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Auditory trace fear conditioning requires perirhinal cortex

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ABSTRACT

The hippocampus is well-known to be critical for trace fear conditioning, but nothing is known about the importance of perirhinal cortex (PR), which has reciprocal connections with hippocampus. PR damage severely impairs *delay* fear conditioning to ultrasonic vocalizations (USVs) and discontinuous tones (pips), but has no effect on delay conditioning to continuous tones. Here we demonstrate that *trace* auditory fear conditioning also critically depends on PR function. The trace interval between the CS offset and the US onset was 16 s. Pre-training neurotoxic lesions were produced through multiple injections of *N*-methyl-D-aspartate along the full length of PR, which was directly visualized during the injections. Control animals received injections with phosphate-buffered saline. Three-dimensional reconstructions of the lesion volumes demonstrated that the neurotoxic damage was well-localized to PR and included most of its anterior–posterior extent. Automated video analysis quantified freezing behavior, which served as the conditional response. PR-damaged rats were profoundly impaired in trace conditioning to either of three different CSs (a USV, tone pips, and a continuous tone) as well as conditioning to the training context. Within both the lesion and control groups, the type of cue had no effect on the mean CR. The overall PR lesion effect size was 2.7 for cue conditioning and 3.9 for context conditioning. We suggest that the role of PR in trace fear conditioning may be distinct from some of its more perceptual functions. The results further define the essential circuitry underlying trace fear conditioning to auditory cues.

1. Introduction

Several lines of evidence suggest that perirhinal cortex (PR) functions to bind separate stimulus elements together into unitary representations (Barense et al., 2005; Buffalo, Bellgowan, & Martin, 2006; Graham et al., 2006; Kholodar-Smith, Allen, & Brown, in press; Lee, Barense, & Graham, 2005; Murray, Bussey, & Saksida, 2007). First, PR damage severely impairs performance on object-recognition tasks (Barense et al., 2005; Barker & Warburton, 2008; Bartko, Winters, Cowell, Saksida, & Bussey, 2007; Buffalo et al., 2006; Bussey, Saksida, & Murray, 2002; Bussey & Sakaside, 2005; Graham et al., 2006; Lee et al., 2005; Murray & Bussey, 1999; Norman & Eacott, 2004; Petrulis & Eichenbaum, 2003; Taylor, Moss, Stamatakis, & Tyler, 2006). PR is argued to support performance on tasks that require the use of complex conjunctions of the features that compose an object or a scene (Kholodar-Smith et al., in press; Murray et al., 2007; Bussey & Sakasida, 2005).

Second, PR damage severely impairs context conditioning (Corodimas and LeDoux, 1995; Bucci, Phillips, & Burwell, 2000; Bucci, Saddoris, & Burwell, 2002; Kholodar-Smith et al., in press; Lindquist, Jarrard, & Brown, 2004; Kholodar-Smith et al., in press). Context conditioning is commonly understood to entail "configural" or "conjunctive" learning about key stimulus features (Bucci et al., 2000; Bucci et al., 2002; Kholodar-Smith et al., in press; Lindquist et al., 2004; Rudy & O'Reilly, 2001; Kholodar-Smith et al., in press). Eichenbaum and colleagues emphasize the role of PR in "stimulus fusion" (Eichenbaum, Schoenbaum, Young, & Bunsey, 1996; Eichenbaum, 1997). Interestingly, rats require one to three minutes to form a representation of a context that can support context conditioning (Fanselow, 1986, 1990; Fanselow, Landeira-Fernandez, DeCola, & Kim, 1994; Landeira-Fernandez, DeCola, Kim, & Fanselow, 2006; Rudy & Matus-Amat, 2005; Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006; Wiltgen, Sanders, Behne, & Fanselow, 2001).

