, 2020a, 2020b).

Here, we examine whether the PER contributes more broadly to fear learning by conditioning rodents to either a visual (light) CS or an auditory (tone) CS. Based on previous research suggesting the continuity of the cue is an essential characteristic that engages the PER in fear learning, the CSs were presented either as a continuous or discontinuous stimulus. Thus, there were a total of four CS groups: continuous tone CS, discontinuous tone CS, continuous light CS, and discontinuous light CS. We predicted that animals with damage to the PER would have impaired cue-elicited freezing compared to control animals when the CS was discontinuous regardless of the modality of the CS. Surprisingly, results contradicted our predicted outcome.

2. Material and Methods

2.1 Subjects

Seventy male Sprague Dawley Derived rats (Simonsen Laboratories, Gilroy, CA; 290-400g) were maintained on a 16/8 light/dark schedule and were provided food and water ad

libitum. Subjects were pair-housed prior to surgery, then single-housed. Animals were handled before and following surgery. All procedures are in accordance with the National institutes of Health guide for the care and use of Laboratory animals, as well as being approved by the Institutional Animal Care and Use Committee (IACUC) at the California State University Sacramento.

2.2 Surgery

Subjects were anesthetized (Acetylpromazine 1mg/kg, Xylazine 6mg/kg, and Ketamine 100mg/kg i.p) and secured in a stereotaxic apparatus. Under aseptic conditions, the dorsal surface of the skull was exposed. Bore holes were drilled above PER target coordinates (A/P: -3.2 mm, -4.5 mm, -6.0 mm; M/L: +/- 4.8 mm 14 degrees in the lateral direction; D/V: - 6.5 mm, relative to bregma; Furtak, Wei, Agster, & Burwell, 2007). At each site a microinjector (Stoelting; Wood Dale, IL) infused 0.19 μL (0.1 μL/min) of either 0.1M phosphate buffered saline (PBS; Sham groups) or N-methyl-D-aspartate (NMDA; 250 μM NMDA in 0.1 M PBS; Lesion groups). The incision site was sutured, Neosporin® was applied, and post-operative analgesic was administered (Ketoprofen 2-3mg/kg, s.c.).

2.3 Behavior

A Pavlovian Fear Conditioning paradigm consisted of three phases (Fear Acquisition, Cue Test, and Context Test) that occurred over consecutive days (Fig. 1A). Stimuli were presented using a Med Associates control box (see Calub, Furtak, & Brown, 2018). Fear Acquisition and the Context Test occurred in Chamber A, while the Cue Test occurred in Chamber B. Chambers differed in wall texture, floor structure, odor, and room lighting. Chambers were equipped at the top with a camera (CB21; Circuit Specialists; Tempe, AZ) that recorded behavior for offline analysis. On Day 1, Fear Acquisition, animals were placed in

Chamber A and after a 2-min baseline received 5 CS-US paired presentations (ITI = 120 sec ± 20 sec, Fig. 1A). One of four CSs were used depending upon CS group: continuous tone (c-Tone groups: 10 kHz; 7.778 sec), discontinuous tone (d-Tone groups; 10 kHz, 7.778 sec, ISI = 132 msec), continuous light (c-Light groups; 50 lux; 9.7 sec), or discontinuous light (d-Light groups; 50 lux; 9.7 sec, ISI = 300 msec). Auditory cues were generated by a Coulbourn Tone/Noise Generator (Coulbourn Instruments; Whitehall, PA). The US was a foot shock (0.8 mA, 0.5 sec; Precision Shock Generator, Coulbourn Instruments; Whitehall, PA) that co-terminated with the CS. On Day 2 and Day 3, in counterbalanced order, the Cue Test and Context Test occurred (Fig. 1A). The Cue Test involved a 2-min baseline followed by a 6-min CS alone presentation. Animals were removed from the chamber 2 min after the CS presentation terminated. In the Context Test, animals were returned to the conditioning context (Chamber A) for 10 min without CS or US presentations.

