Contributions of Postrhinal and Perirhinal Cortex to Contextual Information Processing

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The role of the postrhinal cortex (POR) and the perirhinal cortex (PER) in processing relational or contextual information was examined with Pavlovian fear conditioning. Rats with electrolytic or neurotoxic lesions of the POR or PER were tested in 2 contextual fear conditioning paradigms. In Experiment 1, electrolytic lesions of the POR or PER produced impairments in contextual fear conditioning but not in conditioning to a phasic auditory conditioned stimulus. Neurotoxic lesions of the POR or PER likewise resulted in anterograde (Experiment 2) and retrograde (Experiment 3) deficits in fear conditioning to the training context in an unsignaled shock paradigm. The results suggest that operations performed on sensory information by the POR and PER are necessary to support contextual learning.

Identification of the neural substrates that support encoding, consolidation, storage, and retrieval of configural or relational information is a primary goal of neuroscientific research on memory (see Eichenbaum, Otto, & Cohen, 1994; Maren, Anagnostaras, & Fanselow, 1998; Rudy & Sutherland, 1992; Squire, 1992; Sutherland & Rudy, 1989). Historically, the major emphasis of this research has been on the hippocampus, but researchers are increasingly interested in cortical contributions and corticohippocampal interactions that support memory (e.g., Bucci & Burwell, 1999; Burwell & Phillips, 1998; Bussey, Muir, & Aggleton, 1999; Eichenbaum, 1997; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999; Thornton, Rothsblat, & Murray, 1997). One example of the type of memory of interest is contextual learning, which presumably involves the configuring of multimodal sensory cues present in a given training environment (e.g., visual, spatial, or auditory stimuli). A behavioral model widely used to study this type of learning is contextual fear conditioning in rats (Holland & Bouton, 1999, Maren et al., 1998). In this paradigm, rats learn to associate the contextual cues of a conditioning environment with the occurrence of a brief footshock. Subsequent exposure to the training context alone elicits a fear response, for example, freezing behavior (Fanselow, 1980).

Evidence for hippocampal involvement in processing configural or relational information has accrued from a number of behavioral paradigms including fear conditioning (Good & Honey, 1997; Kim & Fanselow, 1992; Kim, Rison, & Fanselow, 1993; Phillips & LeDoux, 1992, 1994; Young, Bohenek, & Fanselow, 1994). The effects of hippocampal damage on contextual fear conditioning depend on several factors, such as the timing of the lesion and the method used to damage the hippocampus. Electrolytic, but not neurotoxic, lesions of the hippocampus made before training impair the acquisition of contextual fear, which suggests that damage to, or disconnection of, other regions may underlie the deficit produced by electrolytic lesions (Maren, Aharonov, & Fanselow, 1997; Phillips & LeDoux, 1992, 1994; Young et al., 1994). Thus, brain regions outside the hippocampus appear to have some role in processing contextual information. Moreover, neurotoxic as well as electrolytic lesions of the hippocampus made shortly after training disrupt the expression of conditioned fear, whereas contextual conditioning remains largely intact at longer training-to-lesion intervals (Kim & Fanselow, 1992; Maren et al., 1997). Such a time-limited role suggests that the hippocampus contributes to the stabilization of contextual memories that are stored elsewhere in the brain.

The rodent hippocampus receives its primary cortical input from the perirhinal (PER) and postrhinal (POR) cortices (Burwell & Amaral, 1998b; Burwell, Witter, & Amaral, 1995; Kosel, Van Hoesen, & Rose, 1983; Naber, Caballero-Bleda, Jorritsma-Byham, & Witter, 1997; Shi & Cassell, 1997). The input arrives by both direct connections with the hippocampus proper and indirect connections through the entorhinal cortex. Neuroanatomical studies have indicated that the POR and PER are distinguished by the composition of input each receives from sensory association regions (Burwell & Amaral, 1998a). The POR receives input almost exclusively from the visual association cortex and visuospatial association regions such as the posterior parietal and retrosplenial cortices. In contrast, the PER receives input from all sensory modalities, although a large proportion of its visual and spatial input is provided by the POR. The projections from the POR and
PER to the entorhinal cortex and hippocampus are organized such that the information remains somewhat segregated (Burwell & Amaral, 1998b; Burwell & Eichenbaum, 1999; Naber et al., 1997). The POR has reciprocal connections with caudal and medial portions of the entorhinal cortex; conversely, the PER is reciprocally connected with rostrolateral regions of the entorhinal cortex. The corticohippocampal connections suggest that the hippocampal involvement in contextual fear conditioning may depend on higher order polymodal sensory information from POR, PER, or both regions.

Currently little is known about the function of the POR, and there are no published studies of the effects of POR damage on conditioned fear. The results of the few available PER lesion studies have been mixed. Three studies have suggested that pretraining lesions of the PER do not affect contextual conditioning (Herzog & Otto, 1997, 1998; Phillips & LeDoux, 1995). One prior study indicated that PER lesions carried out shortly after training significantly impair the expression of contextual fear (Corodimas & LeDoux, 1995). In support of this finding, a study using reversible inactivation procedures provided evidence that the PER participates in consolidation of contextual fear (Sacchetti et al., 1999). Related research on the effects of entorhinal cortex damage on fear conditioning has also yielded both positive (Maren & Fanselow, 1998b; Burwell & Eichenbaum, 1999; Naber et al., 1997) and negative (Phillips & LeDoux, 1995) results. Although the failure to provide consistent positive evidence for cortical contributions to contextual fear conditioning could be explained by a number of factors including sample sizes, lesion methods, or differences in behavioral procedures, the contradictory results highlight the need for further investigation.