Third, PR damage severely impairs delay fear conditioning to discontinuous but not continuous auditory cues (Kholodar-Smith et al., in press; Lindquist et al., 2004). One plausible inference is that, in order for successful conditioning to occur, the successive segments of these discontinuous conditional stimuli (CSs) must be unitized through a PR-dependent binding mechanism (Kholodar-Smith et al., in press; also discussed in Allen, Furtak, & Brown, 2007; Furtak, Allen, & Brown, 2007a). Unitized representations of chunks of sound have been termed "auditory objects" (see Treisman, 1998; Rauschecker, 1998; Goldstone, 2000; Tian, Reser, Durham, Kustov, & Rauschecker, 2001; Griffiths and Warren, 2004; Furtak, Allen, et al., 2007a; Kholodar-Smith et al., in press). The recognition of both

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auditory and non-auditory objects may entail similar or identical PR-dependent mechanisms (see Murray et al., 2007).

The present study uses a trace fear conditioning paradigm to explore a different type of temporal gap—a "trace interval" between the CS offset and the onset of the unconditional stimulus (US). Although the CS and US were not contiguous in space or time, the US was perfectly predictable across the trace interval. The results reveal what may be a novel aspect of PR function, one that seems not to entail unitization.

2. Materials and methods

2.1. Subjects

Subjects were fifty-six male Sprague–Dawley rats (~320 g weight; Charles River Laboratories, Kingston, NY). Animals were handled for 3 days prior to surgery. Experiments were conducted in accordance with the Society for Neuroscience policies and the Yale Animal Resources Center guidelines on the care and use of animals.

2.2. Surgery

After anesthetizing the rat, the temporal bone and the zygomatic arch of the skull were visualized (Furtak, Allen, et al., 2007a; Kholodar-Smith et al., in press). The trephination was made along the intersection of the two bones, exposing the temporal cortex (TE) and PR. Each rat received ~8 bilateral PR injections (0.07 μL per infusion; 0.05 μL/min; equally spaced at ~0.7 mm) with either 0.01 M phosphate-buffered 0.9% saline (PBS) or 340 mM NMDA (50 mg/mL; Sigma, St. Louis, MO), using a hypodermic (26 gauge) microsyringe at a 45° angle from vertical. Sometimes only 7 injections were made due to the presence of a blood vessel at the intended injection site. Openings on the lateral surface of the skull were covered with a thin layer of bone wax. Rats were given 5–7 days to recover before initiating the behavioral procedures discussed next.

2.3. Behavioral procedures

Two modified Coulbourn chambers (29 cm length × 25.5 cm width × 32 cm height) differed in odorant, lighting, flooring, visual design on the walls, and background noise. Chamber A served as the training and context-testing chamber. Chamber B served as a "shifted" context for testing CS-elicited freezing. A "bat detector" (Mini-3, tuned at 23 kHz; UltrasoundAdvise, London, UK) was positioned inside each sound-attenuating enclosure. Both chambers were equipped with an infra-red camera, which was attached to a video recorder. Video and audio signals that were recorded during cue and context testing were stored for offline analysis of freezing behavior. The latter was done with video-analysis software (see Supplementary Figs. 1 and 2) described elsewhere (Bang, Allen, Jones, Boguszewski, & Brown, 2008; Boguszewski, Bang, & Brown, 2007). For cue and context conditioning, respectively, the correlation between machine and human scoring (n = 12 rats) is 0.99 and 0.91 (Bang and Brown, unpublished).

2.4. Auditory stimuli

The auditory CSs (Fig. 1) were similar to ones that have previously been shown to elicit widespread firing in rat PR neurons (Allen et al., 2007; Furtak, Allen, et al., 2007a). A pre-recorded 10-call 23 kHz ultrasonic vocalization (USV; Fig. 1C) was elicited from a naïve rat by an unsignaled foot shock through the grid floor (1 s, 1 mA). Vocalizations were digitally recorded with an RP2.1 (TuckerDavisTechnologies (TDT), Alachua, FL), sampled at 100 kHz (32-bit), and band-pass filtered (18–26 kHz). Continuous and discontinuous tone cues (10 ms rise/fall time) were produced with a D/A tone generator (RPvds, TDT) used in conjunction with an RP2.1 (Fig. 1A–B). All three auditory cues were matched in loudness (~65 dB).