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2.4 Histology

Upon experimental completion, subjects were euthanized (Beuthanasia®, 100 mg/kg, i.p; Intervet Inc.; Madison, NJ) and transcardiacally perfused using 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin and transferred to 30% sucrose for cryoprotection for at least three days before coronal sections were obtained using a freezing microtome (American Optical Company; Buffalo, NY). Sections were mounted on subbed slides and stained for Nissl. Lesion quantification was conducted at three locations along the rostro-caudal extent of the PER (Paxinos & Watson, 2005; see Fig. 1B) similar to previous experiments (for review see; Bucci et al., 2000; Bucci et al., 2002; Corodimas & LeDoux, 1995). Lesion reconstructions were digitized and quantified for percentage of damage using ImageJ (Rasband, 2012).

2.5 Data Analysis

An automated computer program was used to calculate freezing offline (FreezR, for details see Calub et al., 2018; Potter et al., 2020). Due to the flashing nature of the discontinuous light during the Cue Test, the software overestimated the amount of movement during this phase. Therefore, Cue Test data from all groups were hand scored by researchers who were blind to the experimental condition for this portion of the experiment. In order to assess accuracy and coherence, a subset of Fear Acquisition data was also hand scored by researchers. Correlations between researchers and the software were strong during Fear Acquisition, $r(88) = 0.92$, $p < 0.001$. Group differences were analyzed using SPSS version 27 (IBM, Corporation; Armonk, NY). Descriptive statistics were reported as the mean ± 1 standard error of the mean.

3. RESULTS

3.1 Histological Quantification

A total of 70 subjects were used in this study. Four subjects were excluded from behavioral analysis due to amygdala damage. For the Tone CS groups, the total number of animals by group was c-Tone (Sham, n = 9, and Lesion, n = 8) and d-Tone (Sham, n = 9, and Lesion, n = 8). For the Light CS groups the total number by group was c-Light (Sham, n = 8, and Lesion, n = 8) and d-Light (Sham, n = 8, and Lesion, n = 8). Lesions were quantified as the percentage of damage across three coronal sections of the PER (Fig. 1B). The percentage of total damage to the PER by group were c-Tone Lesion, 76 ± 5%, d-Tone Lesion, 74 ± 6%, c-Light Lesion, 73 ± 5%, and d-Light Lesion, 66 ± 8%. Importantly, there were no significant group differences in lesion size, $F(3, 28) = 0.41$, $p = 0.75$. Seven additional regions were quantified and again no differences were observed among groups (results not reported).

3.2 Modality-Dependent Differences in Sham Animals

Prior to analyzing the effects of perirhinal lesions on fear learning, we first examined whether there were any differences in freezing levels during acquisition or testing that may indicate an inherent difference in cue salience. Only Sham animals were included in this analysis. A two-way ANOVA examined the effect of Modality (Tone vs Light) and Continuity (Continuous vs Discontinuous) of the cue on freezing levels during Fear Acquisition. Results show no main effect of Modality, $F(1, 30) = 1.07$, $p = 0.31$, or Continuity, $F(1, 30) = 0.21$, $p = 0.65$, in the freezing levels during Fear Acquisition. In addition, there was no interaction between these factors, $F(1, 30) = 0.46$, $p = 0.50$. Freezing levels during CS-US presentations for Sham groups were as follows: c-Tone (76% ± 3%), d-Tone (71% ± 5%), c-Light (68% ± 5%), and d-Light (69% ± 3). Results show no significant differences in acquisition based on the modality or continuity of the cue.

A second two-way ANOVA examined these same two factors on freezing levels during the Cue Test. There was a significant main effect of Modality, $F(1, 30) = 12.31$, $p < 0.01$. However, there were no significant difference based on whether the cue was continuous or discontinuous, $F(1, 30) = 3.85$, $p = 0.06$, or interactions between these factors, $F(1, 30) = 2.78$, $p = 0.11$. Freezing levels during the Cue Test for Sham groups were as follows: c-Tone (94% ± 2%), d-Tone (95% ± 2%), c-Light (65% ± 11%), and d-Light (85% ± 4). These results suggest that there may be differences in the salience of tone compared to light cues with tone CS groups freezing at higher levels during cue retrieval than light CS groups. Due to these differences in cue-elicited freezing, groups conditioned to tone cues were analyzed separately from those conditioned to light cues.