On the basis of our knowledge about the source of polymodal associative input to the hippocampus, it seems reasonable to hypothesize that the cortical regions surrounding the hippocampus play a role in transforming sensory input into higher order contextual or relational information. The POR and PER are likely candidates for cortical involvement in contextual learning. Accordingly, the present experiments were designed to examine the effects of POR or PER damage on contextual fear conditioning by using multiple lesion methods and behavioral paradigms. In Experiment 1, the effect of pretraining electrolytic lesions of the POR or PER were examined in one fear conditioning procedure, and the effects of pretraining (Experiment 2) and posttraining (Experiment 3) neurotoxic lesions were subsequently examined in a second paradigm.

Experiment 1

This experiment examined the effects of pretraining electrolytic lesions of the POR or PER in a fear conditioning paradigm used by Phillips and LeDoux (1994). In this paradigm, training is conducted over a 3-day period. After being placed in the conditioning chamber on each of the first 2 days, rats receive three conditioning trials consisting of a brief auditory stimulus followed immediately by delivery of a footshock. On the 3rd day, the auditory stimulus is presented, but no shock is delivered. Prior studies have indicated that fear conditioning to a phasic, auditory cue can be accomplished through either a direct auditory thalamo-amygdaloid route or by a thalamo-cortico-amygdaloid route (Romanski & LeDoux, 1992a, 1992b), and so neither POR nor PER damage was expected to affect conditioning to the tone. On the basis of cortical afferentation and corticohippocampal connectivity, however, either POR or PER lesions were predicted to affect fear conditioning to the contextual cues of the experimental chamber.

Method

Subjects. Thirty-seven male Long-Evans rats (Charles River Laboratories, Wilmington, MA) weighing approximately 300 g at the time of surgery served as subjects in this study. The rats were individually housed in wire mesh cages and maintained on a 12-hr light–dark cycle with free access to food and water. All research subjects were handled in accordance with guidelines approved by the International Animal Care and Use Committee and the American Association for the Accreditation of Laboratory Animal Care.

Surgery. Subjects were brought to a surgical level of anesthesia with halothane gas and secured in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA) with the head level set between bregma and lambda. Under aseptic conditions, an incision was made to reveal the skull and the skin was retracted to the side. A series of holes were then drilled through the skull above the intended lesion site. Electrolytic lesions were made with monopolar electrodes (Teflon-coated wire, 125 μm diameter). For the POR group (n = 13), 2 mA DC was passed through the electrodes for a duration of 13 s at each of three sites: 0.1 mm anterior to lambda, 5.54 mm lateral to bregma, and 3.8 and 2.8 mm ventral to skull surface; and 0.1 mm posterior to lambda, 5.4 mm lateral to bregma, and 2.8 ventral to skull surface. For the PER group (n = 12), 2 mA DC was passed through the electrodes for a duration of 15 s at each of five sites: 3.3, 4.3, 5.3, 6.3, and 7.3 mm posterior to bregma, 5.1 mm lateral to midline at 12° from vertical in the mediolateral plane, and 6.6, 6.6, 6.6, 6.4, and 5.8 ventral to skull surface, respectively. Control subjects (n = 12) received sham operations in which holes were drilled but the electrode was not lowered into the brain. Half of the control subjects received craniotomies to simulate POR lesions; the other half received craniotomies to simulate PER lesions. After all sites had been lesioned, the wound was sutured and an analgesic was administered to relieve pain. Rats were then placed in a warm plastic cage, monitored postsurgically, and returned to the colony after recovery from anesthesia. Rats were allowed to recover for at least 10 days before beginning behavioral training.

Apparatus. Behavioral training was conducted in an operant testing environment (MED Associates, St. Albans, VT) interfaced with a 360° computerized and controlled by MED-PC V2.1 software (MED Associates). Custom software written in the Pascal-based MED-PC notation controlled the behavioral tasks and recorded task events and responses. Experiments were conducted in four 24.0 x 30.5 x 29.0 cm operant test chambers, each of which had modular component aluminum panels in the front and back, Plexiglas side walls and top, and a floor constructed of 0.48-cm rods placed 1.6 cm apart. Scrambled alternating current was delivered through the grid floor by a constant current shock source. A speaker connected to a programmable audio input generator was located at the top right corner of the front panel. A partially shaded houselight (28 V, 100 mA), mounted centrally at the top of the front wall, illuminated the chamber during habituation, training, and testing. Each testing chamber was enclosed in a 62 x 56 x 56 cm sound-attenuating chamber fitted with an exhaust fan that provided air flow to the test chamber and background white noise. A surveillance camera was mounted above and behind each testing chamber on the back wall of the sound-attenuating chamber, and a VCR was used to record behavior in all four chambers simultaneously.

Behavioral procedure. Fear conditioning to discrete stimuli and to contextual stimuli was conducted according to the procedure of Phillips and LeDoux (1994), in which an auditory conditioned stimulus (CS) is paired with a footshock unconditioned stimulus (US). The auditory CS was a 20-s 10-KHz tone at 75 dB, and the US was a 500 ms delivery of a 0.5-mA constant current shock through the grid floor of the operant chamber. On Day 0, rats were placed in the conditioning apparatus for 20 min with no
stimulus presented. On Days 1 and 2, conditioning trials were delivered, during which three CS presentations were given with a 60–120-s intertrial interval. Each 20-s CS coterminated with a presentation of the US. On Day 3, the CS was presented on the same schedule as the preceding days, but without the US.