2.5. Conditioning

Subjects were randomly assigned to receive presentations one of three auditory cues (Fig. 1) that were used as conditional stimuli: a continuous tone ("Tone" Group); a pre-recorded USV ("USV" group); and a discontinuous tone, consisting of 10 tonal segments, whose on/off pattern matched the temporal structure of the USV ("Pips" group). By convention, USVs with root frequencies of 18–35 kHz are collectively termed "22 kHz USVs" (Brudzynski, 2005). Among naïve laboratory rats, USVs and Pips are as "neutral" as continuous tones in terms of the unconditional elicitation of freezing behavior (Bang et al., 2008; Endres & Fendt, 2007). Prior to conditioning, subjects were given 2 min to explore the training chamber. During conditioning, subjects received 10 trace pairings of a 9.7 s auditory CS and a 1.0 s foot shock US (0.8 mA) in Chamber A. The trace interval was 16 s and the ISI was 25.7 s. A recent study suggests that hippocampal participation in trace conditioning diminishes with trace intervals less than 15 s (Misane et al., 2005). The mean inter-trial interval (±SE) was 5 min ± 15 s.

2.6. Testing

Freezing responses to the cue (in Chamber B) and context (in Chamber A) were tested 24 and 48 hr later in a counterbalanced order. Context-elicited freezing was measured for 6 minutes. Cue-elicited freezing was measured in massed trials during the 2-min baseline and 6 min of the cue presentation. Freezing was defined as the cessation of all movement except that required for respiration (Blanchard & Blanchard, 1969; Fanselow, 1997). The freezing recognition software converted the cumulative time spent freezing into a percentage score (100% freezing duration ÷ total duration).

2.7. Statistics

Group data were analyzed using ANOVAs and t-tests. The family-wise α-error rate was maintained at 0.05. The lesion effect size (d) on freezing was computed as follows (Cohen, 1988):

$$d = \frac{\text{mean}_C - \text{mean}_L}{\sqrt{\left(\text{SD}_C^2 + \text{SD}_L^2\right)/2}}, \quad (1)$$

where the numerator is the difference between the mean freezing of control and lesioned animals and the denominator is the standard deviation of a pooled estimates from control and lesioned animals. In psychological studies, d = 0.8 is conventionally regarded as a "large effect size" (Cohen, 1988).

2.8. Histology

Subjects were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg) and perfused intracardially with 0.01 M PBS and 4% paraformaldehyde. Cryoprotected brains were sectioned with a freezing microtome (75 μm) into three sets of immediately-adjacent sections for a Nissl stain, a NeuN stain, and a fiber-specific myelin stain. The conspicuously low level of myelin staining in PR, relative to the adjacent temporal (TE) and entorhinal cortices (EC; Brown & Furtak, 2006; Burwell, 2001), quickly reveals its borders. The Nissl and NeuN stains highlight the distinctive lami-