3.3 Fear Conditioning to Continuous and Discontinuous Tone Cues

During the baseline period in Fear Acquisition, freezing levels ranged from 1% - 3% among the continuous and discontinuous Tone groups (see Fig. 2A, left panel). A two-way ANOVA compared freezing levels during CS-US presentations as a factor of Surgery (Sham vs Lesion) and Continuity (Continuous vs Discontinuous). Results indicated a significant difference in freezing levels between Sham and Lesion groups, $F(1,30) = 22.51$, $p < 0.001$ (Fig. 2A, left panel). Unexpectedly, there was no significant main effect of Continuity, $F(1,30) = 0.00$, $p = 0.99$, and no interaction between these factors, $F(1,30) = 0.87$, $p = 0.36$. Freezing levels during the CS-US presentations were as follows: c-Tone Sham (76% ± 3%), c-Tone Lesion (46% ± 6%), d-Tone Sham (71% ± 5%), and d-Tone Lesion (51% ± 6%; see Fig. 2A, left panel). Damage to the PER significantly impaired freezing to tones during acquisition regardless of whether the tone was discontinuous or continuous.

-- Insert Figure 2 here –

Over the two subsequent days, animals underwent a Cue Test and a Context Test in counterbalanced order. During the Cue Test, baseline freezing levels in the tone CSs groups ranged from 23% - 38% (Fig. 2A, middle panel, dashed line). A two-way ANOVA examined group differences in generalized freezing during the baseline period. Results indicated no significant difference between Sham and Lesion groups, $F(1,30) = 0.33$, $p = 0.57$, no significant differences based whether the cue was continuous or discontinuous, $F(1,30) = 0.94$, $p = 0.34$, and no interaction between these factors, $F(1,30) = 0.15$, $p = 0.67$. An additional two-way ANOVA assessed freezing levels during the 6-min CS presentation as a factor of Surgery and Continuity. Cue-elicited freezing levels significant differed between the Sham and Lesion groups, $F(1,30) = 6.99$, $p < 0.05$ (Fig. 2A, middle panel). However, there was no significant difference in freezing between the discontinuous and continuous tones groups, $F(1,30) = 0.15$, $p = 0.70$, and no

significant interaction of these factors, $F(1,30) = 0.01$, $p = 0.93$. Freezing levels for each group during the 6-min cue presentation were c-Tone Sham (94% ± 2%), c-Tone Lesion (78% ± 10%), d-Tone Sham (95% ± 2%), and d-Tone Lesion (81% ± 6%; see Fig. 2A, middle panel). In contrast to our prediction, damage to the PER impaired freezing to the cue in both the continuous tone CS and discontinuous tone CS groups.

Animals were placed back into the conditioning context for a 10-minute period without any CS or US presentations during the Context Test. Average freezing levels to the context were c-Tone Sham (58% ± 10%), c-Tone Lesion (44% ± 9%), d-Tone Sham (60% ± 9%), and d-Tone Lesion (53% ± 11%; see Fig. 2A, right panel). A two-way ANOVA compared freezing levels based on surgical group and the continuity of the cue. No significant difference was found based on whether the of the PER was lesioned, $F(1,30) = 1.17$, $p = 0.29$, or based on the characteristic of the CS during acquisition, $F(1,30) = 0.37$, $p = 0.55$. Furthermore, no significant interaction of these factors was found, $F(1,30) = 0.17$, $p = 0.68$. Unlike previous reports, here damage to the PER did not impair fear conditioning to the context.