Performance on each trial was videotaped for subsequent analysis. Freezing served as the index of conditioned fear and was readily identified by a characteristic crouching posture and the absence of any movement except respiration (Blanchard & Blanchard, 1969). Freezing to the context was measured only for the first trial, during the 20-s interval before CS onset. Freezing to the CS was assessed during the 20-s CS of the first trial. The relevant measure was the time spent freezing during the 20-s assessment interval. The observer scoring the videotapes was unaware of the treatment conditions.

**Histology.** At the end of behavioral training, subjects were deeply anesthetized with sodium pentobarbital (Nembutal, 100 mg/kg) and transcardially perfused with an automatic pump at a flow rate of 35–40 ml/min. To clear the blood, normal saline at room temperature was first perfused for a maximum of 2 min, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4) at 4 °C for 15 min. After fixation, each brain was removed from the skull and postfixed in the same buffer paraformaldehyde solution for 6 hr at 4°C. Finally, brains were cryoprotected for 24–48 hr at 4 °C, in a solution of 20% glycerol in 0.1 M PB.

Coronal brain sections were cut at 40 μm on a freezing microtome. Sections were collected in two series for POR-lesioned brains and four series for PER-lesioned brains for subsequent processing and storage. One series was collected in a 10% formalin solution in preparation for cell staining. That series was subsequently mounted and Nissl-stained with thionin. The remaining series were collected and stored at −20 °C in cryoprotectant tissue-collecting solution consisting of 30% ethylene glycol and 20% glycerol in 0.1 M PB.

Coronal sections at 240 μm intervals for POR lesions and 480 μm intervals for PER lesions were used to assess the amount of tissue damage. Camera lucida techniques were used to draw section contours, add regional borders, and circumscribe the location of tissue damage. Tissue damage was identified primarily by missing tissue, but obvious necrosis or marked thinning of the cortex was also noted. The resulting drawings were digitized, and a computer program was used to obtain area measurements of the target regions and the tissue damage. For each coronal section, the resulting measures included the total area of the target region and the area of the target region that was damaged. Due to the organization of intrinsic connections of the POR and PER (Burwell & Amaral, 1998b), the amount of damage along the rostrocaudal extent of each region was assumed to be an important factor in the efficacy of the lesion. Thus, the proportion of sections in the rostrocaudal plane that exhibited damage was quantified. A subject was retained in the study if a lesion involved extensive bilateral damage only to the target region.

**Data analysis.** Repeated measures analysis of variance (ANOVA) was used to assess the effects of lesions on acquisition of fear conditioning to the context and to the tone. The variable of interest for contextual conditioning was time spent freezing during the 20-s pre-CS interval for the first trial of the 3 days of training and testing. Thus the day (1–3) was the within-subject variable and group (POR, PER, and control) was the between-subjects variable. In similar analyses performed for fear conditioning to the tone, the variable of interest was time spent freezing during the 20-s CS interval for the first trial of the 3 days of training and testing. Significant main effects and interactions were followed by appropriate contrasts. In addition, Pearson correlation analysis was conducted to determine whether there was a relationship between lesion size and freezing behavior. An alpha level of .05 was adopted for all analyses, which were conducted with SAS V.6.1 (SAS Institute, Cary, NC).

**Results**

**Histology.** Two subjects in the POR group exhibited little or no damage on one side of the brain, and 1 subject exhibited minimal damage to the POR on either side. These subjects were eliminated from the study, leaving a total of 10 subjects in the POR group. In general, the POR lesions were relatively small. Figure 1 shows a schematic of the largest and smallest lesions. The mean percentage of POR surface area damaged in the coronal sections analyzed was 38.0 ± 2.9. Although portions of the POR in each coronal plane were spared, as illustrated in photomicrographs of coronal sections (Figure 2), rostrocaudal involvement was extensive. On average, 85.6 ± 2.9% of the sections analyzed in the rostrocaudal plane exhibited damaged or missing POR tissue. In addition, damage consistently encroached very slightly on the dorsally adjacent ventral temporal association cortex (Te, according to Swanson, 1992). In three cases, the lesion included slight damage to the adjacent lateral entorhinal area (LEA). One case exhibited minor unilateral damage to the angular bundle.

Two subjects in the PER group died during or shortly after surgery, leaving a total of 10 subjects in the PER group. As was true for POR lesions, the PER lesions were small, as illustrated schematically in Figure 3. The mean percentage of POR surface area damage in the coronal sections analyzed was 30.5 ± 3.8. Portions of the PER in the coronal plane were spared, as shown in

![Figure 1. Schematic of the placement of the largest (gray) and smallest (black) postinhalial cortex (POR) electrolytic lesions at three rostrocaudal levels (Experiment 1). Arrows mark the upper and lower boundaries of the POR. LEA = lateral entorhinal cortex; MEA = medial entorhinal cortex; Te, = temporal association cortex, ventral; VISI = lateral visual association cortex.](attachment:image.png)
Figure 2. Photomicrograph of a representative postrhinal cortex (POR) electrolytic lesion at three rostrocaudal levels (Experiment 1). Arrows indicate cytoarchitectonic boundaries of the POR. Scale bar = 1 mm.