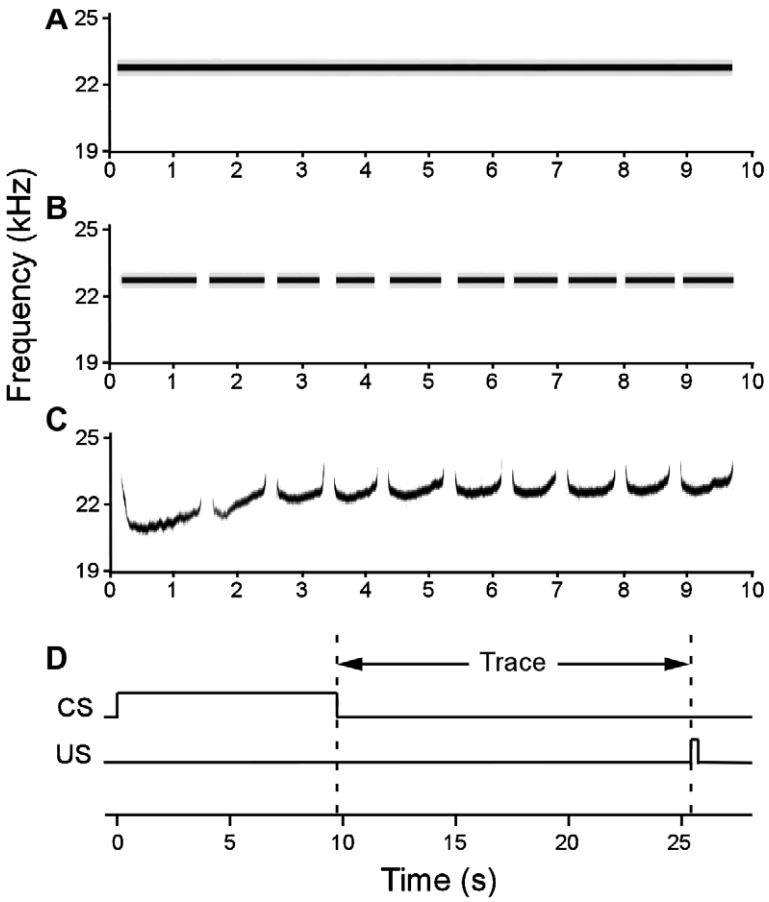

Fig. 1. Training paradigm and spectrograms of the three conditional stimuli (CSs) used as cues. (A) A continuous 23 kHz tone. (B) A discontinuous 23 kHz tone ("pips") whose frequency and on/off temporal pattern was matched to the 23 kHz USV that is shown in part C. (C) A 23 kHz USV recorded from a conspecific. All cues were matched in principle frequency, duration, and average loudness. (D) Training paradigm. Training included 10 trials of 9.7 s CS presentation, followed by a 16 s trace interval, and terminated with a 1 s US footshock presentation.

nar organization of PR around the rhinal fissure. In combination, these stains leave no uncertainty about the borders between PR and adjacent structures.

2.9. Lesion reconstructions

Using a Neurolucida (MicroBrightField, Williston, VT) computer–microscope system, the volume of neurotoxic damage to PR and neighboring regions was estimated using NeuN-stained sections (based on Burwell, 2001; Paxinos & Watson, 1998). The Cavalieri method was used to calculate the percentage damage (by volume) to PR, EC, the ventral hippocampus (vHC), area TE of the temporal cortex (TE1 plus TE2), and the lateral and basolateral subnuclei of the amygdala (LA/BLA).

3. Results

3.1. Histology

None of the 56 rats was excluded based on histology. Fig. 2 compares cell damage in sham-operated (Fig. 2A–C) and PR-lesioned rats (Fig. 2D–F). In sham-operated rats, the PR cytoarchitecture was easily discerned in Nissl- (Fig. 2A) and NeuN-stained sections (Fig. 2C) based on its laminar organization around the curvature of the rhinal sulcus. The myelin stain (Fig. 2B) duplicated previous findings of conspicuously levels of PR myelination relative to TE and EC (Brown & Furtak, 2006; Burwell, 2001). This remarkably low level of staining quickly identifies the boarders between PR and adjacent cortex. The multiple NMDA injections caused neurotoxic damage that included areas 35 and 36 along most of the rostro-caudal extent of PR (Fig. 2D–G).

Fig. 3 shows the average amount of neurotoxic damage that was sustained in each hemisphere of PR and in adjacent structures. There were no significant differences between hemispheres in the amount of damage to PR, EC, vHC and TE, $p > 0.05$ (see Fig. 3). There was also no significant difference in the extent of PR damage among the Cue groups: $F(2,24) = 3.17$, $p = 0.06$. The damage to vHC was minor (3.7% volume). For unknown reasons, there was significantly more damage to the right LA/BLA (9.4 ± 6.6%) than the left LA/BLA (1.5 ± 1.5%), $t(26) = 2.14$, $p = 0.04$. Because the US was applied bilaterally, the small unilateral amygdala damage is unlikely to have had any behavioral effect (Blair et al., 2005). Finally, there was no significant difference in the effect of PR damage among the Cue groups, $F(2,24) = 1.97$, $p = 0.16$.