3.4 Fear Conditioning to Continuous and Discontinuous Light Cues

Prior to CS-US presentations, baseline levels of freezing in all groups were very low, ranging from 1% - 3% (Fig. 2B, left panel). Freezing levels were assessed during Fear Acquisition as a factor of Surgery and Continuity for all light CS groups. Freezing levels were found to differ significantly between Sham and Lesion groups during CS-US presentations, $F(1,28) = 17.01$, $p < 0.001$ (Fig. 2B, left panel). There was no significant difference in freezing levels between continuous and discontinuous light groups, $F(1,28) = 2.14$, $p = 0.16$, and no significant interaction, $F(1,28) = 1.63$, $p = 0.21$. Average freezing levels during acquisition were as follows: c-Light Sham (68% ± 5%), c-Light Lesion (42% ± 4%), d-Light Sham (69% ± 3%),

and d-Light Lesion (55% ± 7%; see Fig. 2A, left panel). Similar to the tone CS, these findings indicate that lesions of the PER reduced freezing levels in animals conditioned to light CS regardless of continuity of the light.

During the 2-min baseline period in the Cue Test, freezing levels ranged from 31% - 37% among groups (Fig. 2B, middle panel, dashed line). There were no significant differences in freezing levels during the baseline period between Sham and Lesion groups, $F(1,28) = 0.00$, $p = 0.98$, or based on whether the light CS was continuous or discontinuous, $F(1,28) = 0.01$, $p = 0.92$. Additionally, there was no significant interaction between these factors, $F(1,28) = 0.20$, $p = 0.66$. A two-way ANOVA assessed freezing levels during the 6-min CS presentation period based on Surgery and Continuity. Interestingly, there was no significant differences in cue-elicited freezing levels between the Sham and Lesion groups, $F(1,28) = 1.32$, $p = 0.26$. The freezing levels did significantly differ, however, between on the continuous and discontinuous light CS groups, $F(1,28) = 4.30$, $p < 0.05$. No interaction was detected between these factors, $F(1,28) = 0.00$, $p = 0.98$. The average cue-elicited freezing levels by group were c-Light Sham (65% ± 11%), c-Light Lesion (54% ± 12%), d-Light Sham (85% ± 4%), and d-Light Lesion (74% ± 9%; see Fig. 2A, middle panel). Overall, these results suggest that the differences in freezing level where not dependent upon lesions to the PER. In contrast, differences relied on the continuity characteristics of the light CS.

During the Context Test, the average freezing levels by group to the conditioning context were as follows: c-Light Sham (48% ± 9%), c-Light Lesion (58% ± 10%), d-Light Sham (61% ± 9%), and d-Light Lesion (59% ± 8%; see Fig. 2A, right panel). Freezing levels were compared between surgical groups and cue continuity. No significant main effects (Surgery, $F(1,28) = 0.21$, $p = 0.66$, and Continuity, $F(1,28) = 0.59$, $p = 0.45$) or interaction effect, $F(1,28) = 0.46$, $p =$

0.50, were found. Animals froze at similar rates to the conditioning context regardless of group assignment.

3.5 Lesion Size as a Determinate of Freezing Level

A series of correlation analyses were conducted assessing the relationship between the percentage of damage to the PER and freezing behavior during acquisition and testing. During Fear Acquisition, there were no significant correlations between the size of the lesion to the PER and the average amount of freezing during CS-US presentations for any CS group. Correlations were c-Tone Lesion group, $r(6) = -0.33$, $p = 0.43$, d-Tone Lesion group, $r(6) = 0.35$, $p = 0.39$, c-Light Lesion group, $r(6) = -0.36$, $p = 0.39$, and the d-Light Lesion group, $r(6) = -0.46$, $p = 0.26$. Correlations were also conducted on the size of the lesion and each minute of acquisition, which also resulted in no significant correlations (results not reported).

The relationship between the percentage of damage to the PER and cue-elicited freezing during the Cue Test was then examined. Again, there were no significant correlations between the size of the lesion and the average freezing level over the 6-min cue presentation. Correlations were as follows: c-Tone Lesion group, $r(6) = -0.003$, $p = 0.99$, d-Tone Lesion group, $r(6) = -0.41$, $p = 0.31$, c-Light Lesion group, $r(6) = -0.25$, $p = 0.54$, and d-Light Lesion group, $r(6) = -0.56$, $p = 0.15$. Correlations were also conducted on the size of the lesion and each minute of cue presentation. These additional analyzes resulted in a significant correlation in the d-Light Lesion group during the first minute of cue presentation, $r(6) = -0.78$, $p < 0.05$. This negative correlation suggests that larger amounts of damage to the PER related to lower freezing levels during the Cue Test specifically in animals conditioned to the discontinuous light CS.