Figure 4, but, again, the rostrocaudal involvement was extensive. Of the total number of sections analyzed in the rostrocaudal plane, damage was observed in 83.4 ± 4.7% of the sections. In six of the cases, slight damage was also observed in area Te. In two cases, slight unilateral damage was observed in the ventrally adjacent LEA. In three cases, slight unilateral damage was observed in the CA1 region of hippocampus at about midseptotemporal levels. In these and two other cases, there was also a small amount of unilateral damage to the external capsule at the same level.

Behavior. Neither POR nor PER damage affected the ability of lesioned rats to associate a tone with a fearful stimulus (see Figure 5, Panel A). This finding was confirmed by ANOVA of group differences in freezing behavior during the first presentation of the tone on Days 1, 2, and 3. There was no significant main effect of group (p > .51) and no Day × Group interaction (p > .60). As would be expected, the main effect of day was highly significant, F(2, 58) = 85.04, p < .0001.

In contrast, damage to the POR or PER resulted in impairment of the ability to associate context with a fearful stimulus; both POR- and PER-lesioned rats exhibited less freezing behavior in the 20-s pre-CS period on Days 2 and 3 compared with the sham-lesioned controls (Figure 5, Panel B). Repeated measures ANOVA revealed a significant main effect of group, F(2, 29) = 5.69, p < .008; and day, F(2, 58) = 20.55, p < .0001. The Day × Group interaction was marginally significant, F(4, 58) = 2.51, p < .052.

Planned group contrasts indicated that each lesioned group exhibited less freezing behavior in the shock context as compared with sham controls, but that expression of contextual fear conditioning was similar in the two lesioned groups. ANOVA revealed that the POR group was significantly different from the control group overall, F(1, 20) = 7.89, p < .011. Additionally, the POR group was increasingly impaired over the course of training, as indicated by a significant Day × Group interaction, F(2, 40) = 4.57, p < .016. The PER group also differed from the control group overall, F(1, 20) = 4.93, p < .038, but the Day × Group interaction was not significant (p > .13). There was no overall difference between the two lesioned groups (p > .35), nor was there a Day × Group interaction (p > .72).

Correlational analyses indicated that lesion size was not related to fear conditioning to the tone or to the context for either lesion group. Neither POR lesion size nor PER lesion size, as measured by surface area or number of sections damaged, was significantly correlated with the amount of freezing behavior on Days 2 or 3. An additional post hoc analysis was conducted to evaluate whether damage to the external capsule might explain the effects of PER lesions on contextual fear conditioning. The PER group was subdivided into the subjects that exhibited slight external capsule damage (n = 5) and those that did not (n = 5). The group with external capsule damage also included the 3 subjects with a small amount of unilateral CA1 damage. The two subgroups were not different on the measure of freezing during the context period. ANOVA revealed no main effect of external capsule damage on freezing (p > .89).

Discussion

Experiment 1 provides evidence that both the POR and PER contribute to the acquisition of conditioned fear to contextual stimuli but not to an explicit auditory cue. The lack of effect of pretraining POR or PER damage on conditioning to the tone is in agreement with previous studies indicating that fear conditioning to phasic acoustic stimuli does not necessarily involve cortical processing but can occur via a direct thalamo-amygdalar connection (Romanski & LeDoux, 1992a, 1992b). As in the present study, a combined lesion of the entorhinal cortex and PER carried out before training failed to impair conditioning to an auditory cue (Phillips & LeDoux, 1995). Interestingly, posttraining PER lesions (Corodimas & LeDoux, 1995) or posttraining reversible inactivation of the PER (Sacchetti et al., 1999) do impair the expression of conditioned fear to auditory stimuli. Thus, acquisition of condi-
These possibilities will be considered further in the General Discussion section. It is also possible that the deficits produced by electrolytic lesions of the POR or PER may have resulted from damage to fibers of passage and not from damage to the target region per se. The present experiment provides some evidence that the deficits exhibited by lesioned subjects cannot be explained by damage to fibers of passage; PER-lesioned subjects that exhibited external capsule damage were not significantly different from subjects without external capsule damage in the amount of freezing to context. It is important, however, to establish more directly that damage restricted to neurons within the POR or PER was responsible for the observed deficits in contextual fear conditioning. Thus, the effect of pretraining neurotoxic damage to these regions was investigated in Experiment 2, in which a different fear conditioning paradigm was used to further assess the effects of POR or PER damage on contextual fear conditioning.

Experiment 2

In this experiment, ibotenic acid was used to produce fibersparing, neurotoxic damage to the POR or PER. Rats were then trained in another commonly used fear conditioning paradigm (e.g., Maren et al., 1997), which involves a single day of training followed by a single test day. On Day 1, rats are placed in the conditioning chambers where, 3 min later, they receive three unsignaled deliveries of footshock over a 3-min period. Twenty-four hours later, rats are returned to the conditioning chambers for an 8-min extinction test (i.e., no shock is delivered) during which the amount of freezing behavior is assessed. This procedure differs in several ways from the task used in Experiment 1. For instance, the delivery of footshock is not signaled by a discrete CS as in Experiment 1. In addition, the present procedure uses a longer observation period, which may be more sensitive in detecting group differences in contextual conditioning (Maren & Fanselow, 1997). Finally, the duration of training in this procedure makes it more amenable to studying the effects of either pretraining or posttraining lesions, allowing for clearer assessment of lesion effects on acquisition versus expression of conditioned fear.