3.2. Cue-elicited freezing

Prior to the cue presentation (during the baseline period), there was no significant effect of Surgery, $F(1,55) = 1.33$, $p = 0.26$; no significant effect of type of Cue, $F(2,55) = 0.32$, $p = 0.73$; and no significant Surgery × Cue interaction, $F(2, 55) = 1.37$, $p = 0.27$. During the CS presentation, there was a significant main effect of Surgery, $F(1, 50) = 72.23$, $p < 0.001$. Fig. 4A shows that freezing during the CS presentation was comparable in the three cue groups. The CS-elicited freezing levels were significantly lower in PR-lesioned rats (39.0 ± 4.3%) than PBS-infused control animals (82.2 ± 2.7%),

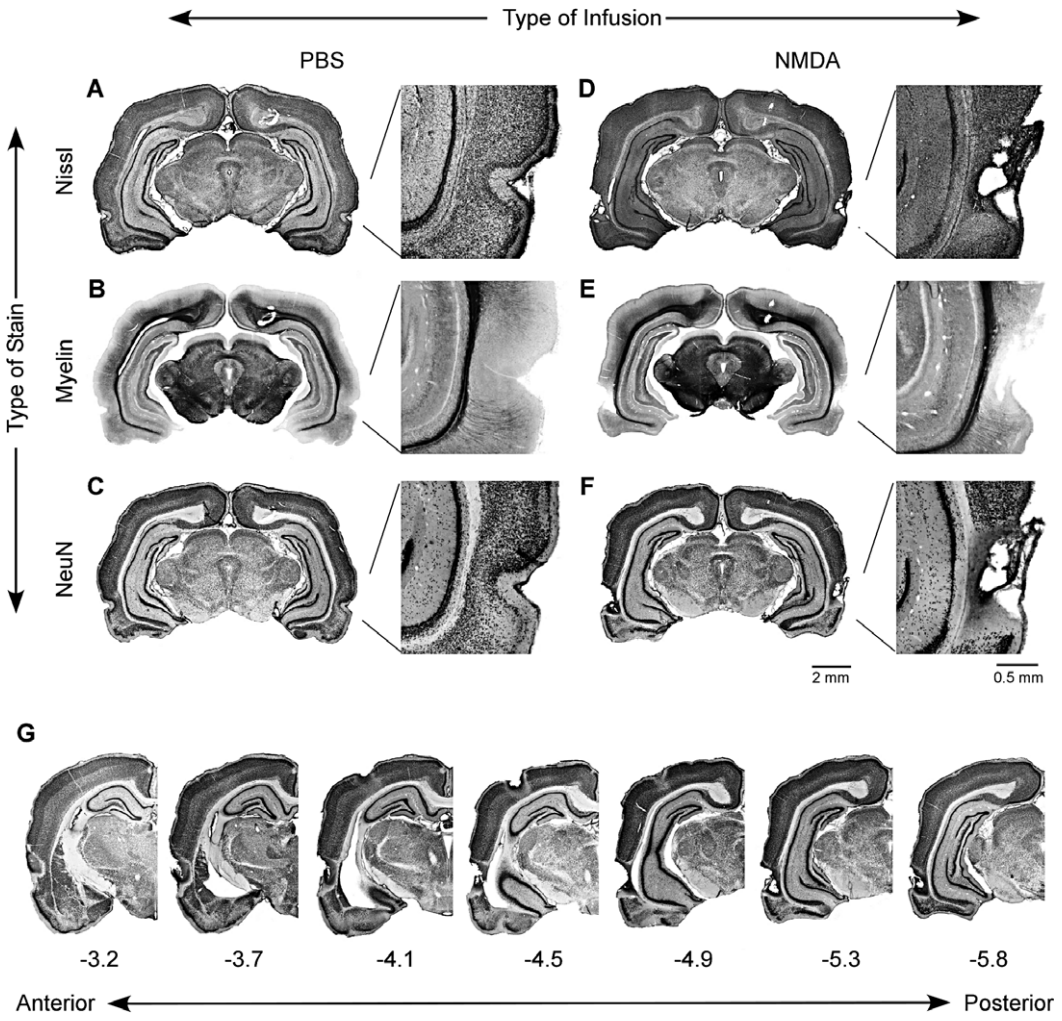

Fig. 2. Examples of histology from a sham-operated subject (A–C) and a PR-lesioned subject (D–F). Coronal slices were reacted using a Nissl stain (A, D), which labels neuronal and glial cell bodies; a gold-chloride stain (B, E), which labels myelin; a NeuN (C, F), which is specific for neurons. The illustrated sections were taken at approximately −5.2 A/P relative to bregma. Multiple injections of PBS resulted in unremarkable damage primarily represented by the needle tracks, while multiple injections of NMDA caused a well-localized neuronal damage. (G) Unilateral images showing the anterior–posterior extent of a representative PR lesion. Brain sections belong to the same subject shown in (D–F).