DISCUSSION

Previous research has suggested that the PER is involved in fear learning to discontinuous auditory cues and contexts. Here, we examined whether the PER contributes more broadly to fear learning by conditioning rodents to either a visual or an auditory CS. Results suggest that the PER is involved in the acquisition of fear regardless of cue modality. Interestingly, in the majority of the literature investigating perirhinal involvement in fear learning, freezing levels during the acquisition phase is not commonly reported. Out of 12 studies examining the PER in fear learning, nine did not report results during the acquisition phase (Bang & Brown, 2009; Herzog & Otto, 1997, 1998; Iordanova, Burnett, Aggleton, Good, & Honey, 2009; Kholodar-Smith, Allen, et al., 2008; Kholodar-Smith, Boguszewski, & Brown, 2008; Lindquist et al., 2004; Otto & Giardino, 2001; Romanski & LeDoux, 1992). Three studies only reported freezing levels during the first presentation of the CS during the acquisition phase, reporting no differences between sham and lesioned animals (Bucci et al., 2000; Bucci et al., 2002; Phillips & LeDoux, 1995). The current results suggest that there should be greater emphasis on analyzing the acquisition phase as well as the test phase and may point to a perceptual function of the PER in fear learning.

Previous studies have determined that temporal discontinuity was necessary to engage the PER in fear learning to a tone cue (Kholodar-Smith, Allen, et al., 2008). This requirement of temporal discontinuity for perirhinal engagement in fear learning led to the "stimulus unitization" hypothesis proposed by Brown and colleagues (Kent & Brown, 2012). In the present study, continuity of the cue was manipulated to discern if the previous pattern of perirhinal involvement in discontinuous cues would be observed with a visual cue. Results did not support the predictions of the stimulus unitization hypothesis, finding that perirhinal lesions produced deficits in both the continuous and discontinuous light groups during acquisition while not

impacting subsequent retrieval during the cue testing. A similar outcome was observed in one study examining the PER in second order conditioning. When the PER was inactivated prior to CS-US presentations using a flashing light CS no impairment in freezing levels occurred during the light CS test (Wong, Westbrook, & Holmes, 2019). While this may aid in the interpretation of current results, second order conditioning paradigms vary in several ways from the current study, making the comparison challenging. Additional studies are needed to understand if modality is an important factor in the role of the PER in fear learning.

Overall freezing levels were reduced in tone groups for both acquisition and subsequent cue testing regardless of cue continuity. This pattern of results was not predicted considering the substantial evidence that the PER serves to unitize discontinuous auditory stimuli (Bang & Brown, 2009; Kent & Brown, 2012; Kholodar-Smith, Allen, et al., 2008; Lindquist et al., 2004). Given these contradictory findings, it is prudent to note that the size of the lesion may hold some explanation for this difference in findings. While the size of the lesions to the PER in the current experiment were similar to previous published studies (for review see; Aggleton, Keen, Warburton, & Bussey, 1997; Heimer-McGinn, Poeta, Aghi, Udawatta, & Burwell, 2017), there was damage to immediately adjacent anatomical brain regions. In particular, there was damage to the ventral portion of TE and the lateral area of the EC. This damage is partially due to the methodological technique of a dorsal surgical approach as compared to a lateral approach, which has previously been used by Brown and colleagues (Furtak, Allen, & Brown, 2007; Kholodar-Smith, Allen, et al., 2008). While the lateral approach does lead to more refined location of the lesion, other more modern neural silencing techniques could be utilized to more accurately identify the location of the affected cells.

In conclusion, the PER appears to play a significant role in the acquisition of the fear memory regardless of the CS modality. One possibility is that the PER works in conjunction with task-specific structures to supply and to update the representation of stimuli, including important attributes of the stimulus such as emotional or motivational relevance. Additional studies examining the contribution of the PER will further elucidate how and when this region is engaged in fear learning.