Method

Subjects. Thirty-seven male Long-Evans rats weighing approximately 350 g were used as subjects. The rats were housed and maintained as described in Experiment 1. Each rat was handled by the experimenter for 1–2 min each day for 1 week before the start of the experiment. Surgery. The general surgical procedures were identical to those used in Experiment 1, with the exception that in the present experiment, bilateral neurotoxic lesions of the POR or PER were made with ibotenic acid (10 μg/μl; Sigma Chemical, St. Louis, MO) dissolved in 0.1 M PB. Ibotenic acid was pressure-injected into the brain through a glass pipette (50-μm tip). For POR lesions (n = 12), the pipette was angled at 22° from vertical, with the tip oriented rostrally. The pipette was lowered through the skull at 2.0 mm posterior to lambda, and an injection of 0.05 μl was made at each of two sites: 0.3 mm lateral from the lateral ridge at 5.92 mm below the skull surface, and 0.2 mm medial to the ridge at 4.85 mm below the skull surface. For PER lesions (n = 10), injections of 0.025–0.050 μl were made at each of the following stereotaxic coordinates: 2.3, 3.3, 4.3, 5.2, 6.4, and 7.1 mm posterior to bregma, at 6.3, 6.4, 6.5, 6.6, 6.7, and 6.1 mm lateral from the midline and 8.0, 8.0, 8.0, 7.0, 6.1, and 5.2 mm below the skull surface, respectively. All injections were made at a rate of 0.03 μl/min, and the pipette was left in place for 30 s before and 2 min after each.
injection. For sham-lesioned controls (n = 8), holes were drilled as described above for either POR or PER lesions, but no injections were made. Another set of control rats (n = 7) did not undergo surgery. Rats were allowed to recover for 2 weeks before beginning behavioral training.

**Apparatus and behavioral procedures.** The behavioral apparatus was identical to that described in Experiment 1. One day before behavioral training began, the rats were habituated to a plastic transporter used for carrying rats from the colony room to the behavioral chambers. Rats were then trained in an unsignaled Pavlovian fear conditioning paradigm as described by Maren et al. (1997). On the training day, the rats were placed in individual conditioning chambers (counterbalanced for lesion group) and, after 3 min, were given three constant current shocks (1.0 mA, 1 s, 64-s intertrial interval) delivered through the grid floor. Rats were removed from the chambers and returned to the colony room 64 s after the final shock was delivered. Twenty-four hours later, rats were returned to the conditioning chambers for an 8-min, 32-s extinction test, during which no shocks were delivered. Behavioral responses during the training and testing days were videotaped for subsequent analysis.

Freezing served as the index of conditioned fear, as in Experiment 1. The amount of freezing was assessed according to the methods used by Fanselow and colleagues (e.g., Maren et al., 1997). On the training day, the incidence of freezing behavior was recorded during the 64-s period prior to delivery of the first shock, and during the 64-s periods following each trial (immediate postshock freezing). During a 64-s observation period, each subject was observed every 8 s (yielding a total of 8 observations per period), and recorded as either freezing or not freezing. On the test day, the 8-min, 32-s observation period was divided into 64-s blocks, and freezing behavior was again recorded every 8 s. The frequency of freezing behavior was converted to a percentage of total observations. A single primary observer scored all of the behavioral data, and, to assess objectivity, a second observer scored a subset of the data. Both observers were unaware of the rats' lesion condition, and their scores were highly correlated \((r^2 = 0.8, p < .0001)\).

**Histology.** The histological procedures were the same as in Experiment 1. In assessing neurotoxic lesions, tissue damage was primarily identified by neuronal loss and decay as well as gliosis, but occasional cortical thinning and missing tissue were also noted.

**Data analysis.** Analysis of freezing behavior on the conditioning day was conducted with ANOVAR, with trial (baseline, Trials 1, 2, and 3) as the within-subjects variable and group (POR, PER, and control) as the between-subjects variable. For the extinction test, block (eight 64-s intervals) served as the within-subjects variable and group was the between-subjects variable. Planned comparisons of significant main effects were assessed with appropriate contrasts. An alpha level of .05 was adopted for all analyses.

**Results**

**Histology.** Photomicrographs of Nissl-stained sections from a POR-lesioned rat are shown in Figure 6. Three rats in the POR group exhibited bilateral damage extending into the PER and were subsequently eliminated from the study, leaving 9 rats in the POR group. In the remaining subjects, POR damage was bilateral and primarily localized to lateral and ventral portions, as illustrated in Figure 7. The mean percentage of POR surface area damage in the coronal sections analyzed was 19.7 ± 3.1. The POR lesion extended throughout the rostrocaudal extent of the region; 66.8 ± 4.8% of the sections analyzed were damaged. In addition, slight damage to the dorsally adjacent Tev region was observed in 1-2 sections analyzed in 4 rats. In six cases, minor damage was also evident in the neighboring entorhinal cortex.

One rat in the PER group died soon after surgery, and another was eliminated from the study due to extensive unilateral damage throughout the cerebral cortex, leaving 8 rats with PER lesions. Neurotoxic damage to the PER is depicted in the photomicrographs in Figure 8. On average, 59.7 ± 7.9% of the total surface area of the PER was damaged in the coronal sections analyzed. The PER lesion extended throughout the rostrocaudal extent of the region, encompassing 82.4 ± 6.7% of the sections analyzed. Additionally, damage consistently extended ventrally into adjacent portions of entorhinal cortex and occasionally into area Tev, as shown in Figure 9. In six cases, there was also slight damage to the most caudal regions of the CA1 field of the hippocampus on 1-2 sections.
Figure 5. A: Fear conditioning to the tone in Experiment 1 as measured by time spent freezing during the 20-s conditioned stimulus (CS) period during training (Days 1 and 2) and testing (Day 3). Control and lesioned groups displayed comparable amounts of freezing to the tone CS. B: Fear conditioning to the context as measured by time spent freezing during the 20-s pre-CS period during training and testing. The postrhinal cortex (POR)-lesioned and perirhinal cortex (PER)-lesioned groups were significantly impaired in exhibiting freezing behavior associated with the context. SH = sham controls.