$t(52) = 8.48$, $p < 0.001$. There was no main effect of the Cue on freezing levels, $F(2,50) = 0.22$, $p = 0.80$. The mean freezing level among sham-operated rats was 82.24 ± 2.73% and there were no significant differences among Cue groups, $F(2,24) = 0.91$, $p = 0.42$. The mean freezing level among PR-lesioned rats was 39.03 ± 4.29% and there were no significant differences in freezing levels among the Cue groups, $F(2,24) = 1.10$, $p = 0.35$. In addition, there was no significant Surgery × Cue interaction, $F(2,50) = 1.87$, $p = 0.17$. The mean (± SE) lesion effect size (from Eq. (1)) on trace cue conditioning was very large ($\bar{d} = 2.7 \pm 0.8$). The effect sizes for each cue group were as follows: Tone-group ($d = 1.9$), USV-group ($d = 1.7$), and Pips-group ($d = 4.6$).

3.3. Context-elicited freezing

The effects of PR damage on context-elicited freezing are summarized in Fig. 4B. The histograms show comparably severe impairment in all three CS groups. The mean lesion effect size on context conditioning ($\bar{d} = 3.9 \pm 0.7$) was extremely large. By group, the lesion effect sizes on context conditioning were as follows: Tone-group ($d = 4.2$), USV-group ($d = 4.4$), and Pips-group ($d = 3.0$). There was a significant main effect of Surgery on context-elicited freezing, $F(1,50) = 183.87$, $p < 0.001$. Overall, PR-lesioned rats froze significantly less to the context (18.4 ± 5.6%) than did the PBS-controls (79.3 ± 5.6%), $t(52) = 6.67$, $p < 0.001$. There was no significant effect of Cue on context conditioning, $F(2,50) = 2.63$, $p = 0.08$, and no significant Lesion × Cue interaction, $F(2,50) = 0.28$, $p = 0.76$. Within each Cue group, the pattern of statistical significance was the same. Specifically, there was significantly less freezing in PR-lesioned rats than in PBS-controls in the Tone group, $t(16) = 8.65$, $p < 0.0001$; the USV group, tthe $C(16) = 9.25$, $p < 0.0001$; and the Pips-group, $t(16) = 4.97$, $p < 0.0001$.

4. Discussion

4.1. Brief summary

The present study explored the role of PR in trace fear conditioning, using three different auditory cues (Fig. 1A–C). In the experimental group, neurotoxic damage was evident along most of the length of PR (Fig. 2). Three-dimensional reconstructions revealed that the volume of neuronal damage was well-localized to PR (Figs. 2 and 3). There was relatively little bilateral damage to either vHC or LA/BLA (Fig. 3). PR lesions profoundly impaired trace fear conditioning to all three auditory cues (Fig. 4A; $\bar{d} = 2.7$) as well as conditioning the training context (Fig. 4B; $\bar{d} = 3.9$). There were no significant differences among the cues in the levels of elicited

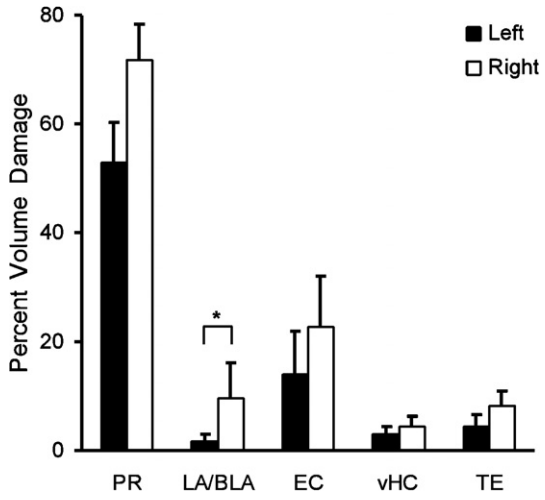

Fig. 3. Average amount of bilateral damage to PR and adjacent structures caused by neurotoxic lesions. The amount of damage is given as a percentage of the total volume. PR sustained extensive bilateral damage, whereas damage to adjacent brain regions was limited. In the LA/BLA region there was significantly more damage to the right side than on the left side. *Abbreviations:* PR, perirhinal cortex; LA/BLA, lateral and basolateral nuclei of amygdala; EC, entorhinal cortex; vHC, ventral hippocampus; TE, temporal cortex. Bars represent the standard error (SE) of the mean.