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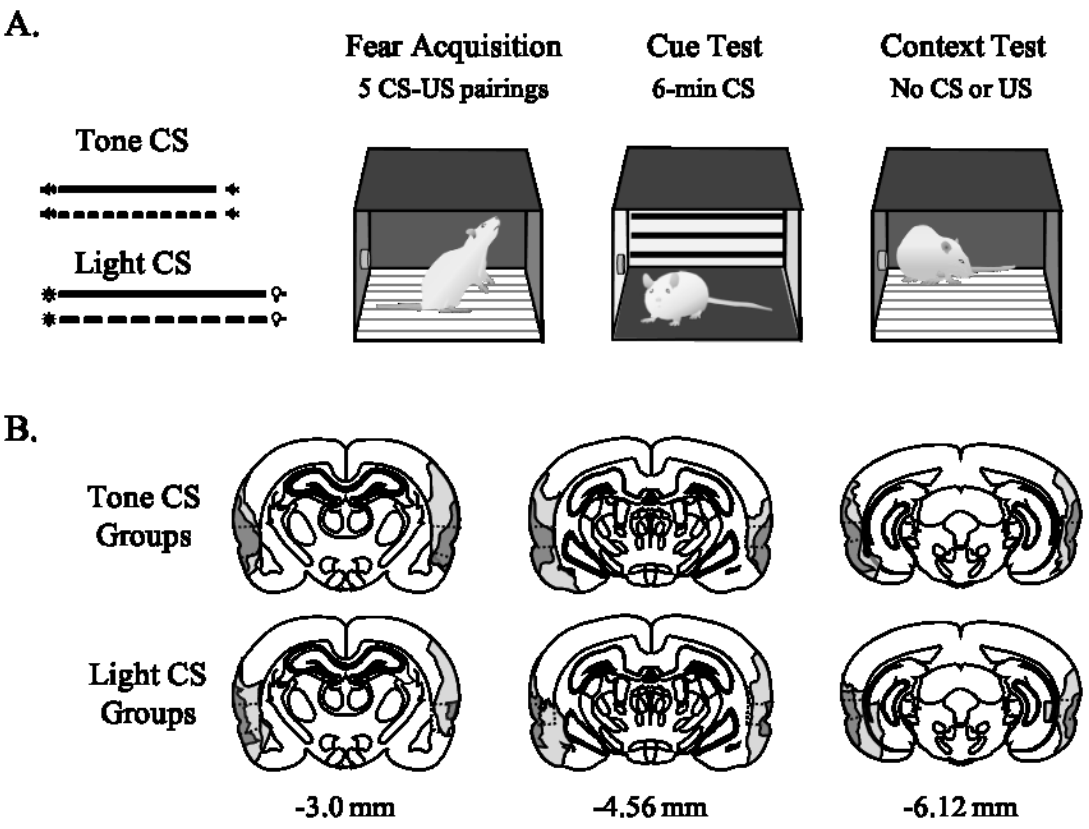

Figure 1. **Overview of the Experimental Design and Lesion Location.** A) Animals were fear conditioned to one of four conditioned stimuli (CSs) that varied based on continuity (continuous or discontinuous) and modality (10 kHz tone or white light, depicted on the left panel). The unconditioned stimulus (US) was a brief foot shock for all groups. The conditioning paradigm included acquisition followed by two test session that evaluated associative learning to the cue and the context. Test sessions occurred in counterbalanced order. B) Histological reconstructions of lesions were drawn at three rostro-caudal levels relative bregma. Lesion were quantified as the percentage of damage to the perirhinal cortex as well as to surrounding regions. The dashed lines denote the boundaries of the perirhinal cortex. Note, light and dark gray shading indicates the largest and the smallest lesion, respectively.