Behavior. Freezing behavior exhibited by the 8 sham-lesioned and 7 unoperated control rats did not differ during training or testing. Thus, the data from the two control groups were combined for all analyses.

On the conditioning day, rats in all groups exhibited increased postshock freezing behavior as training proceeded, as shown in Figure 10 (Panel A). However, less freezing was observed in the PER group compared to both control and POR-lesioned rats. A repeated measures ANOVA revealed a significant main effect of group, $F(2, 29) = 11.14, p = .0003$, and trial, $F(3, 87) = 106.25, p < .0001$, as well as a significant Group × Trial interaction, $F(6, 87) = 5.89, p < .0001$. Planned comparisons of overall freezing indicated a significant difference between the PER group and both the control and POR groups, but no significant difference between the control and POR groups.

Freezing behavior during the extinction test is presented in Figure 10, Panel B. Both POR- and PER-lesioned rats exhibited less fear conditioning to the context than did control rats. These findings were confirmed by a repeated measures ANOVA, which revealed a main effect of group, $F(2, 29) = 9.50, p = .0007$. Subsequent pairwise comparisons indicated that both lesioned groups differed significantly from the control groups but not from each other. There was also a significant main effect of block, $F(7, 203) = 10.21, p < .0001$, but no significant Group × Block interaction, $F(14, 203) = 0.80, p > .67$.

Additional analyses were conducted to determine whether damage to adjacent regions affected contextual fear conditioning in the lesioned rats. An ANOVA revealed no significant difference in freezing between POR-lesioned rats with and without entorhinal cortex damage ($p > .21$). Similarly, POR-lesioned rats with damage to area Te, did not differ from those without damage to that area ($p > .22$). In the PER group, the behavior of rats with slight damage to area CA1 did not differ from the rest of the rats in the PER group ($p > .69$).

Discussion

Neurotoxic lesions of either the POR or PER, carried out prior to training, produced significant deficits in contextual fear conditioning in an unsignaled shock procedure. The results of this experiment, using a different lesion technique and conditioning protocol provide additional evidence that the POR and PER play an important role in contextual fear conditioning. Indeed, pretraining neurotoxic lesions of the hippocampus, in comparison, did not produce deficits in the same task (Maren et al., 1997), suggesting that POR and PER function may be critical for normal fear conditioning to contextual cues. Furthermore, previous studies have suggested that hippocampal involvement in contextual fear conditioning may vary depending on whether the delivery of footshock is preceded by an explicit CS. It has been proposed that the hippocampus is particularly involved in processing contextual information when contextual cues are present in the background during conditioning; that is, when delivery of footshock is signaled by a CS (Phillips & LeDoux, 1994). The results of Experiments 1 and 2 indicate that both POR and PER lesions impair contextual conditioning in both signaled and un signaled versions of the task, suggesting that these cortical regions may have a fundamental role in the processing of sensory information in contextual fear conditioning.

In the present experiment, the deficits produced by neurotoxic damage to the POR differed somewhat from those produced by PER lesions. Damage to either region produced deficits in the expression of conditioned fear to the context during the extinction test; however, only PER-lesioned rats exhibited a statistically significant decrease in postshock freezing during training. It is possible that this difference was due to the relatively small size of the POR lesions compared with the PER lesions (19.7% vs. 59.7% of the surface area damaged, respectively), and that larger POR lesions may have produced deficits in postshock freezing as well.
Figure 6. Photomicrograph of a representative postrhinal cortex (POR) neurotoxic lesion at three rostrocaudal levels (Experiment 2). Arrows indicate cytoarchitectonic boundaries of the POR.

If this is the case, the deficits observed during the extinction test could reflect poor acquisition of contextual fear on the conditioning day, possibly caused by impaired processing of sensory information during conditioning. It is also possible that the POR or PER have a role in the subsequent expression of conditioned fear. This possibility was directly tested by examining the effects of post-training lesions of the POR or PER, as described in Experiment 3.

Experiment 3

Rats were first trained in the same behavioral procedures described in Experiment 2; they then received neurotoxic POR or PER lesions before being returned to the conditioning chambers for the extinction test. Using this paradigm, previous studies have indicated that posttraining hippocampal lesions produce severe retrograde deficits in contextual fear (Kim & Fanselow, 1992; Maren et al., 1997). This effect, however, is only observed at short training-to-lesion intervals, suggesting that other regions are involved in the long-term storage and expression of contextual memory. On the basis of the neuroanatomical connections between the POR, PER, and hippocampus, it would seem likely that the POR and PER may be involved in the extended processing and storage of contextual information and might thus be expected to play a significant role in the subsequent expression of contextual fear. It was predicted that both POR and PER lesions, produced one day after training, would impair the expression of contextual fear.

Method

Subjects. The subjects were 22 male Long-Evans rats weighing approximately 350 g. The rats were housed and maintained as described in Experiments 1 and 2. Before training, each rat was handled by the experimenter for 1–2 min each day for 1 week.

Surgery. Bilateral neurotoxic lesions (ibotenic acid) of the POR (n = 6) or PER (n = 6), or sham surgeries (n = 6) were carried out as described in Experiment 2, with the exception that the surgery took place 24 hr after behavioral training. Rats were allowed to recover from surgery for 2 weeks before being returned to the behavioral chambers for the extinction test.