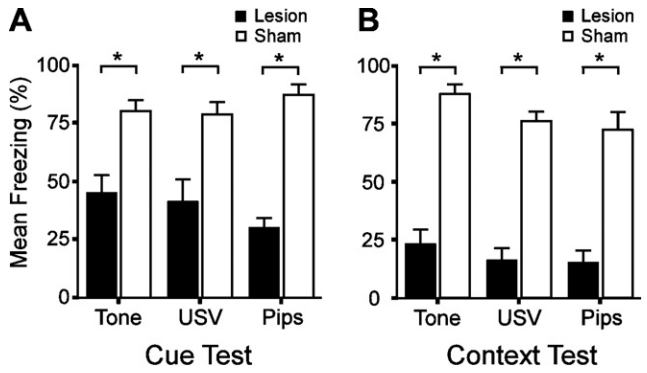

Fig. 4. Effects of the bilateral NMDA lesions on freezing to the cue and training context. (A) Mean freezing levels to the cue in PR-lesioned subjects (black bars) and sham-operated control animals (white bars). All three cues elicited robust freezing in the sham-operated control animals. By contrast, freezing was significantly reduced to all auditory cues in PR-lesioned subjects. (B) Mean freezing levels to the training context were comparable among the three groups of sham-operated subjects. By contrast, freezing was significantly reduced in the three groups of PR-lesioned subjects. Asterisks denote significant differences between the sham-operated and PR-lesioned subjects. Bars represent the standard error (SE) of the mean.

freezing in either PR-lesioned rats or sham-operated control animals. The new and remarkable finding is that PR damage impaired trace fear conditioning to a continuous tone. The observed deficits in context conditioning and conditioning to discontinuous auditory cues were anticipated (see Bucci et al., 2002; Kholodar-Smith et al., in press; Lindquist et al., 2004). These and other results are interpreted in terms of dual mnemonic functions of PR.

4.2. Trace fear conditioning system

4.2.1. Background and contemporary theory

Historically, the dorsal hippocampus (dHC) has been most closely associated with trace conditioning. Damage to the dHC can impair both trace fear and trace eyeblink conditioning (Berger, Rinaldi, Weisz, & Thompson, 1983; McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; McEchron, Tseng, & Disterhoft, 2000; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Weiss, Kronforst-Collins, & Disterhoft, 1996). Recent evidence suggests that the ventral region of the hippocampus (vHC) may be more directly involved in trace conditioning (Rogers, Hunsaker, & Kesner, 2006; Yoon & Otto, 2007). Indeed, the dHC is thought to communicate with the amygdala by means of the vHC (Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000).

For several days after trace conditioning, HC damage severely impairs memory performance. However, at longer time intervals, HC function is no longer required for retrieval (Anagnostaras, Maren, and Fanselow, 1999; Kim & Fanselow, 1992; also see Moyer, Thompson, & Disterhoft, 1996). One contemporary theory argues that HC-independent memories can emerge from repeated activations and strengthening of the synapses between HC and neocortex and within neocortex (Paz, Bauer, & Paré, 2007; Rolls, 1996; Rolls & Kesner, 2006; Rudy, 2008; Teyler & DiScenna, 1986; Teyler & Rudy, 2007; Zola-Morgan & Squire, 1986).