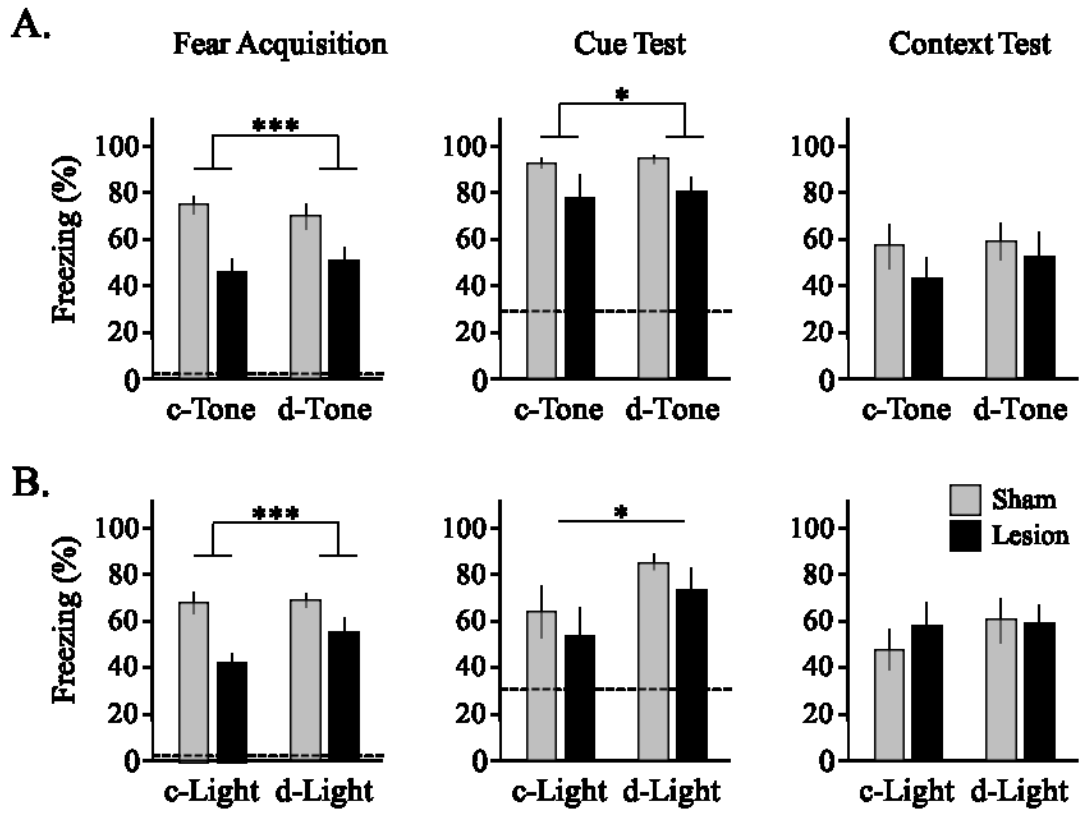

Figure 2. Lesions to the perirhinal cortex impair fear learning to tone and light cues. Freezing levels are graphed over a three-day fear conditioning paradigm consisting of Fear Acquisition, Cue Test and Context Test. A) Fear conditioning to tone cues. During acquisition, freezing levels were significantly reduced in animals with perirhinal lesions (black bars) compared to sham controls (gray bars) in the continuous tone (c-Tone) and a discontinuous tone (d-Tone) groups (left panel). Cue-elicited freezing during the test was attenuated in the lesion groups regardless of the continuity of the tone cue (middle panel). No group differences were detected in freezing levels during the Context Test (right panel). B) Fear conditioning to light cues. Lesions to the perirhinal cortex significantly impaired acquisition in both the continuous light (c-Light) and discontinuous light (d-Light) groups (left panel). During the Cue Test, freezing levels differed based on the continuity of the cue with significantly less freezing in the

continuous compared to the discontinuous light groups (middle panel). However, there were no differences between lesion and sham groups in freezing levels to the cue in either group (middle panel). All groups froze at comparable levels to the conditioning context (right panel). Dashed lines represented the average baseline freezing levels among groups. Data is presented as mean ± 1 SEM. Note, *, $p<.05$, **, $p<0.01$, ‡, $p<0.001$.