Apparatus and behavioral procedures. The behavioral apparatus was identical to that used in the previous experiments. As in Experiment 2, the
rats were habituated to the plastic transporter 1 day prior to conditioning and were trained in the same Pavlovian fear conditioning paradigm. After training, rats were assigned to either the POR or PER lesion group, the sham-lesioned control group, or an unoperated control group (counterbalanced for conditioning chamber and matched for training performance). The extinction test took place 2 weeks after training to allow for recovery from surgery. The behavioral data were scored by two observers who were unaware of lesion condition, and the results were highly correlated ($r^2 = 0.9$, $p < .0001$).

**Histology and data analysis.** The histological procedures and data analyses were identical to those used in Experiments 1 and 2.

**Results**

**Histology.** Both the POR and PER neurotoxic lesions were similar to those described in Experiment 2. The mean percentage of POR surface area damage in the coronal sections analyzed was 37.2 ± 7.5; damage was apparent on 93.2 ± 4.0% of the sections along the rostrocaudal axis. Minor damage extended into the entorhinal cortex and, in one case, into area Te v. In the PER group, 62.5 ± 5.4% of the PER surface area was damaged in the sections analyzed; PER damage was apparent on 89.7 ± 3.8% of the sections. Additionally, there was minor damage to the entorhinal cortex and area Te v and slight damage to area CA1 on the most caudal sections.

**Behavior.** As in Experiment 2, the freezing behavior exhibited by the 6 sham-lesioned rats and 4 naive rats did not differ on any behavioral measure. Data from both control groups were combined for all subsequent analyses.

As shown in Figure 11, Panel A, the level of postshock freezing during training was comparable in all groups and increased as training continued. A repeated measures ANOVA revealed a significant effect of trial, $F(3, 57) = 68.18$, $p < .0001$, but no significant main effect of group, $F(2, 19) = 0.06$, $p > .93$, or Group × Trial interaction, $F(6, 57) = 0.46$, $p > .83$. 

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**Figure 8.** Photomicrograph of a representative perirhinal cortex (PER) neurotoxic lesion at three rostrocaudal levels (Experiment 2). Arrows indicate cytoarchitectonic boundaries of the PER.

**Figure 9.** Schematic of the largest (gray) and smallest (black) perirhinal cortex (PER) neurotoxic lesions at three rostrocaudal levels (Experiment 2). Arrows mark the upper and lower boundaries of the PER. LEA = lateral entorhinal cortex; Pir = piriform cortex; Te v = temporal association cortex, ventral.
Freezing behavior observed during the extinction test is shown in Figure 11, Panel B. As in Experiment 2, both POR- and PER-lesioned rats exhibited significantly less freezing behavior during the extinction test than did control rats. A repeated measures ANOVA revealed significant main effects of group, $F(2, 19) = 25.06, p < .0001$, and block, $F(7, 133) = 14.53, p < .0001$, as well as a significant Group × Block interaction, $F(14, 133) = 2.79, p = 0.001$. Planned comparisons of overall freezing confirmed that both the POR group and PER group differed significantly from the control group, but there was no significant difference between the two lesioned groups.

General Discussion

These experiments were designed to examine the role of the POR and PER in mnemonic processing of contextual or relational information. In the service of this aim, we examined the role of the POR in contextual fear conditioning and more fully assessed the function of the PER in this type of learning. In Experiment 1, electrolytic POR or PER lesions produced deficits in conditioning to the training context but not in fear conditioning to an explicit auditory cue. Experiments 2 and 3 used a different paradigm, in which the behavioral procedures were divided into a distinct training day and testing day and shock delivery was not signaled by a CS. Pretraining neurotoxic lesions of either region disrupted the expression of contextual fear, and damage to the PER, but not the POR, produced significant deficits in postshock freezing during training (Experiment 2). In Experiment 3, neurotoxic POR or PER lesions produced 1 day after training impaired the subsequent expression of contextual fear. Together, the results of these experiments, which used multiple behavioral paradigms and lesion techniques, indicate that the POR and PER make an important contribution to both the acquisition and expression of contextual fear and may thus play a key role in processing contextual information.

To our knowledge, the present study represents the first systematic examination of POR involvement in Pavlovian fear conditioning. Although little is known about the function of the POR, these data suggest that it contributes to contextual or relational information processing, a finding that is consistent with the observed neuroanatomical connections. The finding that PER damage produced both anterograde and retrograde deficits in contextual fear conditioning is in partial agreement with the results of previous studies. A prior investigation of the effects of posttraining POR lesions also revealed retrograde deficits in contextual fear conditioning (Corodimas & Ledoux, 1995). Similarly, posttraining reversible inactivation of the PER impairs the expression of contextual fear (Sacchetti et al., 1999). In contrast, prior studies suggested that pretraining POR lesions do not impair fear conditioning to contextual cues (Herzog & Otto, 1997, 1998; Phillips & LeDoux, 1995). Some of those studies, however, used relatively small lesions of the anterior PER that spared caudal portions of the region (Herzog & Otto, 1997, 1998). Visuospatial input terminates in caudal areas of the PER (Burwell & Amaral, 1998a); thus, for the present task, if those areas were largely spared, conditioning to contextual cues might also have been spared.