4.2.2. Cortical fear conditioning pathways

During and after trace fear conditioning, critical interactions are believed to occur between HC, EC, PR, and the prefrontal cortex (PFC; Dash, Hebert, & Runyan, 2004; Paz et al., 2007). The latter has been proposed to be a long-term memory storage site for both trace fear and trace eyeblink conditioning (Blum, Hebert, & Dash, 2006; Dash et al., 2004; Powell, Skaggs, Churchwell, & McLaughlin, 2001; Quinn, Ma, Tinsley, Koch, & Fanselow, 2008; Runyan, Moore, & Dash, 2004). PR sends direct projections to HC as well as indirect projections via EC (Burwell, 2001; Furtak, Wei, Agster, & Burwell, 2007b; Lavenex, Suzuki, & Amaral, 2004; Pitkänen et al., 2000). The CA1 region of HC in turn projects back to PR through the subiculum and EC (Pitkänen et al., 2000).

Both EC and the subiculum project to LA/BLA (Kerr, Agster, Furtak, & Burwell, 2007; Pitkänen et al., 2000), which are "cortex-like" parts of the amygdala (McDonald, 1982; Swanson & Petrovich, 1998). Reciprocal monosynaptic projections connect PR and LA/BLA. The latter projects to the central nucleus of the amygdala (Furtak Wei, et al., 2007b; Pitkänen et al., 2000), which in turn controls conditional freezing behavior (Choi & Brown, 2003; Fanselow, 1998; LeDoux, 2000). The only monosynaptic projections from rat HC to amygdala occur via subfield CA1 of vHC (Canteras & Swanson, 1992; Pitkänen et al., 2000; Yoon & Otto, 2007). Interestingly, the output from LA/BLA can also control the propagation of activity from PR to EC and HC (Kajiwara, Takashima, Mimura, Witter, & Iijima, 2003), raising the possibility of recursive interactions among these structures.

4.2.3. Functions of PR in trace conditioning

The simplest account of the role of PR in trace fear conditioning is that this "transitional" cortex (Lavenex & Amaral, 2000) serves as a crucial part of the input to and/or output from HC, which houses the essential memory trace (see Eichenbaum, Yonelinas, & Ranganath, 2007; Lee & Kesner, 2003; Rodriguez & Levy, 2001; Rolls & Kesner, 2006; Rudy & O'Reilly, 2001; Shapiro, 2001; Wallenstein, Eichenbaum, & Hasselmo, 1998). However, the role of PR in trace fear conditioning may transcend this purely-passive or non-adaptive conceptualization.

We propose that the critical function of PR in Pavlovian trace conditioning is to enable a representation of the cue in the absence of exteroceptive input. This same PR function is thought to support performance on a delayed non-match to sample (DNMS) tasks. PR damage impairs performance on DNMS tasks in both monkeys (Gaffan & Murray, 1992; Meunier, Bachevalier, Mishkin, & Murray, 1993; Murray & Bussey, 1999) and rats (Otto & Eichenbaum, 1992). Neurophysiological recordings from rat PR have revealed neurons that maintain stimulus-specific activity during the delay period

of an odor-guided version of the DNMS task (Young, Otto, Fox, & Eichenbaum, 1997).

In neurophysiological terms, transient sensory memory could be supported by recirculating neuronal activity (Durstewitz, Seamans, & Sejnowski, 2000; Fellous & Sejnowski, 2003) and and/or by endogenous "persistent firing", which can last for minutes after the termination of the original excitation (Egorov, Unsicker, & von Bohlen und Halbach, 2006). This remarkable single-cell phenomenon is common in LA/BLA (Egorov et al., 2006) and certain layers of PR (Boguszewski, Leung, Zhao, & Brown, 2007; Brown, Zhao, & Leung, 2007) and EC (Egorov, Hamam, Fransén, Hasselmo, & Alonso, 2002; Fransén, Tahvildari, Egorov, Hasselmo, & Alonso, 2006; also see Hasselmo & Stern, 2006), but does not occur HC.

5. Conclusions

PR damage profoundly impaired both trace fear conditioning and context conditioning. We suggest that the function of PR in trace cue conditioning is different from its role in delay cue and context conditioning. The latter two are consistent with the general hypothesis (see Kholodar-Smith et al., in press), reviewed earlier, that PR supports unitization through "configural" or "conjunctive" learning or "stimulus fusion". The role of PR in trace conditioning may reflect a different kind of adaptive function, one that is more intimately associated with HC. Seemingly different higher-level functions may turn out to depend on similar cellular mechanisms, such as endogenous persistent firing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nlm.2008.06.006.

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