The organization of connections within the PER suggests that damage throughout the rostrocaudal extent of this region may be necessary to produce behavioral deficits. Neocortical sensory input to the PER terminates primarily in the dorsal third of the region (dorsal area 36), whereas entorhinal and hippocampal efferents originate in the ventral third (area 35). Perirhinal intrinsic connections are organized such that the flow of information is mainly dorsal to ventral, with fewer associational connections oriented rostrocaudally (Burwell & Amaral, 1998b). One can think of the region as organized into coronal slabs stacked in the rostrocaudal plane; a small amount of damage within a slab would disrupt function at that rostrocaudal level. Thus, as long as some damage in the dorsoventral plane was achieved at all rostrocaudal levels, as...
in the present experiments, normal function of the region would be disrupted.

A previous result that is difficult to reconcile with the present findings is the report that very large pretraining PER lesions had no effect on contextual fear conditioning (Phillips & LeDoux, 1995). In the present experiments, pretraining PER lesions consistently produced deficits in contextual fear conditioning tasks that used multiple behavioral protocols and assessments of conditioned freezing, as well as multiple methods of damaging the entire rostrocaudal extent of the PER. Hence, it seems likely that this cortical area is indeed involved in the acquisition of contextual fear.

A possible limitation of the present study involves the extent of neurotoxic damage observed in Experiments 2 and 3. In both POR- and PER-lesioned rats, damage often extended into the neighboring entorhinal cortex; thus, it is possible that the observed behavioral deficits were due to entorhinal cortex damage and not POR or PER damage per se. Indeed, there is recent evidence that entorhinal cortex damage can produce deficits in contextual fear conditioning (Maren & Fanselow, 1997). Given the present findings, it is not surprising that entorhinal cortex lesions would impair contextual conditioning because a substantial proportion of the POR and PER input to the hippocampus projects through the entorhinal cortex. There are several factors, however, that argue against the possibility that entorhinal cortex damage alone was responsible for the observed impairments. For instance, there was no difference in the performance of POR-lesioned rats with or without entorhinal cortex damage. Additionally, deficits in contextual freezing were observed in Experiment 1, despite the fact that there was little or no damage outside of the target region in either POR- or PER-lesioned rats. Thus the impairments observed in the present series of studies most likely resulted from damage to the POR or PER.

In studies that use Pavlovian fear conditioning, impaired freezing behavior is often attributed to deficits in learning and memory of conditional fear. Some investigators have argued, however, that the observed impairments may instead be due to an inability to perform fear responses (e.g., freezing). In the present experiments, both POR- and PER-lesioned rats exhibited normal freezing to an auditory CS despite severe deficits in contextual freezing. These data argue against performance deficits and support the notion that POR or PER damage produced deficits in the acquisition and expression of contextual fear.

Given the neuroanatomical connections of the POR and PER, a role for these regions in contextual fear conditioning is not surprising. The POR and PER are polymodal association regions that provide the major cortical sensory input to the hippocampal formation and receive strong reciprocal connections from the hippocampus itself (Burwell & Amaral, 1998b; Burwell et al., 1995; Kosel et al., 1983; Naber et al., 1997; Shi & Cassell, 1997). As such, the hippocampal involvement in contextual conditioning may be dependent on input from the POR and PER. Neuroanatomical studies, however, now indicate that sensory input reaches the hippocampus via parallel, redundant pathways (Burwell & Amaral, 1998b; Burwell et al., 1995; Naber et al., 1997). Thus, a POR or PER lesion would not disconnect the hippocampus from all sensory input. The neuroanatomy, together with the present findings that pretraining POR or PER lesions impair contextual fear conditioning, suggests instead that these regions may be involved in a more fundamental role. In other words, the higher order processing of information that occurs in the POR and PER is critical for normal conditioning to contextual cues in this task.

The present data, along with the results of recent inactivation studies, indicate that the POR and PER are also involved in processing contextual information beyond the level of acquisition or encoding. Both POR and PER damage produced 1 day after training disrupted the expression of contextual fear. Similarly, reversible PER inactivation at different time points after training impairs the expression of contextual fear when rats are placed back in the training environment (Sacchetti et al., 1999). Interestingly,
in the Sacchetti et al. study, PER inactivation disrupted expression at longer posttraining intervals compared with inactivation of the hippocampus. Together with the present results, these data indicate that the PER and PO may be involved in the consolidation and possibly the storage of contextual information.

This study provides direct evidence that the PO and PER have a role in contextual or relational memory. This study represents the first published examination of the role of the PO in fear conditioning and one of the few studies of its contribution to mnemonic function. The PO and PER may be involved in the acquisition, encoding, and subsequent processing and storage of contextual information, although further study is needed to assess more completely the mnemonic function of these regions. For example, it will be important to determine the particular operations performed by the PO and PER on polymodal sensory information in support of contextual learning. Further study will also be necessary to examine the functional interaction between the PO and PER and the hippocampus. Given the time-limited role of the hippocampus in contextual fear conditioning, it would also be informative to further investigate the time course of PO and PER involvement in the expression of contextual fear.

References


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**Call for Nominations**

The Publications and Communications Board has opened nominations for the editorships of *Journal of Applied Psychology, Journal of Consulting and Clinical Psychology, Journal of Educational Psychology, Psychological Bulletin*, and *Journal of Personality and Social Psychology: Interpersonal Relations and Group Processes* for the years 2003–2008. Kevin R. Murphy, PhD, Philip C. Kendall, PhD, Michael Pressley, PhD, Nancy Eisenberg, PhD, and Chester A. Insko, PhD, respectively, are the incumbent editors.

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